ACTA MÉDICA PORTUGUESA
A Revista Científica da Ordem dos Médicos

Practical Guidance on the Detection of *NTRK* Fusions in Sarcomas: Current Status and Diagnostic Challenges

Orientações Práticas sobre a Deteção de Sarcomas de Fusão *NTRK*: Estado Atual e Desafios Diagnósticos

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Acta Med Port 2025 Apr;38(4):266-275 ▪ https://doi.org/10.20344/amp.21925

ABSTRACT

Sarcomas are a rare and heterogeneous group of mesenchymal malignant tumors and account for approximately 1% of all adult cancers and around 20% of all pediatric solid tumors in Europe. Technology advances have enabled a more accurate and efficient characterization of the molecular mechanisms underlying the pathogenesis of sarcoma subtypes and revealed novel and unexpected therapeutic targets with prognostic/predictive biomarkers, namely the neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion. The *NTRK* fusion assessment has recently become a standard part of management for patients with unresectable locally advanced or metastatic cancers and has been identified in various tumor types. In the more prevalent adult and pediatric sarcomas, *NTRK* fusions are present in 1% and 20%, respectively, and in more than 90% of very rare subsets of tumors. The inhibition of TRK activity with first-generation TRK inhibitors has been found to be effective and well tolerated in adult and pediatric patients, independently of the tumor type. Overall, the therapeutic benefit to those patients compensates for the difficulties of identifying *NTRK* gene fusions. However, the rarity and diagnostic complexity of *NTRK* gene fusions raise several questions and challenges for clinicians. To address these issues, an expert panel of medical and pediatric oncologists, radiologists, surgeons, orthopedists, and pathologists reviewed the recent literature and discussed the current status and challenges, proposing a diagnostic algorithm for identifying *NTRK* fusion sarcomas. The aim of this article is to review the updated information on this issue and to provide the experts' recommendations and practical guidance on the optimal management of patients with soft tissue sarcomas, infantile fibrosarcoma, gastrointestinal stromal tumors, and osteosarcoma.
Keywords: Gene Fusion; Oncogene Proteins, Fusion/genetics; Receptor, trkA/genetics; Sarcoma/genetics

RESUMO

Os sarcomas são um grupo raro e heterogéneo de tumores mesenquimatosos malignos, e constituem um dos principais grupos de cancros raros na Europa, representando cerca de 1% de todos os cancros em adultos e cerca de 20% de todos os tumores sólidos pediátricos. Os avanços tecnológicos permitiram uma caracterização mais precisa e eficiente dos mecanismos moleculares subjacentes à patogénese dos subtipos de sarcoma e revelaram novos e inesperados alvos terapêuticos e biomarcadores prognósticos/preditivos, nomeadamente o gene de fusão do recetor tirosina cinase neurotrófico (*NTRK*). A avaliação da fusão de *NTRK* foi incluída, recentemente, na gestão de doentes com cancros localmente avançados irressecáveis ou metastáticos e foi identificada em vários tipos de tumores de adultos e pediátricos. Nos sarcomas mais prevalentes diagnosticados em adultos e pediátricos, as fusões de *NTRK* estão presentes em 1% e 20%, respetivamente, e em mais de 90% dos subconjuntos de tumores muito raros. A inibição da atividade de TRK com inibidores de primeira geração tem-se mostrado eficaz e bem tolerada em doentes adultos e pediátricos, independentemente do tipo de tumor. Globalmente, o benefício terapêutico para estes doentes compensa as dificuldades em identificar os genes de fusão de *NTRK*, sendo que a raridade e a complexidade diagnóstica dos genes de fusão de *NTRK* levantam várias questões e desafios para os médicos. Para abordar estas questões, um painel

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Recebido/*Received*: 08/06/2024 - **Aceite/*Accepted*:** 26/12/2024 - **Publicado/*Published*:** 01/04/2025
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de oncologistas médicos e pediátricos, radiologistas, cirurgiões, ortopedistas e patologistas reviram a literatura recente e discutiram o estado atual e os desafios, propondo um algoritmo de diagnóstico para identificar sarcomas de fusão de *NTRK*. Este artigo pretende apresentar uma revisão da literatura atual sobre o tema e fornecer as recomendações dos especialistas e orientações práticas para a gestão de doentes com sarcomas de tecidos moles, fibrossarcoma infantil, tumores do estroma gastrointestinal e osteossarcomas.
Palavras-chave: Fusão Génica; Proteínas de Fusão Oncogénica/genética; Receptor trkA/genética; Sarcoma/genética

INTRODUCTION

Sarcomas are a rare and heterogeneous group of mesenchymal malignant tumors, accounting for approximately 1% of all adult cancers and 20% of all pediatric solid tumors.[1-6] These tumors can occur in virtually any anatomic site, arising in either soft tissue (~80%) or bone (~20%).[1–5]

