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REVIEW ARTICLE

Detecting and Targeting NTRK Fusions in Cancer in the Era of Tumor Agnostic Oncology

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Abstract

Gene rearrangements involving the neurotrophic receptor kinase genes *NTRK1*, *NTRK2*, and *NTRK3* (referred to as TRK, encoding TRKA, TRKB, and TRKC, respectively) result in highly oncogenic fusions. TRK fusions are rare, with a prevalence of < 1% in solid tumors. Detection of TRK fusions can be based on fluorescence in-situ hybridization (FISH), immunohistochemistry (IHC), and next-generation sequencing (NGS), where RNA sequencing is the most sensitive method. Inhibition of TRK fusions with highly selective small-molecule TRK inhibitors (TRKi) such as entrectinib and larotrectinib, results in profound responses in most cancer patients, regardless of cancer histology. Even response in CNS metastases is relatively common. Although responses are often durable, many patients develop resistance to TRKi due to mutations in one of the TRK genes, or due to genetic alterations conferring activation of alternative oncogenic signaling pathways. Second-generation TRKi have been developed, which can overcome some of the TRK resistance mutations. TRKi are well tolerated, with most common adverse events being related to on-target/off-tumor inhibition of TRKs.

Key Points

Neurotrophic receptor kinase (NTRK) fusions are rare but significant genomic alterations in most common solid tumors.

Entrectinib and larotrectinib are US FDA-approved TRK inhibitors (TRKi) with impressive efficacy in patients with TRK fusion-positive cancer.

Second-generation TRKi are in the pipeline for patients developing resistance to first-line TRKi.

1 Introduction

Cancer is a disease of the genome, with driver mutations often having dramatic consequences on the phenotypic behavior of the tumor cell. Developments in molecular profiling over recent decades, together with the increasingly widespread availability of affordable technologies, has made it possible to profile genomic aberrations with high sensitivity and specificity. This has led to new paradigms of cancer care that rely increasingly on tumor molecular profiling throughout the course of care to refine prognosis and inform treatment decisions [1–4].

Consequently, a new generation of drugs targeting specific molecules and activated oncogenic pathways have been transformative to many patients with advanced cancers and exhausted treatment options. With the detection of the *BCR-ABL* gene fusion in chronic myeloid leukemia (CML) and the development of imatinib as a small molecule targeting the resultant fusion protein in CML, the era of genomic-targeted therapies in cancer has begun [5]. The indication for imatinib was expanded to include c-*KIT* or *PDGFR*-mutant gastrointestinal stromal tumors (GIST), and other drugs targeting these and other driver mutations were developed. Several drugs have been US FDA-approved, with one indication harboring a relevant genomic alteration. These include *EGFR* tyrosine kinase inhibitors (TKIs) for *EGFR*- mutant

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non-small cell lung cancer (NSCLC), *ALK* and *ROS1* TKIs for the treatment of NSCLC with *ALK* or *ROS1* rearrangements, *BRAF* and *MEK* TKIs for the treatment of *BRAF V600E*-mutated melanoma, and *BRAF* TKIs in combination with an *EGFR* monoclonal antibody (mAb) for *BRAF V600E*-mutated colorectal cancer. Most recently, the FDA granted approval for the MET TKI capmatinib for NSCLC with *MET* exon 14 skipping mutations, and AMG-510 was granted FDA fast-track designation for *KRAS-G12C* NSCLC [6, 7].

Basket trials have studied the value of these agents in other indications [8–12], and some have been successful and achieved new labels, including the combination of *BRAF* and *MEK* inhibition for *BRAF*-mutated anaplastic thyroid cancer and NSCLC [13–15], while others have shown encouraging response rates and approval is being evaluated [16, 17]. Numerous emerging targeted agents are in development in basket trials for new activating mutations, including human epidermal growth factor receptor 2 (HER2) TKIs for activating *HER2* mutations [18, 19].

