Review

Clinical Cancer Research

Emerging Targeted Therapy for Tumors with *NTRK* Fusion Proteins

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Abstract

The oncogenesis-promoting role of chromosomal rearrangements for several hematologic and solid malignancies is well recognized. However, identifying targetable, actionable, and druggable chromosomal rearrangements remains a challenge. Targeting gene fusions and chromosomal rearrangements is an effective strategy in treating gene rearrangement–driven tumors. The *NTRK* (*N*eurotrophic *T*yrosine *R*eceptor *K*inase) gene family encodes three tropomyosin-related kinase (TRK) receptors that preserve central and peripheral nervous system development and function. *NTRK* genes, similar to other genes, are subject to alterations, including fusions. Preclinical studies have demonstrated that TRK fusion proteins promote oncogenesis by mediating constitutive cell proliferation and survival. Several clinical trials have estimated the safety and efficacy of TRK fusion kinase receptor inhibitors and have demonstrated encouraging antitumor activity in patients with *NTRK*-rearranged malignancies. Specifically, larotrectinib and entrectinib have emerged as potent, safe, and promising TRK inhibitors. Herein, we discuss the potential oncogenic characteristics of TRK fusion proteins in various malignancies and highlight ongoing clinical trials of kinase inhibitors targeting them. *Clin Cancer Res; 24(23); 5807–14. ©2018 AACR.*

Introduction

Tropomyosin-related kinase A, B, and C (TRKA, TRKB, and TRKC) are receptor tyrosine kinases encoded by the genes neurotrophic tyrosine receptor kinase 1, 2, and 3 (*NTRK1*, *NTRK2*, and *NTRK3*), respectively. TRKs are membrane-spanning receptors composed of extracellular ligand-binding, transmembrane, and intracellular ATP-binding domains (1). The extracellular domains of TrkA, TrkB, and TrkC exhibit high structural similarity, composed of three leucine-rich motifs flanked by two cysteine clusters and two immunoglobulin-like I set domains (2). The immunoglobulin-like regions are believed to encompass the ligand-binding sites. TRKs serve as signal receptors for neurotrophins, their cognate ligands. Nerve growth factor, brain-derived growth factor, and neurotrophin 3/4 are neurotrophic factors that activate TrkA, TrkB, and TrkC, respectively. TRKs play a pivotal role in the physiology, development, and function of the peripheral and central nervous systems (3, 4). Ligand–receptor interaction results in receptor dimerization and subsequent phosphorylation of the kinase domain. Activated kinases promote cell proliferation, differentiation, and survival by triggering downstream intracellular signal transduction pathways (refs. 5–9; Fig. 1).

NTRK rearrangements are the most common alterations in *NTRK*-mutated tumors (10). Our discussion herein focuses on the role of *NTRK* fusions in cancer and ongoing clinical trials involving TRK inhibitors.

NTRK Fusions in Carcinogenesis

NTRK oncogenic fusions arise from exact intrachromosomal or interchromosomal rearrangements that juxtapose the kinase domain-containing 3′ region of *NTRK* with the 5′ region of *NTRK*'s gene partner. Chimeric fusion proteins promote tumorigenesis via constitutive ligand-free activation of intracellular biological pathways and signal transduction cascades that control cell-cycle progression, proliferation, apoptosis, and survival (11). Preclinical data demonstrated that chimeric oncogenic fusions may lead to partial or complete deletion of the immunoglobulin-like domain of TRK, which has an inhibitory influence on downstream signaling pathways in the absence of activating ligands (2). Several *NTRK* fusion partners have been identified so far and shown to contribute to the development of various cancer types (Table 1).

Prevalence of *NTRK* Fusions in Solid Tumors

NTRK oncogenic fusions are infrequent but recurrent events observed in various types of congenital and acquired cancers (Table 2). The exact frequency of *NTRK* fusions in solid tumors remains unclear. The variations in frequencies among different studies and tumors subtypes may be biased by screened study cohorts and *NTRK* fusion detection techniques. In an analysis of over 11,000 patients conducted by Caris Diagnostics, TRK fusion proteins were detected by immunohistochemistry (IHC) in only 26 patients (0.23%; Gatalica and colleagues abstract TARG-17-A047). Most common fusions detected were *TPM3* (*Tropom*yosin 3)-*NTRK1*, and

