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Review

NTRK-Fusions – A new kid on the block

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ABSTRACT

The neurotrophic tyrosine receptor kinases (*NTRK*) play an important role in the development and function of the nervous system. Fusions involving *NTRK* and a wide range of genes that act as fusion partners are oncogenic and activate well-known signal transduction pathways like the MAPK-ERK pathway. *NTRK* fusions occur in many very different tumor entities in children and youth as well as in adults. There are a few tumors like secretory breast cancer and congenital fibrosarcoma for which *NTRK* fusions are pathognomonic. At the same time there a large number of tumors in which *NTRK* fusions occur in very rare frequency (e.g., lung cancer). TRK inhibitors offer now the possibility to use *NTRK* fusion as antitumorigenic targets in a tumor agnostic fashion regardless of the basic histology. It is the task of modern pathology to identify such targetable fusions in a highly effective and efficient manner.

1. Introduction

The neurotrophic tyrosine receptor kinases (NTRK) play an important role in the development and function of the nervous system. NTRK is involved in the regulation of pain, thermoregulation, NTRK2 controls movement, memory, cognition, mood, appetite, body weight. Finally, NTRK3 is important for the function of proprioception. There are three receptors (TrkA, TrkB, and TrkC) that are encoded by the three genes *NTRK1, NTRK2*, and *NTRK3*. The main ligands and functions are also summarized in Table 1. The three proteins are structured into an extracellular binding domain, and a transmembrane section and an intracellular domain. The ligand binding results in homodimerization of the receptor and consecutive phosphorylation. That is followed by the downstream activation of well-known signaling pathways like MAPK/ERK, PI3K/AKT and PLCγ/PKC resulting in an enhancement of migration, cell differentiation, synapse formation, and proliferation [1,2] (Fig. 1). Of interest the three ligands also bind p75 neurotrophin receptor ($p75^{NTR}$), which is a transmembrane glycoprotein. The binding with this receptors also cause interaction with proteins important for regulating neuronal functions and survival [3].

The title of this review may suggest that fusions of *NTRK* genes have been detected only very recently. However, the novelty in this field is caused only by its new targetability [4]. Actually, *NTRK* fusions are already known since 1982 [5]. In *NTRK* fusions the 3′-region is fused with a 5′ region of a fusion partner resulting in a chimeric receptor protein with dimerization and consecutively uncontrolled activation of the TRK kinase domain. These oncogenic fusions can occur both intra- or interchromosomal. All three *NTRK* genes are potentially affected. Vaishnavi et al. report in their overview 25 different fusions of which 13 occur in *NTRK1*, eight in *NTRK2* and four in *NTRK3*, respectively [6].

2. Entities

Tumor entities that harbor *NTRK*-fusions can be classified into two groups. The first groups consist of tumors that are very rare and are defined by a specific *NTRK*-fusion which is often diagnostic in those cases. The second group comprises many different tumors that are partly very common but harbor *NTRK* fusions only very rarely. Importantly, *NTRK*-positive tumors are found both in adults and children.

2.1. Tumors with a high frequency of NTRK fusions

Cocco and coworkers defined this group as being driven by NTRK fusions in > 90% of cases. These entities are secretory breast cancer, mammary analog secretory cancer (MASC) of salivary glands, congenital mesoblastic nephroma and infantile fibrosarcomas. These entities mainly show *ETV6-NTRK3* fusions [7]. Lipofibromatosis like neural tumor is a new very recently described entity that carries a *TPR*- and *TPM3-NTRK1* fusion in a very high frequency [8–10]. Haller et al. published two cases of pediatric spindle cell tumors with similar

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Table 1
Physiologic function of the different TRK receptors.

Receptor (*Gen*)	Function
TRKA (*NTRK1*)	Pain, thermoregulation
TRKB (*NTRK2*)	Movement, memory, cognition, mood, appetite, body weight
TRKC (*NTRK3*)	Proprioception

Physiologic function of TRK receptors.

clinicopathological features and an *LMNA-TRK1* fusion [11].