Neurotrophic receptor kinase (*NTRK*) gene fusions are oncogenic somatic chromosomal rearrangements involving the *NTRK1*, *NTRK2,* or *NTRK3* genes. The fusion proteins are oncogenic, and activated TRK tyrosine kinase domains have been identified as therapeutic anticancer targets.

The development of TRK inhibitors (TRKi) has been based on tumor agnostic basket trials as the fusions occur in up to 1% of all solid tumors and have been reported across a wide range of tumor types. In this review, we focused on the biology and detection of TRK fusions and the clinical development of first- and second-generation TRKi.

2 Biology

The neurotrophic tropomyosin receptor kinase family consists of three transmembrane receptor tyrosine kinases, namely TRKA, TRKB, and TRKC, which are encoded by *NTRK1*, *NTRK2* and *NTRK3*, respectively [20]. In normal biology, TRKs play important roles in the survival and plasticity of the sensory and sympathetic nervous system and other compartments of the neuronal system (Table 1) [21]. Upon ligand binding, receptor homodimerization and subsequent intracellular phosphorylation of the tyrosine kinase domain occur. Downstream signaling through the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), and protein kinase C (PKC) pathways induces survival and differentiation. Other signaling pathways may also play a role in the downstream effects [22].

Although fusions involving members of the NTRK family are the most common oncogenic activation affecting these proteins, other genomic alterations in the *NTRK* genes have been described in cancer, such as mutations, amplifications, and splice variants. Known de novo point mutations in cancer are associated with reduced kinase activity compared with wild-type TRKs and do not seem to function as oncogenic drivers [24]. Amplifications have only been described in very rare cases, where transient response to TRK inhibition has been reported [25]. Splice variants of the *NTRK1* gene have been described in neuroblastoma and acute myeloid leukemia. These variants lack regions of the extracellular domain and the mutant protein has a constitutively active kinase domain, independent of ligand binding, and may hence function as an oncogenic driver. Fusions involving *NTRK1*, *NTRK2*, or *NTRK3* typically involve intrachromosomal or interchromosomal rearrangements that form hybrid genes in which 3′ sequences of *NTRK1*, *NTRK2*, or *NTRK3* that include the kinase domain are juxtaposed to 5′ sequences of a different gene. Fusions may occur in any of the three *NTRK* genes, and, except from brain tumors, they more often arise in the *NTRK1* and *NTRK3* genes, with *NTRK2* being less frequently involved [23, 26, 27]. The product of the gene fusion is an oncoprotein with ligand-independent constitutive activation of TRKs. A variety of fusion partners has been described, such as *ETV6*, *TPM3*, and *LMNA* [28]. The fusion partner seems to play a role in the distribution of the TRK fusion protein within the cell [29]. The clinical implications of this are yet to be established.

Table 1 Neurotrophin family of receptors

TRK receptor	Gene (chromosomal location)	Functions	Natural ligands
TRKA	*NTRK1* (1q23.1)	Pain signaling, thermoregulation	NGF, NT-3
TRKB	*NTRK2* (9q21.33)	Regulation of movement, memory, mood, appetite, body weight	BDNF, NT-4, NT-3
TRKC	*NTRK3* (15q25.3)	Proprioception	NT-3

Neurotrophins are important growth factors that promote sympathetic nervous system development [23]

NGF nerve growth factor, *BDNF* brain-derived neurotrophic factor, *NT* neurotrophin