Complete surgical resection with or without pre-and postoperative therapies is the standard treatment for most localized sarcomas.[1,7] In locally advanced, metastatic, or recurrent settings, treatment may involve a combination of strategies, including systemic therapy and local approaches.[1,8] The clinical management of sarcomas remains challenging due to their heterogeneity, aggressive nature, and different responses to current treatment options.[7,9]

Technological advances have enabled a more accurate and efficient characterization of the molecular mechanisms underlying the pathogenesis of sarcomas and revealed novel therapeutic targets and prognostic/predictive biomarkers.[1,7,10–12] The discovery of neurotrophic tyrosine receptor kinase (*NTRK*) gene fusions as sarcoma oncogenic drivers led to new personalized therapies for a subset of patients in the form of tropomyosin receptor kinase (TRK) inhibitors, improving clinical outcomes.[1,12–15]

The *NTRK* fusion assessment has recently become a standard for patients with unresectable locally advanced or metastatic cancers.[16] These fusions can be detected using a variety of methods, including tumor deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) sequencing and plasma cell-free DNA profiling.[1,13,17]

Although rare in most common tumor types, *NTRK* fusions are recurrent in certain tumors such as secretory carcinoma of the salivary gland, secretory carcinoma of the breast, thyroid cancers, congenital mesoblastic nephroma, pediatric melanoma, infantile gliomas and infantile fibrosarcoma, where they can be present in > 90% of cases.[1,15,18] In contrast, *NTRK* fusions have been identified in < 1% of other adult and pediatric sarcomas.[1,15,18]

The rarity and diagnostic complexity of *NTRK* gene fusions raises several questions and challenges for clinicians. To address these issues, an expert panel of Portuguese medical and pediatric oncologists, radiologists, surgeons, orthopedic surgeons, and pathologists reviewed the recent literature and discussed diagnostic challenges of patients with soft tissue sarcomas, infantile fibrosarcoma, gastrointestinal stromal tumors (GIST), and osteosarcoma.

The aim of this article is to present updated information on this issue and the experts' proposal of a diagnostic algorithm for *NTRK* fusion sarcomas for practical guidance on the optimal management of these patients.

OVERVIEW OF *NTRK* FUSION CANCER

Etiology of *NTRK* Cancers

The *NTRK* gene family includes three members: *NTRK1* (chromosome 1q23.1), *NTRK2* (chromosome 9q21.33), and *NTRK3* (chromosome 15q25.3), that encode transmembrane TRK proteins TRKA, TRKB, and TRKC, respectively.[1,19] Tropomyosin receptor kinase proteins are expressed in the adult's peripheral and central nervous systems (CNS), and during embryonic development.[20]

Under normal physiological conditions, TRK proteins bind to neurotrophic family ligands leading to downstream signaling that is critical for the normal development and function of the peripheral and CNS.[1,19,21] However, *NTRK* genes may undergo chromosomal rearrangements due to intra- and inter-chromosomal translocations of the kinase portion of *NTRK1/2/3* with an unrelated gene.[20,22] These gene fusions lead to the constitutive activation/expression of chimeric TRK proteins, which have oncogenic properties by driving uncontrolled cell proliferation and tumor growth in a variety of tissues.[20,22]

Neurotrophic tyrosine receptor kinase fusions were first found in colon carcinoma[23] and later described for the first time in pediatric fibrosarcomas, namely the *ETV6::NTRK3* fusion.[24] Currently, there are over 80 different fusion partners identified in a wide range of tumor types.[23–25] In rare cancers, the most common detected gene fusion is *ETV6::NTRK3*, while in the more common, the *NTRK* genes can be found with a large number of different partners, with the *NTRK1* gene usually having more fusion partners than *NTRK2* and *NTRK3* genes.[26,27]

NTRK gene fusions in soft tissue sarcomas

Soft tissue sarcomas comprise a heterogeneous group of cancers with different responses to treatment, which ultimately confer an aggressive behavior, poor prognosis, and a five-year overall survival (OS) rate of 65%.[28,29] Disease management should be performed by a multidisciplinary team in a sarcoma reference center. Among the underlying causes of soft tissue sarcomas, *NTRK* gene fusions account for only 1% of cases.[28–32]

Infantile fibrosarcoma

Infantile fibrosarcoma is a rare pediatric tumor that usually occurs in the first year of life. It forms in connective tissue and, in almost 50% of cases, arises in the extremities of the body, followed by the head, neck, and trunk.[33,34] The tumor often presents a fast-growing period, but it rarely metastasizes. Resection of the tumor with clean margins is the mainstay of treatment, although 48% - 62% are considered unresectable.[34] Chemotherapy has been shown to improve OS in infantile fibrosarcoma by improving the ability to remove the tumor. Infantile fibrosarcoma are tumors with a high prevalence of *NTRK* fusions.[13] *ETV6::NTRK3* fusion is the most common fusion and, together with other variants, is present in about 90% of infantile fibrosarcomas.[24,35,36] This implies a new therapeutic target for these patients.

