original report

Analysis of *NTRK* Alterations in Pan-Cancer Adult and Pediatric Malignancies: Implications for NTRK-Targeted Therapeutics

abstract

Purpose **Fusions that involve neurotrophic-tropomyosin receptor kinase (*NTRK*) genes are known drivers of oncogenesis. Therapies that target these ultra-rare, constitutionally active *NTRK* fusions have been remarkably effective. Herein, we analyze the prevalence of the full array of *NTRK* alterations—fusions, mutations, copy number alterations, and increased transcript expression—in diverse adult and pediatric tumor types to understand the landscape of *NTRK* aberrations in cancer.**

Methods **We assessed 13,467 samples available from The Cancer Genome Atlas (adult tumors) and the St Jude PeCan database (pediatric tumors) for the prevalence of *NTRK* fusions, as well as associated genomic and transcriptomic co-aberrations in different tumor types.**

Results ***NTRK* fusions were observed in 0.31% of adult tumors and in 0.34% of pediatric tumors. The most common gene partners were *NTRK3* (0.16% of adult tumors) followed by *NTRK1* (0.14% of pediatric tumors). *NTRK* fusions were found more commonly in pediatric melanoma (11.1% of samples), pediatric glioma (3.97%), and adult thyroid cancers (2.34%). Additional genomic and transcriptomic *NTRK* alterations—mutation, amplification, and mRNA overexpression—occurred in 14.2% of samples, whereas the frequency of alterations that implicated *NTRK* ligands and the *NTRK* co-receptor ($p75^{NTR}$) ranged from 3.8% to 5.4%. Among 31 adult samples carrying *NTRK* fusions, co-alterations occurred often and usually involved the downstream phosphoinositide-3-kinase signaling pathway, cell-cycle machinery, other tyrosine-kinase receptors, and mitogen-activated protein kinase signals.**

Conclusion **Whereas *NTRK* fusions are exceedingly rare, other *NTRK* abnormalities affect 14% of patients with cancer. Affecting these alterations has not yet been achievable in cancer. Genomic co-alterations occur frequently with *NTRK* fusions, but it is not known if co-targeting them can attenuate primary or secondary resistance to NTRK inhibitors.**

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INTRODUCTION

NTRK1, *NTRK2*, and *NTRK3* genes encode the neurotrophic-tropomyosin receptor tyrosine kinases (NTRKs) TrkA (NTRK1), TrkB (NTRK2), and TrkC (NTRK3). Ligands for the NTRK receptors are called neurotrophins. Nerve growth factor (NGF) binds to NTRK1; brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) and NT-5 bind to NTRK2; and NT-3 binds both NTRK1 and NTRK3.[1] Binding of neurotrophic factors to their receptors activates the downstream effectors of NTRK: phospholipase C-γ, mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3-kinase (PI3K)/AKT pathways. In addition, neurotrophins also bind to the low-affinity NGF receptor $p75^{NTR}$. $p75^{NTR}$ is a positive regulator of the NGF/NTRK1 system that reduces ligand-induced receptor ubiquitination and delays receptor internalization and degradation.[2]

NTRK receptors promote the proliferation and survival of neuronal cells[3-8] (Fig 1). Of interest,

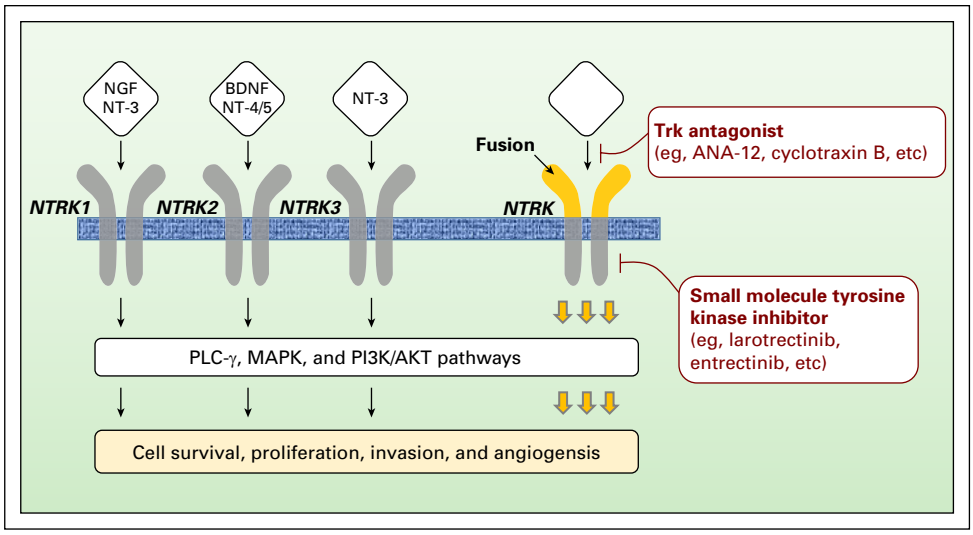

Fig 1. Neurotrophic-tropomyosin receptor tyrosine kinase (NTRK) receptor signaling pathway and inhibitors. The ligands nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and NT-4 bind to their receptors, namely NTRK1 (tropomyosin receptor kinase A or TrkA), NTRK2 (tropomyosin receptor kinase B or TrkB), and NTRK3 (tropomyosin receptor kinase C or TrkC). These receptors are under the regulation of the co-receptor p75 neurotrophin receptor ($p75^{NTR}$). The binding of the ligand to the receptor promotes to the dimerization of the receptor and its subsequent intracellular phosphorylation. Several signaling cascades are further activated—phospholipase Cγ (PLC-γ), mitogen-activated protein kinase (MAPK), and phosphoinositide-3-kinase (PI3K) —and are converging to protumorigenic cell processes, such as proliferation, survival invasion, or differentiation. The hyperactivation of the NTRK signaling pathway induced by *NTRK* alterations—fusions or point mutations—can be overcome by the use of NTRK antagonists (eg, ANA-12 and cyclotraxin B) or small-molecule tyrosine kinase inhibitors (eg, larotrectinib and entrectinib). For now, only small-molecule tyrosine kinase inhibitors are used in the clinic.