3 Prevalence

NTRK fusions are found in multiple tumor types in both adult and childhood cancers. The frequency of NTRK fusions in secretory breast carcinoma, mammary analogue secretory carcinoma (MASC), congenital mesoblastic nephroma, and infantile fibrosarcomas are more than 90%. In a subset of cancers lacking the usual oncogenic driver, such as papillary thyroid cancers lacking driver alterations in BRAF and RET, Spitzoid neoplasms with wild-type RAS, GISTs with wild-type KIT and PDGFR, the frequency of NTRK fusion is 5–25%. In the most common cancer types, including lung cancer, pancreatic cancer, head and neck squamous cell cancer, bile duct cancer, breast cancer, colorectal cancer, renal cell carcinomas, melanomas, primary brain tumors, soft-tissue sarcomas, and acute lymphoblastic and acute myeloid leukemias, the frequency of NTRK fusions is < 1% (Table 2) [26, 28, 30, 31]. Data presented in Table 2 are from a tertiary hospital but are comparable with a similar database generated from The Cancer Genome Atlas (TCGA) [32]. Most data on NTRK fusion prevalence are from tertiary referral hospitals and, to the best of our knowledge, no population-based data have been published. With the increasing use of more extensive sequencing, it is clear that many potential druggable alterations exist in a wider population outside the initial or expected indication.

A recent study by Rosen et al. confirmed that NTRK fusions are mainly present in tumors lacking canonical driver mutations [33]. NTRK fusions were significantly associated with the absence of concurrent oncogenic drivers, as well as lower tumor mutation burden, except from microsatellite instability (MSI)-high colorectal cancer where concurrent NTRK fusions were observed. In addition, NTRK fusions were present at all sequential sampled timepoints in the majority of patients, indicating that NTRK fusions are most often clonal rather than passenger alterations [33].

Table 2 Prevalence of NTRK fusions in adult cancer, based on a retrospective analysis of 38,095 samples from 33,997 patients sequenced by a targeted DNA-based next-generation sequencing panel (MSK-IMPACT) [31]

Tumor type	No. of cases per patients tested	Percentage
Salivary gland carcinoma	13/256	5.08
Thyroid carcinoma	13/571	2.28
Sarcoma	13/1915	0.68
Lung adenocarcinoma	9/3993	0.23
Colorectal carcinoma	9/2929	0.31
Glioma/neuroepithelial tumor	8/1464	0.55
Breast carcinoma	6/4458	0.13
Pancreatic adenocarcinoma	5/1492	0.34
Melanoma	4/1125	0.36
Inflammatory myofibroblastic tumor	3/17	17.7
Cholangiocarcinoma	2/787	0.25
Appendiceal adenocarcinoma	1/208	0.48
Neuroendocrine tumor	1/322	0.31

NTRK neurotrophic receptor kinase

4 Detection

NTRK fusions are detected in tumor tissue by a variety of techniques, including fluorescence in-situ hybridization (FISH), immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RT-PCR), and next-generation sequencing (NGS). The detection of gene fusions is far from trivial, especially where different fusion partners exist, and breakpoints also differ. Furthermore, given the rarity of *NTRK* gene fusions in most common cancer types, widespread screening of tissue from thousands of patients will be necessary to detect the few cases with relevant fusions. This entails a challenge with regard to cost and benefit, and calls for methods for inexpensive screening.

The most comprehensive material investigated for sensitivity and specificity across different diagnostic platforms has been done on material from patients participating in the MSK-IMPACT trial. Formalin-fixed paraffin-embedded tissue from 33,997 patients, of whom 87 had a NTRK fusion, was analyzed for detection of NTRK fusions using IHC, DNA-based NGS, and RNA-based fusion panel [31].

4.1 Immunohistochemistry

Although the sensitivity and specificity of IHC for the detection of gene fusions may not be particularly favorable, efforts are being made to develop IHC tests for the detection of NTRK fusions in solid tumors. IHC is standard in even small pathology facilities and is an inexpensive and fast method for the detection of NTRK expression and refining the population for further molecular testing. The potential of IHC as an inexpensive way of screening for NTRK expression and subsequent validation of the reason for overexpression has been evaluated in different settings using different antibodies. Since TRKs are not expressed in normal tissue other than smooth muscles, testes, and neural tissue, high expression is usually linked to a genomic alteration such as an NTRK fusion or, less frequently, NTRK amplification. In the above-mentioned material from the MSK-IMPACT trial, tissue from 66 patients with known NTRK fusions and 317 fusion-negative patients was tested using IHC for NTRK expression. The antibody used was an mAb clone EPR17341, which recognizes a region conserved across all TRKs. As expected, false positive samples were mainly from cancer types of neuronal or muscle origin. The sensitivity

of pan-TRK IHC in this cohort was 96.9, with sensitivity for NTRK1 of 96.2% (26/27), NTRK2 of 100% (5/5), and NTRK3 with the lowest sensitivity of 79.4% (27/34). Specificity of the pan-TRK IHC was 81.1% (257/317) across all tumor types, with the lowest specificity in gliomas (20.8%), small round cell tumors (45.8%), and salivary gland tumors (52%) [31].