Gastrointestinal stromal tumors

Gastrointestinal stromal tumors are rare tumors characterized by usually small gastroesophageal or duodenal nodules with a progression risk associated with tumor size and mitotic index.[37,38] In adults, GIST typically occurs in patients aged 60 - 65 and frequently harbor *KIT* or *PDGFRA* mutations.[38,39] Pediatric GIST is mainly characterized by loss of function mutations in succinate dehydrogenase (SDH) genes, encoding the subunits of the SDH enzyme.[40] Regarding *NTRK* gene fusion frequency, screening of 24 GIST lacking *KIT* or *PDGFRA* mutations showed one *NTRK* fusion-positive tumor (4.2%).[41] However, another study of targeted sequencing data from 738 GIST did not found cases with *NTRK* rearrangements. More comprehensive large-scale studies are needed to confirm *NTRK* fusion incidence in GIST.

Osteosarcoma

Osteosarcoma has an overall incidence of 0.3 cases per 100 000/year and occurs most frequently around the knee in adolescents and in the craniofacial bones in adults.[42] High-grade osteosarcoma patients usually develop metastases in the lungs and distant bones.[43] *NTRK* fusions appear to be rare in bone sarcomas, as suggested by a study comprising 354 bone tumors that did not find *NTRK* gene fusion after immunohistochemistry screening.[44,45]

ASSESSMENT OF *NTRK* FUSIONS IN SARCOMAS

Technologies for testing *NTRK* fusions

New *NTRK* gene fusions are being discovered regularly, resulting from the emergence of new screening methodologies.[30] The most common technologies to detect, directly or indirectly, *NTRK* fusions in tumor tissues are immunohistochemistry (IHC), reverse transcriptase polymerase chain reaction (RT-PCR), fluorescence *in situ* hybridization (FISH), next-generation sequencing (NGS) of DNA and/or RNA, and NanoString nCounter technique.

Immunohistochemistry

Immunohistochemistry is a valuable screening tool in clinical environments with limited access to NGS platforms. Regarding other methods, IHC has several benefits, namely time- and tissue-efficiency with an overall good cost-effectiveness.[46] This technique is highly sensitive for detecting *NTRK1/2* fusions but sub-optimal for *NTRK3* fusions (sensitivity < 79%).[16] The anti-TRKA and pan-TRK antibodies can be used to spot elevated TRK expression compared to the low TRK levels observed in control cells.[15,16,46] These antibodies enable the detection of the gene fusions at the protein level, allowing to distinguish between expressing (detectable) and non-expressing (non-detectable) *NTRK* fusions. The staining pattern can be correlated with the subcellular location of the *NTRK* fusion partner. However, TRKA/B/C proteins are physiologically expressed in some healthy cells, like neural and muscle tissue, making it difficult to evaluate the presence of *NTRK* fusions in tumors derived from or involving such organ systems. Additionally, sample preparation can lead to false negatives. Hence, internal and external controls, such as endothelial cells and positive cell lines, are highly recommended. The absence of standard criteria for immunohistochemistry evaluation complicates the interpretation of IHC data; thus, positive results should be followed with a molecular method to further confirm the presence of *NTRK* fusion.[16]

Although international guidelines recommend confirmation of positive TRK IHC with a targeted RNA analysis, up-front testing with a targeted RNA analysis should be preferred in some scenarios since there is limited evidence available regarding the use of IHC in detecting *NTRK* gene alterations in routine practice.[1,15,47,48]

Reverse transcriptase polymerase chain reaction

Reverse transcriptase polymerase chain reaction is a well-established technique to measure the expression of fusion transcripts implicated in a wide variety of sarcomas.[49–51] This method employs a 3' primer annealing to an *NTRK* kinase domain and a 5' primer annealing to a fusion partner, flanking the fusion region.[52] In the presence of the targeted region, the aid of fluorescent signaling probes at each PCR cycle allows detecting the DNA amplification with high sensitivity and specificity.[52] Reverse transcriptase polymerase chain reaction can also be used for quantitative reporting of tumor burden or post-treatment monitoring. One disadvantage of RT-PCR is the need to design a set of primers for each gene fusion transcript that, together with an increasing number of 5' fusion partner genes, reduces the applicability of a multiplex RT-PCR assay.[53] Moreover, it is restricted to known fusion partners, which can lead to false negatives in

the case of an unknown fusion partner. Thus, RT-PCR is usually used as a confirmatory test.