NTRK alterations induce tumorigenesis in both neurogenic and non-neurogenic cancers and are targets for therapeutic agents.[9-11] Although the clinical implications of *NTRK* single-nucleotide variants or copy number alterations are unclear, several *NTRK* transcript fusions have been identified. These drive NTRK mRNA and protein overexpression, which further leads to constitutive activation of downstream signaling.[12] The prevalence of *NTRK* fusions is low, but can reach more than 80% in some rare tumors, such as mammary-analog secretory carcinoma of the salivary gland, secretory breast carcinoma, and infantile congenital fibrosarcoma.[12-20] *NTRK* fusions are also found in 40% of pediatric non-brainstem high-grade glioma.[21]

Among all alterations in *NTRK* genes, transcript fusions are currently the best characterized and the most pharmacologically tractable. Nonfusion *NTRK* alterations—for example, mutation or amplification—have been associated with a lack of response with some NTRK inhibitors.[22] Because *NTRK* fusions are rare, the number of patients who can benefit from drugs that target NTRK receptors is relatively low, but the antitumor activity of such agents is remarkable.[23,24] Indeed, larotrectinib, a pan-NTRK inhibitor, demonstrated a response rate of 76% in patients with *NTRK* fusion–positive tumors (17 cancer types).[15,18] Tumor regression has been maintained for more than 1 year in 71% of patients. Entrectinib, an oral pan-NTRK, ROS1, and ALK inhibitor demonstrated a 79% objective response in patients with *NTRK*, *ROS1*, or *ALK* fusions.[22]

In May 2017, a new precedent was set when an immune checkpoint inhibitor—pembrolizumab—was approved by the US Food and Drug Administration (FDA) for use in a tissue-agnostic fashion on the basis of a genomic biomarker (mismatch repair gene deficiency).[25] NTRK-selective inhibitors represent another pharmacology class that has been developed on the sole basis of somatic molecular patterns. Therefore, a comprehensive understanding of individual genomic alterations is becoming crucial.

In the current study, we assessed the landscape of *NTRK* genomic and transcriptomic alterations, as well as co-alterations in common signaling pathways, using a large cohort of samples available from The Cancer Genome Atlas (TCGA; adult, 33 tumor types) and the St Jude PeCan (pediatric, 17 tumor types).

METHODS

NTRK Receptor Fusions

Adult tumor *NTRK*-related transcript fusions were retrieved from The Jackson Laboratory Tumor Fusion Gene Data Portal.[26] These fusions were defined after an integrated analysis of paired-end RNA sequencing and DNA copy number data from TCGA that corresponded to 9,966 adult tumors (33 different tumor types).

Pediatric tumor *NTRK*-related transcript fusions were retrieved from the St Jude PeCan Data Portal database.[27] These fusions were defined after analysis of RNA sequencing data by the CICERO algorithm (Pediatric Cancer Genome

Project) and corresponded to 3,501 pediatric tumors (17 different tumor types).[28,29]

Genomic and Transcriptomic Alterations in NTRK Receptors, Co-Receptor, and Ligands (beyond fusions)

Adult and pediatric tumor *NTRK*-related mutations, copy number variations, and mRNA expression for NTRK receptors (*NTRK1*, *NTRK2*, and *NTRK3*), co-receptor ($p75^{NTR}$), and ligands (*NGF*, *BDNF*, *NT-3*, and *NT-4)* were retrieved from the UCSC Xena Portal.[30] These data include information on 13,467 samples from TCGA (n = 9,966 adults) and St Jude PeCan (n = 3,501 children) pan-cancer cohorts, of which 11,621 (n = 9,966 TCGA and n = 1,655 PeCan) had comprehensive information on fusions, mutations, and copy number alterations. Data were available without restriction of use on the date of February 1, 2018. All data used in this study respected the TCGA's Human Subjects Protection and Data Access Policies[31] and the St Jude Cloud Terms of Use.[32]

Lists of significant variants were generated using whole-genome somatic mutation data and the MutSig2CV algorithm (http://www.broadinstitute.org/cancer/cga/mutsig), taking into account the somatic background mutation rate for each gene and its neighbor genes.[33]

Focal copy number variations that correspond to genome-wide single-nucleotide polymorphism array data were normalized and assessed at the gene level using the GISTIC2 protocol,[34] where a deep loss was documented by the value (–2), a single-copy loss by the value (–1), a low-level gain by the value (+1), and an amplification by the value (+2). Only *NTRK*-related gene amplifications were kept for the analysis.

Sequencing-based mRNA expression signals were integrated and normalized for each gene per sample using the RNA-Sequencing by Expectation Maximization protocol. The standard score (z-score) for each gene per sample was calculated using the mean values and standard deviation found in all similar tumors—same tumor type—that are diploid for the said gene. A z-score of ≥ 1.96 standard deviation was used as the threshold of overexpression, whereas a threshold of ≤ –1.96 standard deviation was used to qualify underexpressed genes. Only *NTRK*-related mRNA overexpression was considered for the analysis.

Genomic and Transcriptomic Co-Alterations Occurring in *NTRK* Fusion–Positive Adult Tumors (n = 31 patients)

Comprehensive co-alteration data were not available in pediatric tumors. In adults, co-alterations within signaling cascades, such as *TP53*, MAPK, PI3K, tyrosine kinase receptor, or cell-cycle signaling pathways, were curated from TCGA. All nonsynonymous missense, nonsense, nonstop, deletion/insertions, frameshift, or splicing site mutations within the genes of interest, as well as deep losses or amplifications and mRNA under- or overexpressions, were kept for analysis.

RESULTS

Prevalence of *NTRK* Fusions in TCGA (adult) and St Jude PeCan (pediatric) Databases

Fusion Frequency in Adults Of the 9,966 adult tumor samples in the TCGA database, 0.31% (n = 31 samples) presented an *NTRK* fusion. This alteration was most common in thyroid cancer (2.34% of samples), colon adenocarcinoma (0.97%), and low-grade glioma (0.94%). Twenty-two adult tumor types had no *NTRK* fusions. (There were 5,023 patient samples with these 22 *NTRK* fusion–negative tumor types [samples per tumor type = 36 to 541].) *NTRK3* fusions were the most common (n = 16), followed by *NTRK1* (n = 9) and *NTRK2* (n = 6) fusions in adults (Table 1).

Fusion Frequency in Children Of the 3,501 pediatric tumor samples (St Jude PeCan database), 0.34% (n = 12) presented an *NTRK* fusion. Of interest, *NTRK* fusions were found in one of nine melanomas. *NTRK* fusions were also found in glioma (high and low grade [3.97%]) and B-cell acute lymphoblastic leukemia (0.14%). Thirteen pediatric tumor types (n = 2,524 patient samples) had no *NTRK* fusions (samples per tumor type = 26 to 714). Of 12 pediatric tumor samples with *NTRK* fusions, the most common partner gene was *NTRK1* (n = 5) followed by *NTRK2* (n = 4) and *NTRK3* (n = 3; Table 1).