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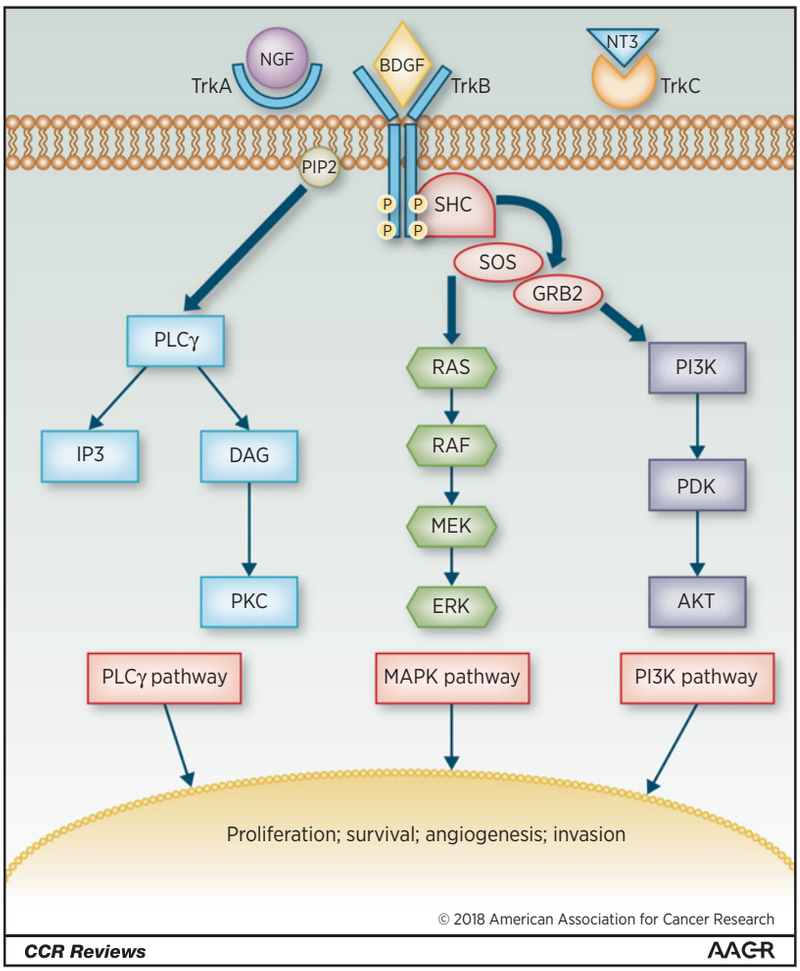

Figure 1.
The TRK signaling pathways. Interaction between TRK and its cognate ligand will lead to downstream signal transduction, resulting in activation of intracellular pathways responsible for cellular proliferation, survival, and invasion. BDGF, brain-derived growth factor; DAG, diacylglycerol; ERK, extracellular signal-regulated kinase; GRB2, growth factor receptor-bound protein 2; IP3, inositol trisphosphate; MEK, mitogen-activated protein kinase kinase; NGF, nerve growth factor; NT3, neurotrophin 3; PDK, phosphoinositide-dependent kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLCγ, phospholipase C-γ; RAF, rapidly accelerated fibrosarcoma kinase; RAS, rat sarcoma kinase; SHC, Src homology 2 domain containing; SOS, sons of sevens.

ETV6 (ETS Variant 6)-NTRK3 (6 cases each). Furthermore, Stransky and colleagues (12) reported various *NTRK* fusions in 9 of 20 screened cancer samples. The estimated prevalence varies among histologic subtypes and fusion partners. The annual incidence of *NTRK* fusion–driven tumors is estimated to be 1,500 to 5,000 cases in the United States (13). Specific findings for *NTRK* fusions by tumor type are described below.