4.2 Fluorescence In-situ Hybridization

FISH is widely used as a routine method for the detection of oncogenic gene fusions in specific indications, such as *ALK* and *ROS1* gene fusions in lung cancer. It is not as inexpensive as IHC and is not as widely available. The method is dependent on appropriate probes detecting the relevant gene fusion and breakpoint. Fusions with unknown fusion partners, or fusion partners not covered by the probes, are not detected. Furthermore, the relevant breakpoint needs to be within the area covered by the probes. Detecting gene fusions with unknown partners is possible using break-apart FISH probes. However, false negative rates as high as 36% have been reported in a single series when detecting ETV6-NTRK3 fusions [34].

4.3 Next-Generation Sequencing (NGS)–DNA

DNA-based NGS is effective in detecting gene rearrangements and predicted fusions; however, it is not as effective in fusions involving *NTRK2* and *NTRK3*, where large intronic regions reduce the sensitivity of DNA-based NGS [35]. Furthermore, DNA-based NGS may detect *NTRK* fusions of unknown functional significance, requiring confirmation by another assay [35]. The European Society for Medical Oncology (ESMO) guidelines recommend that DNA-based targeted sequencing assays are often supplemented with RNA-sequencing methods.

4.4 NGS–RNA

Although the FISH and RT-PCR/Sanger approaches are widely adopted in routine diagnostics, current experience with targeted RNA-based NGS is limited. Pfarr et al. reported on an analysis of the major commercially available assays (TruSight TST170 and TruSight RNA Fusion [Illumina]; Archer FusionPlex Solid Tumor, Archer FusionPlex Lung, and Archer FusionPlex Oncology [Archer]; Oncomine Comprehensive Assay v3 RNA and Oncomine Focus RNA [Thermo Fisher Scientific]). The data supported implementation of targeted RNA sequencing in routine diagnostics as the optimal approach [36].

4.5 Diagnostic Strategy

The diagnostic strategy of NTRK fusion detection depends on the diagnosis and the probability of an NTRK gene fusion being present. In recently published ESMO guidelines, it is recommended that in tumors with a high prevalence of NTRK fusions, FISH, RT-PCR, or RNA-based sequencing methods can confirm the diagnosis. In common cancers, where NTRK fusions are uncommon, either front-line sequencing (preferentially RNA-sequencing) or IHC followed by NGS is recommended [35]. As a consequence, NGS-based methods are used in the majority of cases, which is a challenge due to the cost of the test. Even though availability is increasing and the cost is dropping, prioritization is required, especially in public health care systems with limited resources [37].

As NTRK fusions are mainly present in tumors lacking canonical driver mutations [33], testing for NTRK fusions may be omitted in tumors with other known canonical driver mutations.

5 Therapy

Several TKIs with varying degrees of activity against TRKA, TRKB, and/or TRKC are available. They can be broadly grouped into multikinase inhibitors with activity against a range of targets, including TRKs, more-selective TRKi, and second-generation TRKi targeting TRKs harboring resistance mutations. The multikinase inhibitor group includes entrectinib, crizotinib, cabozantinib, lestaurtinib, altiratinib, foretinib, ponatinib, nintedanib, merestinib, MGCD516, PLX7486, DS-6051b, and TSR-011. Most of these agents target multiple tyrosine kinases and have limited activity on TRK; however, entrectinib is selective for TRK, ROS1, and ALK, with half maximal inhibitory concentration (IC50) values in the nanomolar range and very limited off-target effects [38]. Larotrectinib is a selective TRKi targeting all three TRK proteins, with IC50 values in the nanomolar range [39]. In November 2018, larotrectinib was approved by the FDA for the treatment of adult and pediatric patients with tumors harboring *NTRK* gene fusions, and, in August 2019, the FDA granted accelerated approval to entrectinib for adults and adolescents aged 12 years or older with solid tumors with *NTRK* fusions [40].