Therapeutic or Experimental Molecules With Activity Against NTRK Receptors

Overall, 32 molecules have demonstrated preclinical inhibition activity against one or more

Table 1. Frequency of NTRK Receptor Transcript Fusions in TCGA (n = 9,966 adult tumor samples) and St Jude Pediatric Cancer Database (n = 3,501 pediatric tumor samples), and Specific Tumors With High Incidence of *NTRK* Fusions in the Literature

Tumor Sample	No. of Samples	No. of Tumors (%)			
		Any *NTRK* Fusion	*NTRK1* Fusion	*NTRK2* Fusion	*NTRK3* Fusion
Adult tumors (TCGA)*					
Total	9,966	31 (0.31)	9 (0.09)	6 (0.06)	16 (0.16)
Thyroid cancer	513	12 (2.34)	5 (0.97)	—	7 (1.36)
Colon adenocarcinoma	310	3 (0.97)	—	—	3 (0.97)
Low-grade glioma	534	5 (0.94)	1 (0.19)	3 (0.56)	1 (0.19)
Sarcoma	263	2 (0.76)	2 (0.76)	—	—
Glioblastoma multiforme	180	1 (0.56)	1 (0.56)	—	—
Pancreatic adenocarcinoma	179	1 (0.56)	—	—	1 (0.56)
Head and neck SCC	522	2 (0.38)	—	1 (0.19)	1 (0.19)
Cervical cancer	306	1 (0.33)	—	—	1 (0.33)
Melanoma	476	1 (0.21)	—	—	1 (0.21)
Breast cancer	1119	2 (0.18)	—	1 (0.09)	1 (0.09)
Lung adenocarcinoma	541	1 (0.18)	—	1 (0.18)	—
Pediatric tumors (St Jude PeCan)†					
Total	3,501	12 (0.34)	5 (0.14)	4 (0.11)	3 (0.09)
Melanoma	9	1 (11.11)	1 (11.11)	—	—
High-grade glioma	132	7 (5.3)	4 (3.03)	2 (1.52)	1 (0.76)
Low-grade glioma	120	3 (2.5)	—	2 (1.67)	1 (0.83)
B-cell ALL	716	1 (0.14)	—	—	1 (0.14)

Illustrative Tumor Types With High Prevalence of *NTRK* Fusion			
Tumor Type	**Reported Prevalence of *NTRK* Fusion (%)**	**Comment**	**Reference**
Mammary-analog secretory carcinoma of the salivary gland	93-100	*ETV6-NTRK3* fusion	Skálová et al,[14] Skálová et al,[15] Bishop et al[16]
Secretory breast carcinoma	92	*ETV6-NTRK3* fusion	Tognon et al[17]
Infantile congenital fibrosarcoma	86-91	*ETV6-NTRK3* fusion	Bourgeois et al,[18] Orbach et al,[19] Rubin et al[20]
Pediatric high-grade glioma	40	Fusions in *NTRK1*, *NTRK2*, or *NTRK3* (in those age < 3 years)	Wu et al[21]

Abbreviations: ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; NTRK, neurotrophic-tropomyosin receptor tyrosine kinase; SCC, squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

*Adult tumor types exempt from *NTRK* fusions (22 tumor types): adrenocortical carcinoma (n = 79), bladder urothelial carcinoma (n = 414), cholangiocarcinoma (n = 36), B-cell lymphoma (n = 48), esophageal carcinoma (n = 185), renal chromophobe tumor (n = 66), renal clear cell carcinoma (n = 541), renal papillary cell carcinoma (n = 291), AML (n = 179), hepatocellular carcinoma (n = 374), lung squamous cell carcinoma (n = 502), mesothelioma (n = 87), ovarian serous carcinoma (n = 428), pheochromocytoma or paraganglioma (n = 184), prostate adenocarcinoma (n = 502), rectal adenocarcinoma (n = 95), gastric adenocarcinoma (n = 414), testicular germ cell tumors (n = 156), thymoma (n = 120), endometrial carcinoma (n = 185), uterine carcinosarcoma (n = 57), and uveal melanoma (n = 80).

†Pediatric tumor types exempt from *NTRK* fusions (13 tumor types): T-cell ALL (n = 567), AML (n = 310), mixed leukemia (n = 26), medulloblastoma (n = 714), ependymoma (n = 92), choroid plexus carcinoma (n = 29), neuroblastoma (n = 382), Ewing sarcoma (n = 123), Wilms tumor (n = 91), rhabdomyosarcoma (n = 58), osteosarcoma (n = 53), adrenocortical carcinoma (n = 40), and retinoblastoma (n = 39).

NTRK receptors[35-70] (Table 2). Surprisingly, five of these small inhibitors are already approved by the FDA for other indications, namely cabozantinib (Cabometyx; Exelixis, South San Francisco, CA; IC_{50} against NTRK2, 7 nM), crizotinib (Xalkori; Pfizer, New York, NY; IC_{50} against NTRK1 and NTRK2, 1 nM), midostaurin (Rydapt; Novartis, Basel Switzerland; IC_{50} ranging from 11 to 51 nM), nintedanib (Ofev; Boehringer Ingelheim, Ingelheim am Rhein,

Germany; IC_{50} ranging from 17 to 264 nM), and regorafenib (Stivarga; Bayer, Leverkusen, Germany; IC_{50} against NTRK1, 74 nM). It is not known if these five molecules exhibit clinical activity in patients who harbor *NTRK*-aberrant tumors. Sixteen molecules are currently being evaluated in clinical trials, with the most advanced being larotrectinib (Loxo Oncology, Stamford, CT; IC_{50} for NTRK1, NTRK2, and NTRK3 fusions ranging from 4 to 9 nM). The new drug application was submitted to the FDA in December 2017 and granted priority review status on the basis of remarkable clinical activity[23] (Table 2).

Types of *NTRK*-Related Alterations in Adult and Pediatric Tumors and Sensitivity to NTRK Inhibitors

To understand the potential benefits of selective NTRK inhibitors for the treatment of adult and pediatric patients with cancer, we first aimed to describe the prevalence and type of *NTRK*-activating pathway alterations, including point mutations, gene copy number amplifications, and mRNA overexpression of NTRK receptors, co-receptor, and ligands, within a large cohort of pan-cancer samples (Figs 1 and 2). The number of samples with comprehensive data for this analysis was 11,621 (9,966 adults and 1,655 children).

Alterations in NTRK Receptors and Ligands

Genomic and/or transcriptomic NTRK receptor alterations were found in 14.2% (1,648 of 11,621) of samples, with gene amplification and mRNA overexpression being the most frequent alterations. The three NTRK receptors were equally impacted, with frequencies of alterations ranging from 4.1% to 6.2%. In addition, the co-receptor $p75^{NTR}$ presented one or more presumably activating alteration in almost 5% (579 of 11,621 samples) of tumors. NTRK ligands presented an alteration in 3.8% to 5.4% of samples. Transcript fusions were observed in NTRK receptor genes only, with the exception of two samples that presented one transcript fusion of *BDNF* ligand and one transcript fusion of *$p75^{NTR}$* (positive regulator of the NGF/NTRK1 machinery; Fig 2).

-Transcript Fusion Types

NTRK-transcript fusions that were observed in the pan-cancer cohort and/or described in the literature are listed in Table 3. The *ETV6-NTRK3* rearrangement was the most frequently observed (0.09% of samples). This variant is a known biomarker of sensitivity to larotrectinib and entrectinib.[71,72] Variants *TPM3-NTRK1* (0.04%), *IRF2BP2-NTRK1* (< 0.01%), and *SQSTM1-NTRK1* (< 0.01%) are also sensitive to larotrectinib; however, the sensitivity of the remaining 22 unique variants observed in the pan-cancer cohort is not currently known. Nine rearrangements previously described in the literature were not found in the TCGA and St Jude PeCan databases (Table 3).