2.2. Tumors with lower frequencies of NTRK-fusions

Cocco et al. suggested to divide the group of tumors with lower frequencies of *NTRK*-fusions into two subgroups with corresponding rates of *NTRK* fusions of 5–25% and less than 5% [7]. The first subgroup comprises thyroid cancers, spizoid melanomas, and gastrointestinal stroma tumors (GIST) [4,12–16]. In addition, there is a relatively long list of tumors where *NTRK* fusions are found in a very low frequency in less than 5% but often in less than 1%. Under these entities are very common ones like non small cell lung cancers (NSCLC), colorectal cancers, head and neck cancers (salivary gland tumors) and astrocytic tumors of all grades [17–20]. In a recently published study, Gatalica et al. reported a low frequency of NTRK fusions in such entities, and only gliomas convincingly exceeded the 1% threshold [21]. Vaishnavi et al. reported in their analysis of the literature at least for some entities considerably higher numbers – e.g., for lung adenocancer 3.3% [6].

3. Other molecular aberrations involving *NTRK* genes

Besides fusions which display by far the majority of aberrations in *NTRK* genes, somatic mutations activating splice variants and TRK overexpression have been reported and are reviewed in other publications. Briefly: The role of somatic mutations is unclear and its potential to promote tumorigenesis is yet to be clarified. Splice variants and overexpression are oncogenic, and the latter has been shown to be drugable by TRK inhibitors [4,7,22].

4. Relevance for targeted therapy

Currently, there is one substance available that already achieved FDA approval for the therapy of *NTRK* positive cancers. This substance is larotrectinib, a selective TRK inhibitor that has been tested in adults and children [23–25]. One major novelty of this therapy is – besides the target - its tumor-agnostic approach. Larotrectinib can be applied in all cancers with proven *NTRK* fusion regardless of the tumor entity and the age of the patients. In phase 1/2 basket trials, a total of 55 patients were enrolled harboring different *NTRK* fusions. All three *NTRK* genes were represented, and the range of age was 4 months to 76 years. The overall response rate was 75%. After one year, the response was ongoing in 71% of the responding patients. At this point, 55% of the patients showed progression-free survival [23].

Enetrectinib is another first-generation TRK inhibitor. It has been tested in three patients with tumors that were not only driven by *NTRK* fusions but also by *ROS1* or *ALK* aberrations [26]. All three patients with *NTRK* fusions responded to the therapy. Larotrectinib, as well as Enetrectinib, are ATP-competitive inhibitors. The ATP-binding sites of all three TRK receptors are very similar, which explains the lacking specificity regarding the three receptors which however might be advantageous in cancer given the rarity of *NTRK* fusions. Other experimental compounds bind outside of the ATP-binding pocket and belong therefore to the type III inhibitors [4,27,28].

Other established multi-target tyrosine kinase inhibitors (TKIs) that are indicated in other entities show response against tumors with *TRK* aberrations [7]. Besides that, there are several TKIs in an early phase of clinical testing [29]. This is important because acquired resistances to first-generation TKIs are limiting factors and can be overcome by second and third generation substances.

There are at least some indications that TRK inhibition could be effective in cancers with TRK overexpression like a subset of neuroblastomas [30,31].

5. Acquired resistance against TRK inhibitors

The currently known acquired resistance mechanisms refer to TRKA and TRKC and involve the corresponding *NTRK* genes (on target mechanism). Mechanisms that modulate other targets (off-target mechanism) are currently not known. However, both the exclusion of TRKB and off-target mechanisms might be identified as the experience in this field will grow.

Mutations in *NTRK1* and 3 results in substitutions within the TRK proteins that change the steric features of the binding sites of the first generation inhibitors. This causes a decrease of sensitivity with an increasing half maximal inhibitory concentration (IC50) [32]. Second generation TRK inhibitors are currently under investigation to answer this issue. It could be shown that LOXO-195, as well as Repotrectinib (TPX-0005), are effective in cases of an acquired resistance to first generation inhibitors [26,29,33]. Foretinib a multitarget TKI could be shown to be effective in an experimental model using cells bearing the NTRK1-G667C mutation [34].

6. Diagnostics of *NTRK* aberrations

There are four options to test for NTRK fusions. These options are in the order of their complexity and costs: immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS). All three techniques have their specific advantages and limitations that will be discussed briefly in the following. *NTRK* fusions were thought to be mutually exclusive [18] therefore it is considered questionable to search for *NTRK* fusions in tumors with already known driver mutations. However, very recently Ling et al. reported the simultaneous occurrence of NTRK fusions and activating EGFR. The authors identified in their collective of more than 3.700 solid cancers 12 cases with NTRK-fusions including six cases of lung cancer. Three of those NSCLCs harbored additional EGFR mutations [35]. Gatalica et al. also identified other aberrations in *NTRK* positive cancers including targetable amplifications of EGFR, MET, VEGFR2 and also a mutation of VEGFR2 [21].