Lung cancer

NTRK fusions are rare in lung cancer. Using next-generation sequencing (NGS) and FISH, Vaishnavi and colleagues (14)

Table 1. *NTRK* gene family fusion partners and associated cancers

Tumor	*NTRK1*	*NTRK2*	*NTRK3*
CRC	*TPM3* (16, 18), *LMNA* (19), *TPR* (51), *SCYL3* (20)		
NSCLC	*CD74* (14), *MPRIP* (14), *SQSTM1* (15)	*TRIM24* (12)	
GBM	*ARHGEF2* (27), *BCAN* (52), *NFASC* (53), *TPM3* (54)		*ETV6* (27, 54)
Pilocytic astrocytoma		*NACC2* (55), *QKI* (55)	
Spitzoid melanoma	*TP53* (56), *LMNA* (56)		
Papillary thyroid cancer	*TPM3* (57),*TFG* (58), *TPR* (59)		
MASC			*ETV6* (60, 61)
SBC			*ETV6* (34)
Infantile fibrosarcoma	*LMNA* (62)		*ETV6* (63)
HNSCC		*PAN3* (12)	
Mesoblastic nephroma			*ETV6* (64)
GIST			*ETV6* (28, 29)

Abbreviations: *ARHGEF2*, rho/rac guanine nucleotide exchange factor 2; *BCAN*, brevican; CRC, colorectal cancer; *ETV6*, ETS variant 6; GBM, glioblastoma multiforme; GIST, gastrointestinal stromal tumor; HNSCC, head and neck squamous cell carcinoma; *LMNA*, lamin A/C; MASC, mammary analog secretory carcinoma; *MPRIP*, myosin phosphatase rho interacting protein; *NACC2*, NACC family member 2; *NFASC*, neurofascin; NSCLC, non–small cell lung cancer; *PAN3*, poly(A)-specific ribonuclease subunit; *QKI*, KH domain containing RNA binding; *SBC*, secretory breast carcinoma; *SCYL3*, SCY1 like pseudokinase 3; *SQSTM1*, sequestosome 1; *TFG*, TRK-fused gene; *TP53*, tumor protein P53; *TPM3*, tropomyosin 3; *TPR*, translocated promoter region; *TRIM24*, tripartite motif containing 24.

Table 2. Prevalence of *NTRK* gene fusions in solid tumors

Tumor type	Prevalence (%)	Detection methods	References
Appendiceal cancer	2/97 (2%)	MSK-IMPACT/sequenum	Braghiroli et al. (65)
Cholangiocarcinoma	1/28 (4%)	DNA seq	Ross et al. (66)
CRC	13/346 (4%)	NGS	Pietrantonio et al. (22)
CRC MSI-H	10/13 (76.9%)	NGS	Pietrantonio et al. (22)
Melanoma	1/374 (0.3%)	RNA-seq	Stransky et al. (12)
GBM	3/115 (3%)	AMP	Zheng et al. (27)
HNC	2/411 (0.5%)	RNA-seq	Stransky et al. (12)
Infantile fibrosarcoma	2/4 (50%)	FISH	Knezevich et al. (63)
Low-grade glioma	2/461 (0.4%)	RNA-seq	Stransky et al. (12)
Lung adenocarcinoma	3/91 (3.3%)	NGS/FISH	Vaishnavi et al. (14)
MASC	2/3 (66 %)	FISH/RT-PCR	Skalova et al. (35)
PTC	4/33 (12%)	RT-PCR	Brzezianska et al. (67)
PHGG	28/127 (22%)	NGS	Wu et al. (54)
Polycystic astrocytoma	3/96 (3%)	WGS	Jones et al. (55)
SBC	12/13 (92%)	RT-PCR	Tognon et al. (34)
Spitzoid melanoma	23/140 (16%)	NGS	Wiesner et al. (56)

Abbreviations: AMP, anchored multiplex polymerase chain reaction; CRC; colorectal cancer; DNA seq, DNA sequencing; GBM, glioblastoma multiforme; HNC, head and neck cancer; MASC, mammary analog secretory carcinoma; MSI-H, microsatelite instability-high; NGS, next-generation sequencing; PHGG, pediatric high-grade glioma; PTC, papillary thyroid carcinoma; RNA-seq, RNA sequencing; RT-PCR, reverse transcriptase polymerase chain reaction; SBC, secretory breast carcinoma; WGS, whole-genome sequencing.