Point Mutations

Several point mutations are acquired resistant variants to first-generation NTRK inhibitors (larotrectinib or entrectinib), but not to LOXO-195, specifically designed to overcome secondary resistance. These variants, namely *NTRK1* G595R, *NTRK1* G667C, *NTRK3* G696A, and *NTRK3* G623R, were not observed in any of the 13,467 combined adult and pediatric tumors reviewed (treatment-naïve samples; Table 3).

Co-Alterations Observed in *NTRK* Fusion-Positive Adult Tumor Samples

Among 31 adult tumors presenting *NTRK* fusions, 61.3% (19 of 31) harbored one or more co-alteration that activated the downstream PI3K signaling pathway; 58.1% (18 of 31) harbored one or more co-alteration within cell-cycle–associated genes; 58.1% (18 of 31) harbored one or more co-alteration within other tyrosine kinase receptors; 32.2% (10 of 31) harbored one or more co-alteration within the MAPK signaling pathway; and 35.5% (11 of 31) harbored one or more co-alteration within *TP53*-associated genes. *NF2*-activating mutations were associated with *NTRK* fusions in 42% (13 of 31) of samples, and *TP53* (10 of 31), *RB1* (six of 31) and *CDKN2A* (five of 31) occurred in more than 15% of the *NTRK* fusion–positive samples (Fig 3 and Appendix Table A1). (Adequate data to comprehensively assess co-alteration data in children was not available.) Samples bearing *NTRK* fusions were significantly associated with NTRK mRNA overexpression compared with samples without the fusion (Appendix Fig A1). Moreover, tumors with *NTRK* fusions were significantly associated with lower tumor mutational burden compared with the fusion-negative cases (Appendix Fig A2).

Table 2. Target Specificity and IC_{50} of NTRK-Targeting Inhibitors

Drug Name (company)	IC_{50} (nM) NTRK1	NTRK2	NTRK3	Other Targets (IC_{50} < 500 nM)	Reference
FDA-approved drugs					
Cabozantinib (XL-184; Exelixis)	NA	7	NA	ALK, AXL, BLK, BTK, EPHA4, EPHB4, FAK, FLT1, FLT3, FLT4, FYN, KDR, KIT, LYN, MAP2K1, MET, PDGFRB, RAF1, RET, RON, SAPK4, TIE2, YES	US Food and Drug Administration[35]
Crizotinib (PF-02341066; Pfizer)	1	1	NA	ABL, ALK, ARG, AXL, FES, LCK, LYN, MER, MET, RON, ROS1, SKY, TIE2, YES	US Food and Drug Administration[36]
Midostaurin (PKC-412; Novartis)	11	51	15	AURKA, BRSK1, CSF1R, FLT3, MAP3K9, PDGFRA, PDGFRB, PHKG1, PKN1, PRKCA, PRKCB2, RPS6KA1, RPS6KA2, RPS6KA3, STK4, SYK, TBK1	US Food and Drug Administration[37]
Nintedanib (BIBF-1120; Boehringer Ingelheim)	17.1	263.9	142.5	FGFR, FLT3, LCK, LYN, PDGFR, SRC, VEGFR	Nishiyama et al,[38] Hilberg et al[39]
Regorafenib (BAY 73-4506; Bayer/ Onyx)	74	NA	NA	ABL, DDR2, EPHA2, FGFR1, FGFR2, FLT1, FLT3, HCK, KDR, KIT, LYN, MER, PDGFRA, PTK5, RAF1, RET, SAPK2A, SAPK2B, TIE2	US Food and Drug Administration[40]
Drugs in development (ongoing clinical trials)					
Altiratinib (Deciphera Pharmaceuticals)	0.9	4.6	0.8	MET, TIE2 VEGFR2	Smith et al[41]
Belizatinib (TSR-011; Tesaro)	< 3	< 3	< 3	ALK	Weiss et al[42]
BMS-754807 (Bristol-Myers Squibb)	7	4	NA	AURKA, AURKB, FLT3, IGF1R, INSR, MET, RON	Carboni et al[43]
BMS-777607 (Bristol-Myers Squibb)	290	190	NA	AURKB, AXL, FLT3, KDR, LCK, MER, MET, RON, TYRO3	Schroeder et al[44]
Danusertib (Nerviano)	31	NA	NA	ABL, AURKA, AURKB, AURKC, FGFR1, RET	Carpinelli et al[45]
DS-6051b (Daiichi Sankyo)	< 2	< 2	< 2	ALK, ROS1	Kiga et al[46]
ENMD-2076 (CASI)	24	NA	NA	ABL1, AURKA, AURKB, BLK, CSF1R, FAK, FGFR1, FGFR2, FLT3, FLT4, FYN, JAK2, KDR, KIT, LCK, PDGFRA, RET, SRC, YES1	Fletcher et al[47]
Entrectinib (RXDX-101; Ignyta/ Nerviano)	2	0.1	0.1	ALK, ROS1	Braud et al,[48] Rolfo et al[49]
Larotrectinib (LOXO-101; Loxo Oncology)	9	4	4	—	Drilon et al,[50] Ghilardi et al[51]
Lestaurtinib (CEP-701; Cephalon/ Kyowa)	25	25	25	FLT3, JAK2	Shabbir et al,[52] Miknyoczki et al[53]
LOXO-195 (Loxo Oncology)	4	2	1	—	Drilon et al[50]
Merestinib (LY2801653; Eli Lilly)	15-320	15-320	15-320	AXL, DDR1, DDR2, FLT3, MET, MERTK, MKNK1, MKNK2, MST1R, ROS1, TEK	Yan et al,[54] Konicek et al[55]
MK-5108 (Merck/Vertex)	2	13	NA	ABL, AURKA, AURKB, AURKC, AXL, BRK, EPHA1, EPHA2, FLT1, FLT4, GSK3A, JNK3, KDR, LOK, MER, PTK5, ROS, TIE2, YES	Shimomura et al[56]
Milciclib (PHA-848125; Nerviano /Tiziana)	53	NA	NA	CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E, CDK4/cyclin D1, CDK5/p35, CDK7/cyclin H	Brasca et al[57]

(Continued on following page)

Table 2. Target Specificity and IC_{50} of NTRK-Targeting Inhibitors (Continued)