5.1 Efficacy

5.1.1 Larotrectinib in Clinical Trials

Updated data from three different clinical trials exploring the activity of larotrectinib in adult and pediatric populations were published recently [28]. The publication included

data from 159 patients participating in three trials enrolling TRK fusion-positive, advanced, or metastatic solid tumors: a phase I trial in adults (NCT02122913), the ongoing phase I/II trial in pediatric patients (SCOUT, NCT02637687), and the ongoing phase II trial enrolling adults and adolescents (NAVIGATE, NCT02576431). The analysis only included data from patients with TRK fusion-positive, advanced, or metastatic solid tumors that were not of CNS origin. Most patients were treated at the approved doses of 100 mg capsules twice daily for adults, and a liquid formulation of 100 mg/m^2 twice daily for the pediatric population. In general, patients were treated until progression, and treatment beyond progression was allowed based on specific criteria in the different clinical trials. Eight percent of patients had brain metastases at baseline, 26% had received three or more previous systemic treatment regimens, and 15% had an Eastern Cooperative Oncology Group (ECOG) performance status (or equivalent Lansky performance status score) of 2 or 3. Most prevalent tumor types were soft tissue sarcoma, thyroid cancer, and salivary gland cancer; however, lung cancer, colorectal cancer, and melanoma were also represented. The overall response rate (ORR) according to the Response Evaluation Criteria in Solid Tumors (RECIST) was 77% (95% confidence interval [CI] 72–85), with complete response in 16% and partial response in 63% of patients. ORR was 73% (74 patients) in the adult population and 92% (47 patients) in the pediatric population. No difference in response was observed across different NTRK fusions. There was an early onset to response (median time to response was 1.8 months) equivalent to the first scheduled evaluation, and responses were durable, with a median duration of response of 35.2 months (95% CI 22.8–not evaluated [NE]). The median progression-free survival (PFS) was 28.3 months (95% CI 22.1–NE) and the median overall survival (OS) was 44.4 months (95% CI 36.5–NE).

5.1.2 Entrectinib in Clinical Trials

Data from a pooled analysis of three different ongoing phase I and II trials (ALKA-372-001, STARTRK-1, and STARTRK-2) of entrectinib in adult patients with NTRK fusion-positive advanced or metastatic cancer were recently published [40, 41]. Patients ($n = 59$) included in the analysis were treated with entrectinib at least at the recommended phase II dose of a 600 mg capsule daily. Twenty-two percent of patients had brain metastasis at baseline, 16% had received three or more previous systemic treatment regimens, and all patients had an ECOG performance status of 0–2 (11%, ECOG 2). The most prevalent tumor types were sarcoma (24%), lung cancer (19%), and salivary gland cancer (13%); however, breast cancer (11%), thyroid cancer (9%), and colorectal cancer (7%) were also represented. The ORR, according to RECIST, was 57% (95% CI 43.2–70.8), with complete response in 7% and partial response in 50% of patients. No difference in response was observed across different NTRK fusions, however only one patient had a tumor with an NTRK2 fusion. Responses were durable, with a median duration of response of 10 months (95% CI 7.1–NE) and a median PFS of 11 months (95% CI 8.0–14.9). The estimated median OS was 21 months (95% CI 14.9–NE).