6.1. Immunohistochemistry

IHC is for sure the technique that has the highest availability, is relatively inexpensive and easy to establish. There are currently two antibodies commercially available. One is specific for TRKA (EP 1058Y – Abcam) while the other is a panTRK antibody (clone EPR17341-Abcam and Ventana). Using the pan-Trk monoclonal antibody (mAb) clone EPR17341 (Abcam, Cambridge, MA), Hechtman et al. reported high sensitivity (95.2%) and specificity (100%) compared to the results of MSK-IMPACT, a DNA-based next-generation sequencing assay and transcription analysis with the Archer Dx fusion assay. The authors enrolled 41 cases with 21 TRK positive cases (intestinal, brain, lung, secretory carcinoma, melanoma, and sarcoma) [36]. In concordance, Rudzinski et al. calculated a sensitivity of 97% and a specificity of 98% using also the EPR17341 clone from Abcam. Using the TrkA IHC (EP1058Y) also from Abcam the sensitivity was 100%, and the specificity was 63%. The collection comprised 79 pediatric mesenchymal tumors including 30 *NTRK* positive cases [37]. Gatalica et al. investigated a large cohort including 4136 cases with 28 *NTRK* fusions using the EPR17341 clone from Abcam found a lower sensitivity of 75%

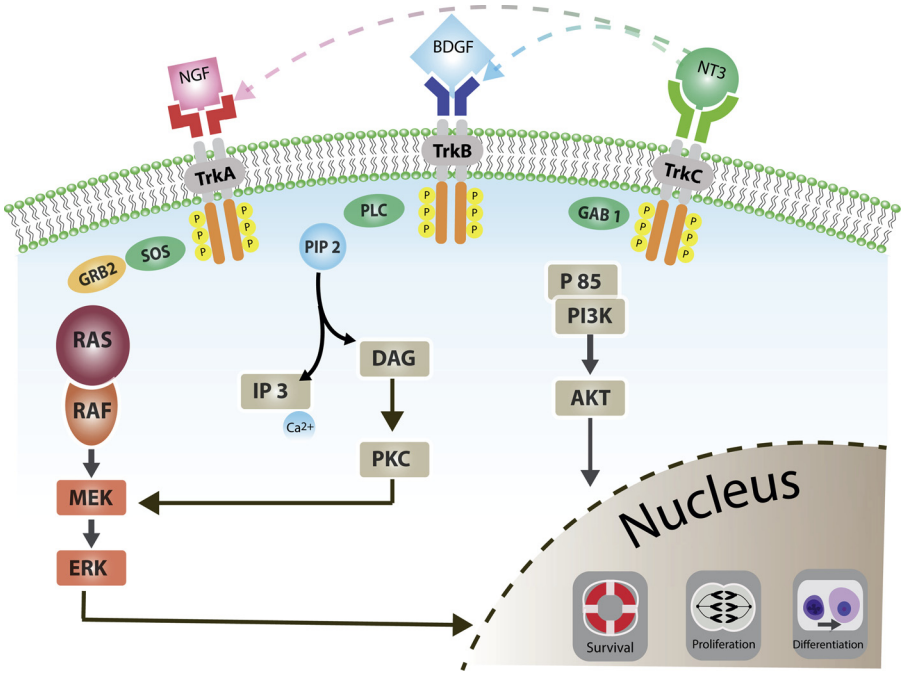

Fig. 1. TRK signaling involving the Ras-ERK-, PI3K-AKT and PLC- pathways. The activation of these pathways promotes survival, differentiation and proliferation. Binding of the ligands with TrkA and TrkB receptors can activate all three pathways while TrkC binding activates the PI3K-AKT pathway. The ligands NGF (nerve growth factor) and BDGF (brain-derived growth factor) bind with the TrkA- and the TrkB receptor, respectively. NT3 (neurotrophin-3) binds with TrkC but also with lower affinity with TrkA and TrkC. AKT, protein kinase Bv-akt murine thymoma viral oncogene homologue; BDGF, brain-derived growth factor; DAG, diacyl-glycerol; ERK, extracellular signal-regulated kinase; GAB1, GRB2-associated-binding protein 1; GRB2, growth factor receptor-bound protein 2; IP3, inositol trisphosphate; MEK, mitogen-activated protein kinase; NGF, nerve growth factor; NTF-3, neurotrophin 3; P85, P85 subunit of, phosphoinositide 3-kinase PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; RAF, rapidly accelerated fibrosarcoma kinase; RAS, rat sarcoma kinase; SHC, Src homology 2 domain containing; SOS, son of sevenless homolog.

and a comparable specificity of 95.9% [21].