Drug Name (company)	IC_{50} (nM) NTRK1	NTRK2	NTRK3	Other Targets (IC_{50} < 500 nM)	Reference
PLX-7486 (Plexxikon)	< 10	< 10	< 10	AURKA, AURKB, CSF1R, MAP3K2, MAP3K3	ECMC Network[58]
Sitravatinib (MGCD516; Mirati Therapeutics)	5	9	NA	RET, CBL, CHR4q12, DDR, AXL, DDR1, DDR2, EPHA2, EPHA3, EPHA4, EPHB2, EPHB4, FLT1, FLT3, FLT4, KDR, KIT, MER, MET, PDGFRA, RET, RON, ROS, SRC	Patwardhan et al[59]
Preclinical drugs					
ANA-12	NA	10	NA	—	Ivanov et al[60]
AZD-7451 (AstraZeneca)	0.2	< 3	< 3	—	Cazorla et al[62]
Cyclotraxin B (Tocris Biosciences)	NA	0.3	NA	—	Cazorla et al[61]
Dovitinib (TKI-258; Novartis)	69	NA	NA	CSF1R, FGFR1, FGFR3, FLT1, FLT3, FLT4, KDR, KIT, PDGFRB	Chong et al[63]
Foretinib (formerly GSK-1363089/ XL880; GlaxoSmithKline/ Exelixis)	34.8	118.2	258.2	FLT1, FLT4, FLT3, KDR, KIT, MET, PDGFRA, PDGFRB, RON, TIE2, VGFR	Nishiyama et al,[38] Qian et al[64]
GNF-5837 (GNF)	8	12	7	KIT, PDGFR	Albaugh et al[65]
GW-441756 (Tocris Biosciences)	2	NA	NA	—	Wood et al[66]
PF-03814735 (Pfizer)	30	NA	NA	AURKA, AURKB, FAK, FLT1	Jani et al[67]
PF-06273340 (Pfizer)	6	4	3	—	Skerratt et al[68]
RXDX-102 (Ignyta/Nerviano)	< 5	< 5	< 5	—	Ignyta[69]
SNS-314 (Sunesis)	12	5	NA	AURKA, AURKB, AURKC, AXL, CSF1R, DDR2, FLT4, RAF1	Gamo et al[70]

Abbreviations: FDA, US Food and Drug Administration; GNF, Genomics Institute of the Novartis Research Foundation; IC_{50}, half-maximal inhibitory concentration; NA, not applicable; NDA, new drug application; NTRK, neurotrophic-tropomyosin receptor tyrosine kinase.

DISCUSSION

Along with recent advances in sequencing technology, a histology-agnostic, matched, targeted approach has emerged as a newer strategy by which to manage malignancies.[80-84] Targeting activated gene mutations or amplification/overexpression has demonstrated some remarkable successes—for example, targeting of *KIT* mutations for GI stromal tumors and targeting of *EGFR* mutation for non–small-cell lung cancer, *BRAF* V600E mutation for melanoma and lung cancer, and human epidermal growth factor receptor 2 overexpression for breast cancer—although in some cases the responses may be short lived.[85-91] Tumor heterogeneity and co-alterations result in resistance to targeted therapeutics.[92] Thus, for many cancers, combination therapy may be necessary.[93-96]

In some instances, targeting fusions—even with monotherapy—has shown more marked antitumor activity than targeting other alteration types. Examples include the suppression of aberrant Bcr-Abl kinase enzymatic activity that characterizes chronic myeloid leukemia. Exploitation of imatinib, dasatinib, or nilotinib leads to near-universal responses, and life expectancy increases from approximately 5 years before the imatinib era to a near-normal life span currently; however, it is also conceivable that, in this case, the success of Bcr-Abl–targeted agents is attributable to their deployment in patients with newly diagnosed disease, as advanced chronic myeloid leukemia responds poorly to single-agent Bcr-Abl kinase inhibitors.[97,98] Conversely, in patients with advanced non–small-cell-lung cancer, targeting *ALK* fusions demonstrates a median progression-free survival of 25.7 months with an 83% response rate, and targeting the *ROS1* fusion demonstrates a median progression-free survival of 19.2 months with an approximate 70% response rate.[99,100] In addition, larotrectinib, an NTRK inhibitor, resulted in a 76% response rate in patients with an *NTRK* fusion.[23] These observations indicate that certain fusions act as strong drivers of tumorigenesis in specific cancers that are likely addicted to this type of founder alteration.

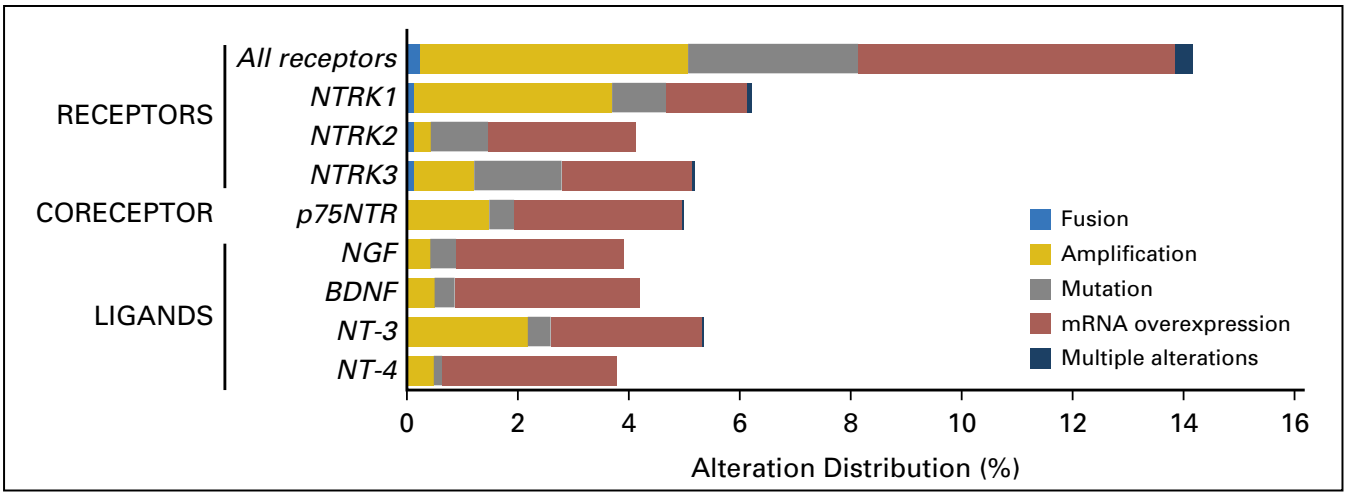

Fig 2. Distribution of molecular alterations leading to the hyperactivation of the neurotrophic-tropomyosin receptor tyrosine kinase (NTRK) signaling pathway in human tumors (N = 11,621 samples with comprehensive molecular data). All samples that presented a nonsilent mutation, gene copy amplification, gene fusion, or mRNA overexpression of NTRK receptors (NTRK1, NTRK2, and NTRK3), co-receptor ($p75^{NTR}$), or ligands (nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin 3 [NT-3], and NT-4) were retrieved from a large adult and pediatric pan-cancer cohort (The Cancer Genome Atlas and St Jude's PeCan databases; N = 11,621samples). Among the *NTRK* fusion cases (n = 31 from TCGA cohort), four cases had concomitant alteration within the genes that code the *NTRK* pathway members—ligands, co-receptor, and receptors—as follows: low-grade glioma, *NTRK3* fusion plus NTF3 amplification (n = 1); low-grade glioma, *NTRK1* fusion plus *NTRK1* amplification (n = 1); glioblastoma, *NTRK1* fusion plus *NTRK1* amplification (n = 1); head and neck squamous cell carcinoma, *NTRK3* fusion plus *NTRK3* amplification (n = 1).