detected two novel *NTRK1* gene fusion partners: *M*yosin *P*hosphatase *R*ho *I*nteracting *P*rotein (*MPRIP*) and *CD74*. The estimated frequency of *NTRK* fusion in this study was 3.3%, with 3 of 91 patients having *NTRK1* fusions (14). The screened cohort, however, did not exhibit any other chromosomal alterations except for *NRTK* gene rearrangements. Furthermore, in a phase I study, Farago and colleagues (15) performed an anchored multiplex polymerase chain reaction (AMP) test to screen 1,378 cases of non–small cell lung cancer (NSCLC) for *NTRK1*, *NTRK2*, *NTRK3*, *ALK* (*A*naplastic *L*ymphoma *K*inase), and *ROS1* (*ROS* Proto-Oncogene*1*) fusions. Utilizing FISH, *NTRK1* gene fusions were detected in 2 patients: one with a *TPM3-NTRK1* rearrangement, whereas the second patient, who had stage VI lung adenocarcinoma, harbored a novel *NTRK1* gene partner, *SQSTM1* (*S*equestosome *1*). *NTRK* fusions were estimated to occur at a rate of 0.1% (95% confidence interval, 0.01%–0.5%; ref. 15). Because FISH was used to confirm the presence of these genetic alterations in both studies (14, 15), the discrepancy in the reported frequencies between these two studies may be attributed to their difference in sample size and screened patient populations.

Colorectal cancer

NTRK gene fusions are also uncommon in colorectal cancer, and their estimated frequency likewise varies across different studies (0.5%–2.0%; refs. 16, 17). Several studies have demonstrated *TPM3-NTRK1* fusions in colorectal cancer patients (16–18). Creancier and colleagues (17) performed IHC and quantitative reverse transcriptase-PCR tests to detect *NTRK* rearrangements in 408 cases of patients belonging to all clinical stages of colorectal cancer (I, II, III, and IV). Two cases (0.5%) were *NTRK* fusion positive. A *TPR* (*T*ranslocated *P*romoter *R*egion)-*NTRK1* oncogenic fusion was identified in a 53-year-old female with stage II, poorly differentiated adenocarcinoma. This patient also harbored wild-type (wt) *KRAS* (*K*irsten *RA*t *S*arcoma viral oncogene homolog), *NRAS* (*N*euroblastoma *RAS*), and *BRAF* (*B R*apidly *A*ccelerated *F*ibrosarcoma), but is MSI-positive/microsatellite instability high (MSI-H) with loss of *MLH1* (*M*ut*L H*omolog *1*)/*PMS2* (*Postme*iotic *S*egregation Increased 1 Homolog *2*) and no *MLH1* promoter methylation. In addition, *TPM3-NTRK1* fusion was detected in a 66-year-old male with moderately differentiated adenocarcinoma of the left colon, also having wt *KRAS*, *NRAS*, and *BRAF*, and is MSI positive. A novel gene fusion with oncogenic potential, *LMNA* (*Lamin A*/C)-*NTRK1*, was detected by FISH in a patient with liver and adrenal gland metastases of colorectal cancer (19). In another study (20), a 61-year-old colorectal cancer patient with high MSI-H, wt *RAS*, *BRAF*, and EGFR harbored a novel *SCYL3* (*SCY1 L*ike pseudokinase protein *3*)-*NTRK1* rearrangement. Moreover, a retrospective study found *NTRK* fusions occurring in 2.5% of 2,044 heavily pretreated patients with metastatic colorectal cancer (21). This is in contrast with a study by Pietrantonio and colleagues (22), wherein they identified *NTRK* gene rearrangements in 13 of 346 (4%) metastatic colorectal cancer patients. Ten of the 13 patients [(76.9%), $P < 00.1$] with *NTRK*-rearranged tumors also had MSI-H status. Genetic alterations were detected using a targeted NGS technique utilizing Foundation One, MSK-IMPACT, and Minerva panel (22). Although *NTRK* gene fusions were screened and detected using highly specific techniques, the sample size was small and may have overestimated the exact prevalence of *NTRK* fusions in patients with MSI-H metastatic colorectal cancer.

Papillary thyroid carcinoma

In general, the estimated prevalence rate of *NTRK* fusions in patients with papillary thyroid carcinoma (PTC) does not exceed 12% (10) and varies among study populations according to geographical distribution and methods of detections (23). *NTRK* fusion oncogenes were also detected in 7 of 27 (26%) PTC patients in a pediatric population. Patients having *NTRK*-rearranged PTC presented with extensive disease and had worse prognosis than those with *BRAF* mutations (24). Although *ETV6-NTRK3* is a rare somatic gene fusion in sporadic thyroid cancers, it was found to be more common in radiation-related tumors (25).