According to Rudzinski, the staining pattern differs between *NTRK1/2* fusions versus *NTRK3* fusions. NTRK1/2 positive cases showed only cytoplasmic staining, while NTRK3 fusions showed nuclear +/- cytoplasmic staining [37]. Hechtman et al. postulate that the staining pattern is determined by the *NTRK* fusion partner. For example, *NTRK1-LMNA* fusions showed pronounced staining of the nuclear membrane. This is explained by the authors by the encoding of lamin A/C which is a component of the nuclear membrane [36] (Figs. 2 and 3).

6.2. Fluorescence in situ hybridization

FISH is a robust and widely available method that has a stable position with the diagnostic panel of pathologists. The accepted advantages are relatively low costs, speed, high sensitivity and specificity [38]. The pathognomonic *ETV6-NTRK3* fusions can be identified using probes for *ETV6* or *TRK3* [39–41]. Break-apart probes also can identify unknown fusions. Regarding NTRK fusions it is important that for all three NTRK gene separate probes are available and necessary. This causes additional efforts regarding analytic work and the number of paraffin sections needed. An exception again is the confirmation of one of the rare entities that harbor specific and known fusions. A disadvantage is that break apart probes cannot discriminate between in- and out-of-frame mutation. The latter ones will not be translated and therefore are not functional [24].

6.3. Reverse transcription polymerase chain reaction

RT-PCR also seems especially suitable for the confirmation of diagnosis with a high frequency of known fusions like secretory breast cancer, MASC or congenital fibrosarcoma with knowledge of the involved NTRK gene and fusion partner. RT-PCR is inexpensive, fast and established in many laboratories. The disadvantages are the limited

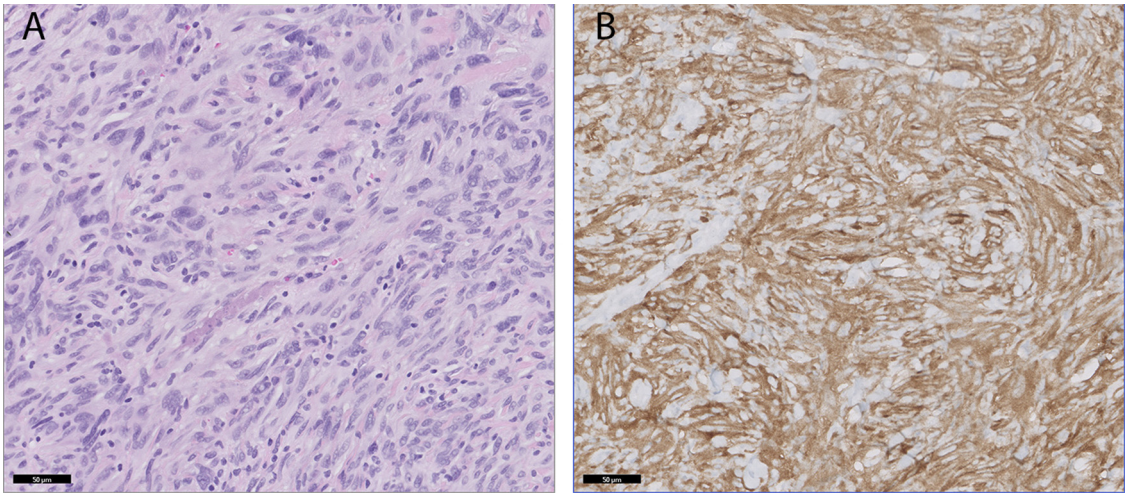

Fig. 2. Histology image of a pediatric mesenchymal spindle cell sarcoma with a *TPM3-TRK1* fusion. **A)** HE. A spindle cell proliferation with moderate atypia, fibroblast-like features, low mitotic activity and fascicular growth pattern is appreciated. Scale bar = 50 µm **B)** IHC with panTRK reveals a diffuse cytoplasmic expression. Scale bar = 50 µm - TPM3 (Tropomyosin 3) is an intracellular plasma protein. The immunohistochemical expression is therefore in concordance with the functional location of the protein of the fusion partner of *TRK*.