We reviewed data from 13,467 tumor samples in the TCGA (adult tumors) and St Jude PeCan (pediatric tumors) databases and found *NTRK* fusions in 0.3% of pan-cancer tumors (Table 1). Although the prevalence of these alterations is low, *NTRK* fusions are more often found in specific and rare tumors, such as mammary-analog secretory carcinoma of the salivary gland (93% to 100% of tumors presenting an *ETV6-NTRK3* fusion), secretory breast carcinoma (*ETV6-NTRK3* fusions in 92% of tumors), infantile congenital fibrosarcoma (*ETV6-NTRK3* fusions in 86% to 91% of tumors), and pediatric non-brainstem high-grade glioma[14-21] (40% of tumors presenting an *NTRK* fusion; Table 1).

Of importance, various NTRK inhibitors are in clinical development and have differential activities (Table 2). Drugs with established clinical trial data and the ability to affect *NTRK1*, *NTRK2*, and *NTRK3* fusions at low IC_{50} include, but are not limited to, larotrectinib (76% response rate in diverse malignancies bearing *NTRK* fusions) and entrectinib, which also affects *ALK* and *ROS1* rearrangements (79% response rate), and some of these responses are durable and occur with remarkable speed[22,23] (Table 2). Of interest, 32 molecules have demonstrated inhibition activity against one or more NTRK receptor (Table 2). Furthermore, five of these small inhibitors are already approved by the FDA for other indications: cabozantinib (IC_{50} against NTRK2, 7 nM), crizotinib (IC_{50} against NTRK1 and NTRK2, 1 nM), midostaurin (IC_{50} against NTRK1, -2, and -3 ranging from 11 to 51 nM), nintedanib (IC_{50} against NTRK1, -2, amd -3 ranging from 17 to 264 nM), and regorafenib (IC_{50} against NTRK1, 74 nM). Even so, it is not known whether these five medications have anti-NTRK activity in patients. Multiple other molecules that target NTRK are also in clinical trials (Table 2).

Resistance to NTRK inhibitors is now emerging. *NTRK* mutations that are associated with larotrectinib or entrectinib resistance include *NTRK1* F589L G595R, G667C, G667S, V573M, and *NTRK3* G696A, G623R (Table 3). (These mutations were not detected in TCGA, likely because these patients had not been previously treated with NTRK inhibitors.) The resistant alterations are targetable with LOXO-195, a next-generation, selective NTRK inhibitor with promising preliminary clinical activity[50] (Fig 2). Other mechanisms of resistance may include the presence or emergence of genomic co-alterations. In the current study, *NTRK*-associated co-alterations were commonly discerned in genes that are involved in PI3K signaling (61% of patient samples), tyrosine kinase families (58% of patient samples), cell-cycle machinery (58% of patient samples), and MAPK pathways (32% of patient samples; Fig 3 and Appendix Table A1). Moreover, cases with *NTRK*

Table 3. *NTRK* Alterations, Frequency in TCGA/St Jude PeCan Databases, and Clinical Response to Illustrative NTRK-Targeting Inhibitors

NTRK Alteration	Type of Alteration	Frequency of Observation (%)	Larotrectinib	Entrectinib	LOXO-195	Reference
AFAP1-NTRK2	Fusion	< 0.01	NA	NA	NA	
AGBL4-NTRK2	Fusion	< 0.01	NA	NA	NA	
AKAP13-NTRK3	Fusion	< 0.01	NA	NA	NA	
BCAN-NTRK1	Fusion	0	NA	Sensitive	NA	Drilon et al,[22] Cook et al[73]
CTRC-NTRK1	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
EML4-NTRK3	Fusion	< 0.01	NA	NA	NA	
EPHB2-NTRK1	Fusion	< 0.01	NA	NA	NA	
ETV6-NTRK3	Fusion	0.09	Sensitive	Sensitive	NA	Khotskaya et al,[11] Hyman et al,[71] Nagasubramanian et al[74]
FAT1-NTRK3	Fusion	< 0.01	NA	NA	NA	
GSN-NTRK1	Fusion	< 0.01	NA	NA	NA	
IRF2BP2-NTRK1	Fusion	< 0.01	Sensitive	NA	NA	Hyman et al[71]
LMNA-NTRK1	Fusion	0	Sensitive	Sensitive	NA	Hyman et al,[71] Doebele et al[75]
LYN-NTRK3	Fusion	< 0.01	NA	NA	NA	
NAV1-NTRK2	Fusion	< 0.01	NA	NA	NA	
NFASC-NTRK1	Fusion	< 0.01	NA	NA	NA	
NTRK1-DYNC2H1	Fusion	< 0.01	NA	NA	NA	
NTRK2-LAP3	Fusion	< 0.01	NA	NA	NA	
NTRK3-ETV6	Fusion	< 0.01	NA	NA	NA	
PAN3-NTRK2	Fusion	< 0.01	NA	NA	NA	
PDE4DIP-NTRK1	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
PPL-NTRK1	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
RBPMS-NTRK3	Fusion	< 0.01	NA	NA	NA	
SLMAP-NTRK2	Fusion	< 0.01	NA	NA	NA	
STRN-NTRK2	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
SQSTM1-NTRK1	Fusion	< 0.01	Sensitive	Sensitive	NA	Hyman et al,[71] Farago et al[76]
SQSTM1-NTRK2	Fusion	< 0.01	NA	NA	NA	
SSBP2-NTRK1	Fusion	< 0.01	NA	NA	NA	
TFG-NTRK1	Fusion	< 0.01	NA	NA	NA	
TPM3-NTRK1	Fusion	0.04	Sensitive	NA	NA	Hyman et al[71]
TPM4-NTRK3	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
TPR-NTRK1	Fusion	0	Sensitive	NA	NA	Khotskaya et al,[11] Hyman et al[71]
TRIM24-NTRK2	Fusion	< 0.01	NA	NA	NA	
TRIM63-NTRK1	Fusion	0	Sensitive	NA	NA	Hyman et al[71]
VCL-NTRK2	Fusion	< 0.01	NA	NA	NA	
VPS18-NTRK3	Fusion	< 0.01	NA	NA	NA	

(Continued on following page)

Table 3. *NTRK* Alterations, Frequency in TCGA/St Jude PeCan Databases, and Clinical Response to Illustrative NTRK-Targeting Inhibitors* (Continued)

NTRK Alteration	Type of Alteration	Frequency of Observation (%)	Larotrectinib	Entrectinib	LOXO-195	Reference
NTRK1 F589L	Mutation	0	Resistant	Sensitive	Sensitive	Drilon et al,[50] Wei et al[77]
NTRK1 G595R	Mutation	0	Resistant	Resistant	Sensitive	Drilon et al,[50] Hyman et al,[71] Russo et al[78]
NTRK1 G667C	Mutation	0	Resistant	Resistant	Sensitive	Drilon et al,[50] Russo et al[78]
NTRK1 G667S	Mutation	0	Resistant	Sensitive	Sensitive	Drilon et al,[50] Wei et al[77]
NTRK1 V573M	Mutation	0	Resistant	Sensitive	Sensitive	Drilon et al,[50] Wei et al[77]
NTRK3 G696A	Mutation	0	Resistant	NA	Sensitive	Drilon et al[50]
NTRK3 G623R	Mutation	0	Resistant	Resistant	Sensitive	Drilon et al,[50,79] Hyman et al[71]
Total		0.32	NA	NA	NA	

NOTE. Frequencies of alterations were computed using a large adult and pediatric pan-cancer cohort (The Cancer Genome Atlas and St Jude's PeCan databases; N = 13,467 samples). Sensitivity and resistance criteria presented in this table correspond to objective clinical responses or nonresponses observed in fusion-positive or mutation-positive patients who received the drug.