Brain tumors

The rate of occurrence of *NTRK* fusions in brain tumors varies based on age group: 40% in pediatric versus 3% in adult-type tumors (12, 26, 27). *NTRK2* fusions are most frequently detected in glioblastoma multiforme (GBM; Gatalica and colleagues abstract TARG-17-A047), whereas *NTRK1* rearrangements were detected in only 3 of 115 (3%) patients with GBM so far (27).

Sarcomas

Sarcomas represent a wide spectrum of uncommon tumors. Yet, in phase I and phase II studies which included 17 different *NTRK* fusion–positive tumor types detected by FISH or NGS, 21 of 55 (38%) patients were diagnosed with sarcoma including 3 patients with gastrointestinal stromal tumor (GIST; ref. 13). Drilon and colleagues (13) showed that sarcoma including soft tissue, infantile fibrosarcoma, and GIST comprises the largest cohort of cancer patients to harbor *NTRK* fusions in their study. Moreover, two other studies identified 1 patient each with *ETV6-NTRK3* fusion GIST (28, 29). Interestingly, both patients exhibited WT *KIT/PDGFR/BRAF* disease. Overall, the estimated prevalence rate of *NTRK* fusions in sarcomas ranges from 1% in adult-type sarcomas to 92% in patients with congenital fibrosarcoma (12, 13, 30). Furthermore, Doebele and colleagues (31, 32) detected a novel *LMNA-NTRK1* fusion using the Foundation One Heme assay in a 41-year-old woman with metastatic soft-tissue sarcoma to the lungs.

Other rare tumors

Secretory breast cancer and mammary analog secretory carcinoma (MASC) of the salivary gland are rare tumors with distinct clinical and pathologic features. However, they harbor the same underlying pathognomonic genetic alteration, *ETV6-NTRK3*, resulting from the chromosomal rearrangement t(12;15)(p12;q26.1) (33–35). *ETV6-NTRK3* fusion is detected in 92% and 100% of secretory breast cancer and MASC cases, respectively (34, 36).

Detection of *NTRK* Fusions

NGS provides a precise method to detect *NTRK* gene fusions (37). In addition to high sensitivity and specificity, it detects gene partners that might have clinical implications in future studies. Although NGS has changed the landscape of detecting chromosomal rearrangements driving tumors, several challenges remain for NTRK fusion testing (38). For example, the most popular commercially available DNA NGS panels, such as Foundation One, may not detect certain *NTRK* fusions. The addition of RNAseq to NGS testing has shown high sensitivity and specificity rates, 93% and 100% respectively, in detecting clinically actionable gene fusions (39). Data showed that RNAseq had led to unbiased results as well. In addition, RNAseq requires no prior knowledge of fusion partners or intronic/exonic break points. RNAseq use is now beyond research goals and has been incorporated into clinical practice (40).

Although FISH is considered the gold standard in detecting gene fusions, it can only detect a single target at a time. For instance, commonly used break apart FISH probe scan detect gene fusion but not the fusion partner. In addition, designing multiple probes for detecting *NTRK* fusions partners is cost ineffective and time consuming, making it not amenable for high-throughput screening (38).

Hechtman and colleagues (41) showed that pan-TRK fusion IHC test had sensitivity and specificity rates of 95.2% and 100%, respectively. Authors concluded that the pan-TRK fusion IHC test is a time- and tissue-efficient method for detecting *NTRK* fusions. However, researchers at MD Anderson Cancer Center were not able to replicate these findings (unpublished data).

A two-step diagnostic method incorporating rapid IHC screening that uses a cocktail of antibodies including anti–pan-Trk antibodies, followed by anchored multiplex PCR (AMP; ref. 38), showed that IHC screening had a 100% negative predictive value for excluding samples devoid of gene rearrangements (38).

TRK Fusion Protein Inhibitors in Clinical Trials

The estimated prevalence rates of chromosome rearrangements range from 17% to 20% in cancer (12, 42). Over the past few years, various TKIs targeting the TRK family members have been developed and tested in clinical trials. The most promising thus far are summarized below, with a complete list provided in Table 3.

MGCD-516 is a novel small-molecule multikinase inhibitor that targets MET, AXL, MER, as well as members of the VEGFR, platelet-derived growth factor receptor (PDGFR), discoidin domain receptor tyrosine kinase 2 (DDR2), and TRK families (43). A phase Ia/II trial (NCT02219711) enrolling patients with advanced solid tumors is also ongoing, with MGCD-516 administrated at escalating doses within a 21- or 28-day cycle.