5.1.3 Efficacy in CNS

The efficacy of systemic antineoplastic therapy in the CNS is a significant challenge. Due to the structure of entrectinib, it is anticipated that entrectinib could have significant efficacy in the CNS [42]. This is supported by in vitro and in vivo experiments, demonstrating a lower binding to P-glycoprotein than larotrectinib, and a higher concentration of entrectinib in cerebrospinal fluid in animal models than larotrectinib [42]. However, clinical data, although sparse, support the efficacy of both larotrectinib and entrectinib in patients with CNS disease and do not support the superiority of entrectinib [28, 41]. An effect of larotrectinib has previously been reported in a patient with primary brain cancer [43]. Patients with brain metastases were enrolled in trials with both larotrectinib and entrectinib. There are different criteria for the enrollment of patients with brain metastases across clinical trials. In the entrectinib trials, the brain metastases had to be previously treated and clinically stable or untreated and asymptomatic [41]. The larotrectinib trials enrolled patients with treated or untreated asymptomatic brain metastases. Twelve patients (22%) with CNS disease at baseline were enrolled in the pooled analysis of clinical trials with entrectinib. Response was recorded in 50% of patients and stable disease in 33% of patients. Thirteen patients (8%) with CNS disease at baseline were enrolled in the pooled analysis of clinical trials with larotrectinib. Response was recorded in 75% of patients and stable disease in 17% of patients [28].

Data on the efficacy in CNS were not well-described in either publication, and the number of patients with brain metastases in the two publications was low; however, both entrectinib and larotrectinib seem to have efficacy in brain metastases [28, 41, 44].

5.2 Resistance

Even though many patients experience long-term response to TRKi, resistance occurs over time in many patients, and generally occurs by either a mutation in the target gene or genomic alterations activating alternative signaling pathways. Data from patients treated with TRKi and preclinical data have identified kinase domain mutations affecting the NTRK genes, leading to steric changes in the drug-binding site decreasing the inhibitory properties and potency of the

selective TRKi. These include amino acid substitutions involving solvent front mutations, gatekeeper mutations, and mutations in the xDFG domains, similar to those described for resistance mutations in other classes of kinase inhibitors [45, 46]. Second-generation TRKi have been developed to address these resistance mutations [45].

An alternative resistance mechanism to NTRK inhibition is off-target activation of downstream signaling pathways caused by genomic alterations such as activation mutations or amplifications. Activation of the MAPK pathway has been described as resistance mechanisms in patients treated with TRKi. The genomic alterations include *KRAS* activation mutation, *ERBB2* activation mutation, *MEK1* activation mutation, and *MET* amplifications [47].

5.3 Second-Generation Tyrosine Kinase Inhibitors

5.3.1 Repotrectinib (TPX-0005)

Repotrectinib is an oral ROS1, pan-TRK, and ALK TKI [48]. In preclinical studies, repotrectinib had high potency against ROS1, TRKA, TRKB, and TRKC [49] and efficacy in cell lines harboring *ROS1* resistance mutations, including solvent front mutations similar to those detected as TRKi resistance mutations. The efficacy of repotrectinib has been described in a patient with NTRK fusion-positive cancer harboring a *TRKA* G623E solvent front mutation after treatment with entrectinib [48]. A study of repotrectinib in patients with advanced solid tumors harboring ALK, ROS1, or NTRK1-3 rearrangements is ongoing (TRIDENT-1; NCT03093116). Preliminary data showed that a patient with an NTRK fusion-positive salivary gland tumor and an acquired *TRKC* G623E mutation after treatment with an NTRK TKI responded to repotrectinib as a clinical proof of concept [50].

5.3.2 Selitrectinib (BAY2731954, formerly LOXO-195)

Selitrectinib is an oral, liquid formulated, highly potent, and selective TRK kinase inhibitor that was designed to overcome resistance mediated by acquired *NTRK* kinase domain mutations. Preclinical development was performed by Loxo in parallel with the clinical development of larotrectinib in order to be ready for the first patients who progressed on larotrectinib to potently inhibit the clinically observed TRK resistance mutations. The first two patients with developing acquired resistance mutations on larotrectinib were treated with selitrectinib with rapid-dose titration based on pharmacokinetic assessments and obtained durable tumor responses [45]. A study to test the safety of selitrectinib in adult and pediatric subjects with previously treated NTRK fusion cancers is ongoing (NCT03215511). Preliminary data from 31 patients were presented at the 2019 American Association for Cancer Research (AACR) annual meeting, showing a response rate of 50% in patients with TRK kinase mutations, whereas patients with bypass mutations did not respond [51].