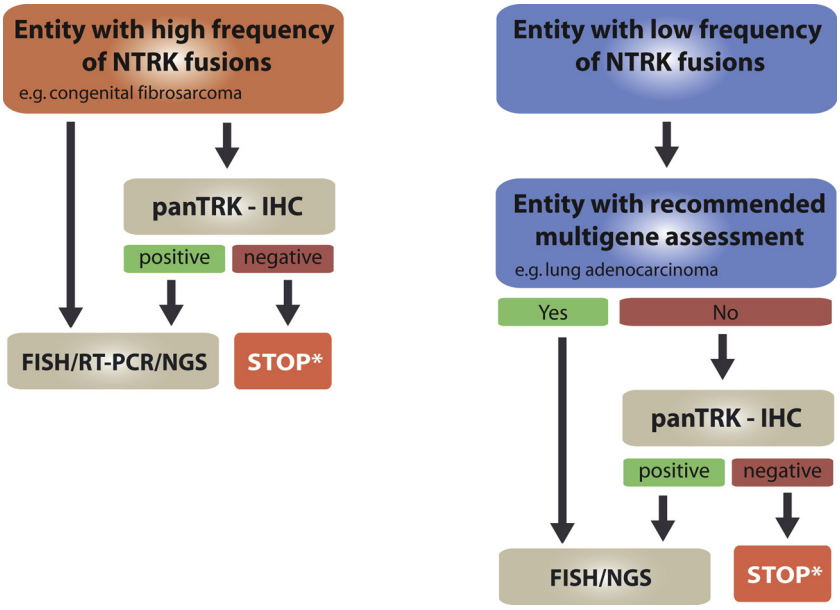

Fig. 3. Suggestion for a diagnostic algorithm to identify *NTRK* fusions stratified according to the pre-test probability for a *NTRK* fusion. Note: despite its high sensitivety negative IHC does not rule out a *NTRK* fusion in 100%.

multiplex capabilities of this technique.

6.4. Next-generation sequencing

NGS is the most powerful tool to search for aberrations including fusions. It has been widely used in the current clinical trials [23–25]. It provides high sensitivity and the opportunity to detect further molecular aberrations. The turn around times are relatively long and amplicon based panel can fail unknown aberrations. The NGS technique is currently only in a minority of pathology laboratories established because the investments for these technologies are high and the establishment itself is complex and needs special experiences and well-trained staff.

6.5. Recommendations for a diagnostic algorithm

Outgoing from a tumor that is suspected to be one of the few and very rare ones that show a high frequency of specific and pathognomonic *NTRK* fusions an initial immunohistochemical investigation is an inexpensive and fast way to screen for it. A confirmation by a second method like RT-PCR, FISH or NGS should be performed in our opinion.

In institutions were NGS is available and the used panel includes *NTRK* aberrations, this technique can also be applied upfront for cases with a low pre-test probability for an *NTRK* aberration. Where this technique is not easily accessible, IHC using a panTRK-antibody is a sensitive method to screen for *NTRK* fusions. Positive cases should be confirmed again by a second technique.

Albert et al. also recommended a frequency adapted approach in pediatric cases. In suspected entities with a known high frequency of *ETV6-NTRK3* fusions, they recommend starting with IHC/FISH (*ETV6* and/or *NTRK3*)/RT-PCR. Negative results need to be re-evaluated by NGS. In intermediately frequent entities (spizoid melanoma, papillary thyroid carcinoma) and inflammatory myofibroblastic tumors IHC/NGS. High-grade gliomas should be tested with NGS technique because glial tissues can express TRK on a physiologic basis. Undifferentiated or spindle cell sarcoma should also be evaluated with NGS upfront [42].

7. Conclusions

NTRK aberrations are tumor drivers in a small portion of very different malignancies. Modern pathology offers with IHC, FISH, RT-PCR, and NGS a wide range of different diagnostic methods to screen for these diagnostic tools with high sensitivity and specificity. Despite its rarity, these aberrations – main fusions – are highly relevant due to the new treatment options with effective TRK inhibitors. The tumor-agnostic approach is a fascinating novelty in oncology. In our opinion, however, this does not mean that classical morphology might be obsolete [43]. Up to now, histology including the special techniques is unbeatable in terms of speed, costs, sensitivity and specificity, and availability and the clear basis of each tissue based diagnosis.

Declaration of Competing Interest

Bruno Märkl received a reimbursement of travel costs and an honorarium for a lecture in 2018 from the Bayer AG, Berlin. Klaus Hirschbühl and Christine Dhillon have no cometing interests to declare.ss

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