TSR-011 is an oral dual ALK (IC_{50}, 0.7 nmol) and pan-TRK (IC_{50}, <3 nmol) inhibitor that had been tested in a phase I/IIa clinical trial (NCT02048488) to determine its safety, tolerability, RP2D, and antitumor activity in patients with advanced tumors refractory to previous treatment with ALK inhibitors (44). TSR-011 was administered orally in dose escalation (30–480 mg 2 or 3 times a day) to 23 patients. This trial demonstrated that TSR-011 was safe and well tolerated at a fractionated dose of 60 mg daily. Dose-limiting toxicities (DLT) were prolonged QTc and dysesthesia. Three of 5 patients with *ALK*-rearranged NSCLC achieved partial response. TSR-011 efficacy is being investigated in *ALK*- and *NTRK*-rearranged tumors.

Entrectinib is a novel, highly potent oral ATP-competitive, pan-TRK, ROS1, and ALK TKI with low to sub-nanomolar antienzymatic efficacy (IC_{50}, 0.1–1.7 nmol/L; ref. 45). Two large multicenter 3+3 phase I clinical trials, ALKA-372-001 and STARTRK-1, were designed and conducted to determine the safety, efficacy, and antitumor activity of entrectinib in patients with advanced or metastatic solid tumors harboring *NTRK1/2/3*, *ALK*, and *ROS1* rearrangements (46). A total of 119 patients (54 in ALKA-372-001, 65 in STARTRK-1) received treatment using different doses and schedules. Of them, only 60 patients possessed the aforementioned gene rearrangements. The majority of the patients (82%, 98/119) received three or more prior lines of treatment. Entrectinib was given to patients in the ALKA-372-001 on three different schedules, whereas entrectinib was administered daily for 28 days to patients in the STARTRK-1 trial. No DLTs were observed in the ALKA-372-001 trial, whereas grade 3 fatigue and grade 3 cognitive disturbances were observed in the STARTRK-01 trial with a daily entrectinib dose of 800 mg. The most common adverse events (AE) of any grade were fatigue (46%, 55/119), dysgeusia (42%, 50/119), paresthesia (29%, 34/119), nausea (28%, 33/119), and myalgia (23%, 27/119). A daily entrectinib dose of 600 mg was determined to be the maximum tolerated dose as well as the RP2D (46).

Twenty-five patients were enrolled in the phase II portions of the trials, four of whom had *NTRK* fusions. The median progression-free survival duration in patients with *NTRK*-rearranged tumors was not reached as of the data cutoff date (95% confidence

Table 3. Current clinical trials of TRK fusion inhibitors

NTRK inhibitor	Gene target	Company	Population	Disease	Phase	NCTID
LOXO-101	*NTRK1/2/3*	Loxo Oncology	Pediatric	Solid tumor	I	NCT02637687
				CNS	II	
			Pediatric	Solid tumor, NHL, histiocytic tumor	II	NCT03213704[a]
			Adult	Solid tumor	II	NCT02576431[b]
			Adult	Solid tumor	I	NCT02122913
			Pediatric	CNS	II	NCT03155620[a]
			Adult	HNC	II	NCT02465060[a]
Entrectinib	*NTRK1/2/3, ALK, ROS1*	Ignyta	Adult	Solid tumor	II	NCT02568267[b]
			Adult	Solid tumor	I	NCT02097810
			Pediatric	Solid tumor, neuroblastoma, CNS	I	NCT02650401
			Adult	Melanoma	II	NCT02587650[b]
LOXO-195	*NTRK1/2/3*	Loxo Oncology	Adult	Solid tumor	I, II	NCT03215511
TSR-011	*NTRK1/2/3, ALK*	Tesaro	Adult	Solid tumor	I	NCT02048488
				Lymphoma	II	
PLX-7486	*NTRK1/2/3, CSF1R*	Plexxikon	Adult	Solid tumor	I	NCT01804530
MGCD-516	*NTRK1/2/3, KDR, MET, KIT, PDGFR, DDR2*	Mirati Therapeutics	Adult	Solid tumor	I	NCT02219711
			Adult	Urinary tract tumor	I, II	NCT03015740[c]
			Adult	Liposarcoma	II	NCT02978859
			Adult	NSCLC	II	NCT02954991
DS-6051b	*NTRK1/2/3, ROS1*	Daiichi Sankyo	Adult	Solid tumor	I	NCT02675491
			Adult	Solid tumor	I	NCT02279433
DCC-2701	*MET, TRK, VEGFR2, TIE2*	Deciphera Pharmaceuticals	Adult	Solid tumor	I	NCT02228811
Cabozantinib	*NTRK2, RET, KIT, FLT3, MET, KDR, FLT1, FLT4, AXL*	Exelixis	Adult	NSCLC	II	NCT01639508
Merestinib	*NTRK1/2/3, MET, AXL, ROS1, MKNK1, MKNK2, FLT3, TEK, DDR1, DDR2*	Eli Lilly	Adult	Solid tumor	II	NCT02920996