Abbreviations: NA, not available; NTRK, neurotrophic-tropomyosin receptor tyrosine kinase.

fusions were significantly associated with NTRK mRNA overexpression (Appendix Fig A1), which is consistent with a previous report.[101] Of interest, in the adult cohort, *NTRK* fusion–positive samples were significantly associated with a lower mutational burden compared with fusion-negative tumors ($P < .001$; Appendix Fig A2). This observation echoes a previous report that demonstrated that tumors harboring a driver fusion tend to have a lower number of point mutations.[101] In contrast, high microsatellite unstable metastatic colorectal tumors have been shown to preferentially bear *ALK*, *ROS1*, or *NRTK* fusions.[102] In our cohort, three *NTRK* fusion–positive colon cancer samples were observed and two of them presented with microsatellite instability-high status (data not shown). Finally, we found that nonfusion *NTRK* gene alterations, such as mutation, amplification, and mRNA overexpression, were found in approximately 14% of pan-cancer samples (Fig 2). Nonfusion *NTRK* alterations have not yet demonstrated druggability.

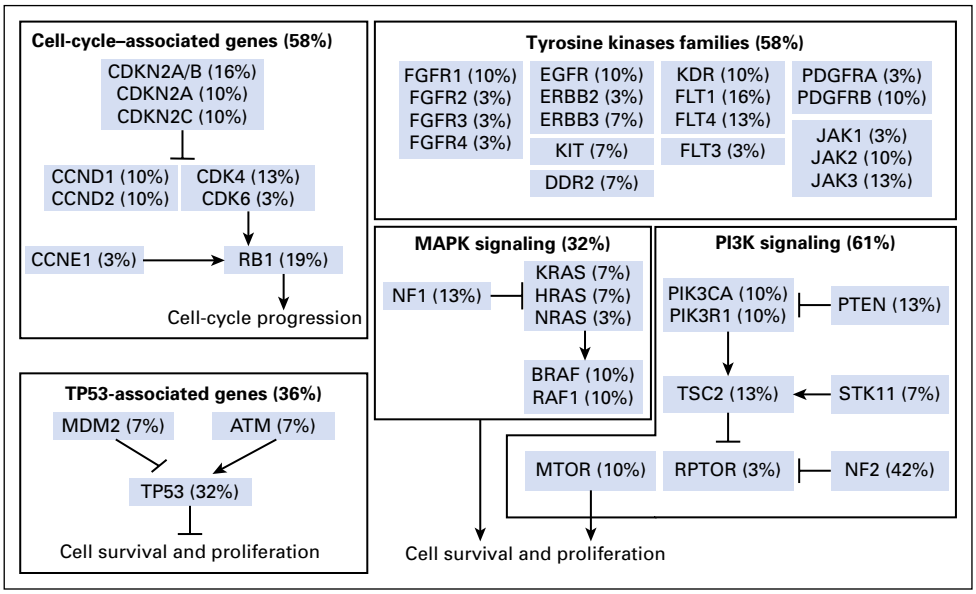

Fig 3. Co-alterations associated with neurotrophic-tropomyosin receptor tyrosine kinase (NTRK) fusions in adult tumors (from The Cancer Genome Atlas). All samples that presented a gene fusion of *NTRK* receptors—*NTRK1*, *NTRK2*, and *NTRK3*—were retrieved from a large adult pan-cancer cohort (The Cancer Genome Atlas database; n = 9,966 samples). Among 31 patients with *NTRK* fusions, some patients also harbored co-alterations that can lead to tumorigenesis. Those co-alterations include *TP53*-associated genes, cell cycle–associated genes, tyrosine kinase families, and mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) signaling alterations.

There are several limitations to the current study. First, clinical correlation with disease outcome among patients with *NTRK* alterations was not feasible because the data were not fully clinically annotated. Second, the possibility of sample size bias cannot be excluded because the number of tumor cases depended on the number of specimens submitted by investigators. Third, direct comparison between the TCGA and St Jude PeCan databases is challenging as a result of the use of different sequencing methods. Finally, we did not observe *NTRK* fusions in a number of cancer types, which may be a result of low sample size. Despite these limitations, the current report provides a comprehensive portrait of the genomic landscape of *NTRK* alterations among pan-cancer tumors using large databases.

In conclusion, *NTRK* fusions were observed in 0.31% (31 of 9,966) of adult tumors and 0.34% (12 of 3,501) of pediatric cancers, mostly in *NTRK3* (0.16% of adult tumors) and *NTRK1* (0.14% of pediatric tumors); however, some tumor types had more frequent *NTRK* fusions (Table 1). Additional genomic and transcriptomic *NTRK* alterations—mutation, amplification, and mRNA overexpression—occurred in 14.2% of samples. Genomic co-alterations were commonly observed in *NTRK* fusion–positive cancers, including in genes involved in PI3K signaling, tyrosine kinase families, cell-cycle–associated regulators, and the MAPK pathway (Fig 3). Additional investigation is needed to elucidate whether these genes mediate resistance to NTRK inhibition and if co-targeting them augments anti-NTRK antitumor activity. Furthermore, it would be of interest to determine whether the salutary effects of NTRK inhibitors in patients who harbor cancers with *NTRK* fusions can be extended via rational compound design to any of the more common *NTRK* alterations, such as mutation, amplification, and overexpression. Finally, the rarity of *NTRK* fusions, but their remarkable tractability in multiple cancer types, further expands the paradigm of tissue-agnostic genomic drug development.

DOI: https://doi.org/10.1200/PO.18.00183
Published online on ascopubs.org/journal/po on November 15, 2018.