5.4 Safety

5.4.1 Larotrectinib

Safety data have been obtained from patients treated with larotrectinib across the adult phase I trial, the pediatric phase I–II trial (SCOUT), and the adult and adolescent phase II basket trial (NAVIGATE). Larotrectinib was well tolerated and adverse events were, in general, mild and similar across the adult and pediatric age groups. A total of 101 (39%) and 17 (7%) of 260 patients experienced grade 3 or 4 treatment-emergent adverse events, respectively. Anemia (10%) was the most common grade 3 or 4 treatment-emergent adverse event, and grade 3 or 4 larotrectinib-related adverse events were rare (13% and < 1%, respectively). Thirteen patients (5%) had related serious adverse events, with the most common being elevated alanine aminotransferase (< 1%). Dose discontinuation because of treatment-related adverse events (TRAEs) occurred in 2% of patients [28]. On-target adverse events are often observed with drugs that have potent anti-TRK activity [26, 52].

5.4.2 Entrectinib

In the NTRK fusion-positive safety-evaluable population of 68 patients from the STARTRK-1, STARTRK-2, and ALKA-372–001 trials (receiving at least one dose of entrectinib), most TRAEs were mild and reversible. The most commonly reported serious treatment-related event was cognitive disorder, with three serious treatment-related events being reported (one each for cognitive disorder, cerebellar ataxia, and dizziness). The most common grade 3 or 4 TRAEs were increased weight (10%) and anemia (12%). Serious TRAEs were reported in seven (10%) patients with NTRK fusion-positive cancer. These serious adverse events included nervous system disorders (4%). Entrectinib was discontinued in 4% of patients due to TRAEs [41].

TRKs are involved in the development and maintenance of the nervous system, and, as a result, TRK inhibition induces on-target neurological adverse events. These include weight gain, dizziness, and decreased nociception, including withdrawal pain. A recent retrospective study of patients treated with NTRK inhibitors at Memorial Sloan Kettering Cancer Center from 2013 to 2019 analyzed this phenomenon in order to propose optimal management and dose modifications [53]. Most on-target toxicities seem to be manageable with pharmacologic intervention and dose modification. More than half of all patients (53%) receiving

NRTK inhibitors experienced weight gain, which was associated with time on TRK inhibition. Metformin or glucagon-like peptide 1 analogs could induce stabilization or loss of weight. A total of 41% of patients experienced dizziness, with or without ataxia, which could be reduced by dose reduction. Finally, withdrawal pain was observed in 35% of patients and was associated with longer TRKi use. Re-initiation of TRK inhibition could reduce withdrawal pain. Other on-target adverse events include dysgeusia, memory and mood disorders, and paresthesia [53]. These data suggest that the long-term administration of NTRK inhibitors is feasible.

6 Conclusions

NTRK gene fusions occur frequently in certain rare tumors, whereas the prevalence is only around 1% in common cancers. The NTRK inhibitors larotrectinib and entrectinib have been shown to be generally well tolerated and highly active in patients with NTRK fusion cancer, irrespective of tumor type, patient age, and fusion type. Second-generation NTRK inhibitors are in development to overcome NTRK kinase domain mutations. The differences in the prevalence of NTRK gene fusions between rare and common cancers warrants clinical diagnostic strategies to identify patients who may benefit from the therapy. In common cancers, where *NTRK1/2/3* fusions are uncommon, either front-line sequencing (preferentially RNA sequencing) or IHC followed by NGS are recommended.

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Author contributions KSR and UL contributed equally to this manuscript.

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