Abbreviations: CNS, central nervous system; CSF1R, colony-stimulating factor 1 receptor; DDR1/2, discoidin domain receptor tyrosine kinase1/2; FLT1, fms related tyrosine kinase 1; HNC, head and neck cancer; KDR, kinase insert domain receptor; MKNK, mitogen-activated protein kinase-interacting serine/threonine protein kinase; NCTID, ClinicalTrial.gov identifier; NHL, non-Hodgkin lymphoma; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection; VEGFR2, vascular endothelial growth factor receptor 2.

[a]National Cancer Institute MATCH Trials.

[b]Basket trials.

[c]Combined with nivolumab.

interval, 3.5 months–not reached; ref. 46). The objective response rate was 100% (95% confidence interval, 44%–100%) in patients with *NTRK*-rearranged tumors, which include NSCLC (*SQSMT1-NTRK1*), metastatic colorectal cancer (*LMNA-NTRK1*), MASC (*ETV6-NTRK3*; refs. 15, 19, 46, 47), and glioneuronal tumor *BCAN* (*Brevican*)*-NTRK1* (48). The majority of the responses occurred within the first two cycles of treatment. The authors concluded that entrectinib is a safe, well-tolerated pan-TRK/ROS1/ALK inhibitor, with patients having NTRK fusion–rearranged malignancies exhibiting the most clinically promising responses (46).

Larotrectinib is a highly selective, potent, ATP-competitive, and small-molecule pan-TRK inhibitor with an IC_{50} in the low nanomolar range (31). The safety and efficacy of larotrectinib in treatment of locally advanced or metastatic solid tumors were investigated in a series of multicenter phase I and II clinical trials. A total of 55 patients with 17 different types of *NTRK* fusion–driven solid tumor [median age, 45 years (range, 0.3–76.0 years); Eastern Cooperative Oncology Group score $\leq$3] were enrolled in three trials: 8 in an adult phase I trial, 12 in the SCOUT pediatric phase I/II trial, and 35 in the NAVIGATE phase II basket trial (13). Larotrectinib was administered at 100 mg twice daily. Fifty-five percent of the patients were treatment-naïve or had received one prior line of treatment, whereas 31% had received at least three lines. As of the data cutoff date (July 17, 2017), the objective, partial, and complete response rates according to investigator assessment were 80% (95% confidence interval, 67%–90%), 64%, and 16%, respectively. Nine percent of the patients had stable disease, and 71% of responses were ongoing at 1-year follow-up. The median duration of response and progression-free survival had not been reached after a median follow-up durations of 8.3 months and 9.9 months, respectively. The median time to first response was 1.8 months. The one-year progression-free survival was 55% (13).

Eight patients (15%) needed dose reductions, with tumor regression maintained in all of them (one complete response, five partial responses, and one stable disease). Majority of AEs (93%) were grade 1or 2. Grade 3 or 4 AEs were anemia (11%), fatigue (5%), increased alanine transaminase or aspartate transaminase level (7%), nausea (2%), and dizziness (2%). Grade 3 treatment-related AEs were noted in less than 5% of the patients. Overall, larotrectinib is a safe, well-tolerated pan-TRK inhibitor in adults and children, and may be a new standard of care for *NTRK*-rearranged tumors.

Larotrectinib and entrectinib are the most clinically effective TKIs that target TRK fusion proteins. Although entrectinib also targets *ROS1* and *ALK* fusion proteins, larotrectinib is, by far, the only highly selective pan-TRK inhibitor in clinical trials. Both drugs are safe and well tolerated, have ability to cross the blood–brain barrier, and control brain metastatic disease (13, 46). Although entrectinib's antitumor activity was tested in two phase I trials, responses were limited to 25 patients and only 4 had *NTRK*-rearranged tumors with partial response.