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Data analysis and interpretation: All authors

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

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No relationship to disclose

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No relationship to disclose

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No relationship to disclose

Jason K. Sicklick
Consulting or Advisory Role: BioTheranostics, Loxo Pharmaceuticals, Grand Rounds
Research Funding: Novartis, Foundation Medicine, Blueprint Medicines

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Speakers' Bureau: Genentech, AstraZeneca, Pfizer
Research Funding: BeyondSpring Pharmaceuticals

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Speakers' Bureau: Roche
Research Funding: Guardant Health (Inst), Sequenom (Inst), Merck Serono (Inst), Genentech (Inst), Pfizer (Inst), Foundation Medicine (Inst), Incyte (Inst), Konica Minolta (Inst)

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Support

This work was supported in part by the Joan and Irwin Jacobs Fund, and NIH P30 CA023100 (R.K.). The authors also acknowledge the support of the Jon Schneider Memorial Cancer Research Fund NIH K08 CA168999 and R21 CA192072 (J.K.S.)

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Appendix

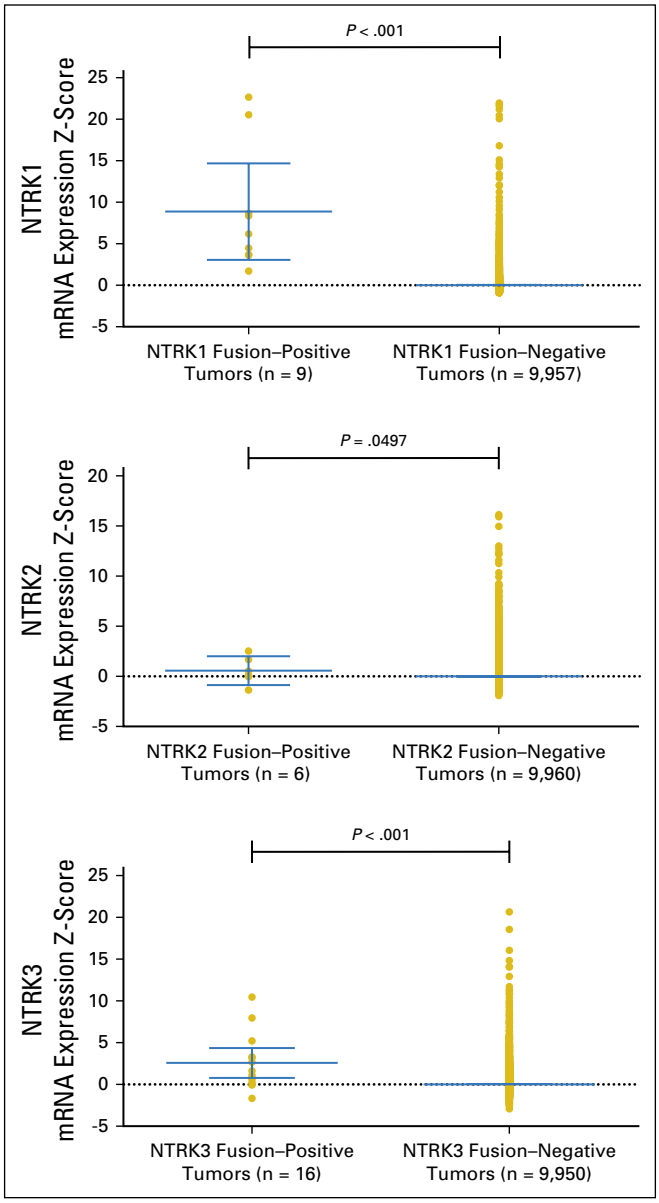

Fig A1. Association of neurotrophic-tropomyosin receptor tyrosine kinase (*NTRK*) fusions and NTRK mRNA overexpression in adult tumors.

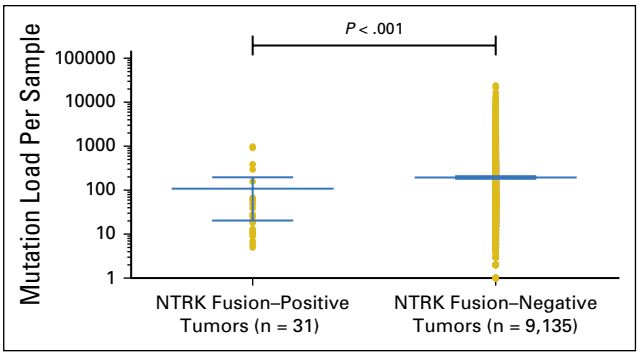

Fig A2. Association of neurotrophic-tropomyosin receptor tyrosine kinase (*NTRK*) fusions and mutational burden in adult tumors. The mutational burden corresponds to the total number of nonsynonymous mutations detected by whole-exome sequencing in each sample.

Table A1. Details of Co-Alterations With *NTRK* Fusions in Adult Tumors From The Cancer Genome Atlas

Alteration	No. of Samples Presenting						
	Nonsynonymous Mutation	Copy Number Gain	Copy Number Loss	mRNA Overexpression	mRNA Underexpression	Multiple Alterations	Total, No. (%)
Cell-cycle associated alterations							18 (58)
CCND1		2		1			3 (10)
CCND2		1		2			3 (10)
CCNE1				1			1 (3)
CDK4		3		1			4 (13)
CDK6				1			1 (3)
CDKN2A	2		6				8 (26)
CDKN2B			5				5 (16)
CDKN2C			3				3 (10)
RB1					3	3	6 (19)
TP53-associated alterations							11 (36)
ATM	2						2 (7)
MDM2		2					2 (7)
TP53	4			1		5	10 (32)
MAPK signaling alterations							10 (32)
BRAF	1	1				1	3 (10)
HRAS				1		1	2 (7)
KRAS	1			1			2 (7)
NF1	2			2			4 (13)
NRAS				1			1 (3)
RAF1	1			1			2 (7)
PI3K signaling alterations							19 (61)
MTOR				2		1	3 (10)
NF2	1			7	1	4	13 (42)
PIK3CA	1			1		1	3 (10)
PIK3R1				1	1	1	3 (10)
PTEN	1			3			4 (13)
RPTOR				1			1 (3)
STK11	1			1			2 (7)
TSC2	2			1	1		4 (13)

(Continued on following page)

Table A1. Details of Co-Alterations With *NTRK* Fusions in Adult Tumors From The Cancer Genome Atlas (Continued)

Alteration	No. of Samples Presenting						
	Nonsynonymous Mutation	Copy Number Gain	Copy Number Loss	mRNA Overexpression	mRNA Underexpression	Multiple Alterations	Total, No. (%)
Tyrosine kinases families							18 (58)
DDR2				2			2 (7)
EGFR		1		1		1	3 (10)
ERBB2				1			1 (3)
ERBB3		1		1			2 (7)
FGFR1		1		1		1	3 (10)
FGFR2				1			1 (3)
FGFR3				1			1 (3)
FGFR4		1					1 (3)
FLT1	1	1		2		1	5 (16)
FLT3		1					1 (3)
FLT4		1		3			4 (13)
JAK1						1	1 (3)
JAK2		1		2			3 (10)
JAK3	1	1		2			4 (13)
KDR	1			1		1	3 (10)
KIT				1		1	2 (7)
PDGFRA				1			1 (3)
PDGFRB	1			2			3 (10)

Abbreviations: MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3-kinase.