Table 4. Acquired *NTRK* mutation–mediated resistance to treatment with TRK inhibitors (13)

Oncogenic fusion	Mutation	Tumor type
TPM3-NTRK1	*NTRK1* p.G595R[a]	Colorectal cancer[c]
	NTRK3 p.F589L[b]	
LMNA-NTRK1	*NTRK1* p.G595R[a]	Colorectal cancer[c]
TPR-NTRK1	*NTRK1* p.G595R[a]	NSCLC
	NTRK1 p.G667S[d]	
ETV6-NTRK3	*NTRK3* p.G623R[a]	Infantile sarcoma
LMNA-NTRK1	*NTRK1* p.F589L[b] + *GNAS* p.Q227H	Cholangiocarcinoma
CTRC-NTRK1	*NTRK1* p.A608D	Pancreas
IRF2BP2-NTRK1	*NTRK1* p.G595R[a]	Thyroid
ETV6-NTRK3	Not tested	Salivary gland
TPM3-NTRK1	*NTRK1* p.G595R[a]	Soft-tissue sarcoma
ETV6-NTRK3	*NTRK3* p.G623R[a]	GIST
	NTRK3 p.G696A[d]	

Abbreviations: CTRC, chymotrypsin c; IRF2BP2, interferon-regulatory factor 2 binding protein 2.
[a]Solvent-front mutations
[b]Gatekeeper mutations.
[c]Treated with LOXO-195 (second line).
[d]xDFG mutations.

Larotrectinib, on the other hand, showed robust outcomes in a series of phase I and II trials, which enrolled 55 patients with 17 unique NTRK fusion–positive solid tumors, who achieved overall response rate (ORR) and complete response rates of 80% and 16%, respectively (13). The FDA awarded Orphan Drug Designation to Entrectinib in 2015. Likewise, in May 2018, the FDA granted Priority Review for larotrectinib for the treatment of adult and pediatric NTRK-rearranged tumors.

Acquired *NTRK* Mutations and Resistance to TKIs

Resistance to larotrectinib is driven by three different categories of mutations: (1) Solvent front mutations (*NTRK1* p.G595R, *NTRK3* p.G623R); (2) Gatekeeper mutations (*NTRK1* p.F589L); and (3) xDFG (*NTRK1* p.G667S, *NTRK3* p.G696A; ref. 13). Solvent front and xDFG mutations involve the nucleotide-binding and activating loop of the kinase domain, respectively, and sterically change the larotrectinib-binding site that decreases larotrectinib's inhibitory properties and potency (13). Two patients with colorectal cancer who experienced resistance to larotrectinib treatment were found to have the *NTRK* p.G595R mutation (Table 4; ref. 13)

NTRK1 p.G595R and *NTRK1* p.G667C are point mutations in the ATP-binding pocket of TrkA chimeric fusion proteins. These mutations were described in a colon cancer patient with *LMNA-NTRK1* rearrangement who had developed resistance to entrectinib. Whereas higher, clinically achievable doses of entrectinib can overcome *NTRK1* p.G667C–mediated resistance in cancer cells, no other TRK inhibitors available in clinical trials (e.g., larotrectinib, TSR-011) have demonstrated activity against *NTRK1* p.G595R.

NTRK3 p.G623R is a point mutation that mediates the resistance of *ETV6-NTRK3*–rearranged tumors to treatment with either entrectinib or larotrectinib (13, 49, 50).

LOXO-195 is a novel and highly selective second-generation pan-TRK inhibitor developed to overcome *NTRK1* p.G595R–mediated resistance to TRK inhibitors. NCT03215511 is a multicenter, open-label phase I/II clinical trial designed to evaluate the safety and efficacy of LOXO-195 in patients with *NTRK*-rearranged solid tumors.

Conclusions

Patients with *NTRK*-rearranged tumors have achieved robust and durable responses to treatment with TRK inhibitors in clinical trials. Hence, targeting *NTRK* fusion proteins is an effective strategy to improve outcomes in patients with *NTRK*-rearranged malignancies, and incorporating molecular and mutational analysis results into cancer treatment planning is crucial.

Disclosure of Potential Conflicts of Interest

D.S. Hong is a consultant/advisory board member for Bayer. No potential conflicts of interest were disclosed by the other author.

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