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization is a quick and inexpensive conventional technique to study chromosomal rearrangements and is widely available in many laboratories.[49,54] In FISH, fluorescently labeled DNA probes anneal to specific regions within or flanking a gene(s) of interest to detect the fusion gene events.[54] To bypass the need to develop many FISH probes for each fusion partner gene, a break-apart FISH probe for each *NTRK* gene is used. This approach allows the observation of several fusion targets in one sample using different fluorochromes and the detection of novel fusions with yet uncharacterized fusion partners.[55] This is a sensitive and powerful technique, and a positive FISH result is sufficient to establish the diagnosis of a *NTRK*-positive tumor.[56] However, the fusion partner gene is not identified, and the expression of *NTRK* fusions is not confirmed. Moreover, false positives may occur due to unproductive rearrangements or aberrant probe hybridization, and false negatives may result from FISH probes failure to detect some rearrangements derived from small genomic deletions.[16,55,57]

Next-generation sequencing of DNA and/or RNA

Next-generation sequencing allows precise and simultaneous evaluation of multiple genomic events including detection of NTRK gene fusions. In some assays, it can also detect novel fusion partners.[30] This method has variable levels of sensitivity depending on the assay and computational pipelines.[16] RNA-based NGS is currently the gold standard for the identification of *NTRK* gene fusions because, with the splicing out of introns, it becomes more precise, specific, highly sensitive, and simplified in terms of technical requirements.[58,59] Conversely, DNA-based NGS is less accurate, especially in *NTRK2* and *NTRK3* genes.[58] Besides identifying novel fusion partner genes, the exon involved, and the precise breakpoint, RNA-based NGS also allows to discriminate between in-frame and out-of-frame rearrangements, inferring transcript functionality, and gene expression levels. Additionally, this method relies on sample preparation and RNA integrity and quality, which is particularly critical in bone sarcomas, where fixation and demineralization procedures can result in RNA degradation.[60]

NanoString nCounter technique

NanoString nCounter is a multiplex nucleic acid hybridization technology that enables reliable and reproducible assessment of the expression of up to 800 genes or 228 gene fusions in 12 samples in a single assay. The technique works well with a variety of starting materials from fresh or formalin-fixed tissues, cell lysates or biological fluid samples.[61]

nCounter is a cost-effective technique, with high specificity and sensitivity for detecting *NTRK* fusions, with a high concordance rate with RNA-based NGS assays.[62] However, it is unsuitable for biomarker discovery and, when compared to other methods, may be less sensitive to gene expression variability.[63]

Strategy and screening algorithms

Considering the vast number of *NTRK* gene fusions already identified and the high heterogeneity of tumor types and stages, efficient identification of *NTRK* fusions can be demanding and of utmost importance in order to select the patients that are more likely to benefit from therapy and to rule out other potential drivers of tumorigenesis. Several screening algorithms and recommendations have been developed to provide a reliable diagnosis without unnecessary further testing and improve the time to treatment.[1,2] In Fig. 1, we propose a diagnostic algorithm adapted to real-world clinical practice to identify such patients.

Generally, in histologically confirmed localized sarcomas detected during early stages with complete surgical excision, *NTRK* fusion testing is not fundamental for disease management and may not be considered routine clinical practice. The exception is infantile fibrosarcoma, which has a high prevalence of *NTRK* fusions[7] and should have a confirmatory test such as NGS or FISH. If NGS is not possible, a first screen using IHC pan-TRK can be performed.

In the case of tumors with an elevated recurrence risk, *NTRK* fusion testing using NGS platforms should be conducted to plan the most appropriate therapies.

For locally advanced, unresectable tumors and/or metastatic disease, where alternative therapies are insufficient, testing for *NTRK* gene fusions is certainly advantageous. Patients with identified *NTRK* fusions can benefit from first-line TRK inhibitors.

Rare cancer types that commonly harbor *NTRK* gene fusions, such as infantile fibrosarcoma and inflammatory myofibroblastic tumors should always be tested for *NTRK* fusions. In these cases, a positive result by NGS or FISH is sufficient to provide a diagnosis, whereas a positive staining by IHC pan-TRK should be confirmed by NGS to exclude false positives.

In sarcomas with other genetic drivers, *NTRK* fusion testing is mainly performed for academic and research purposes rather than clinical practice.

A two-step approach involving an IHC pan-TRK screening and confirmatory NGS testing provides a reliable and cost-effective way of detecting *NTRK* gene fusions. However, pathologists should always adapt these algorithms whenever needed for individual clinical circumstances for

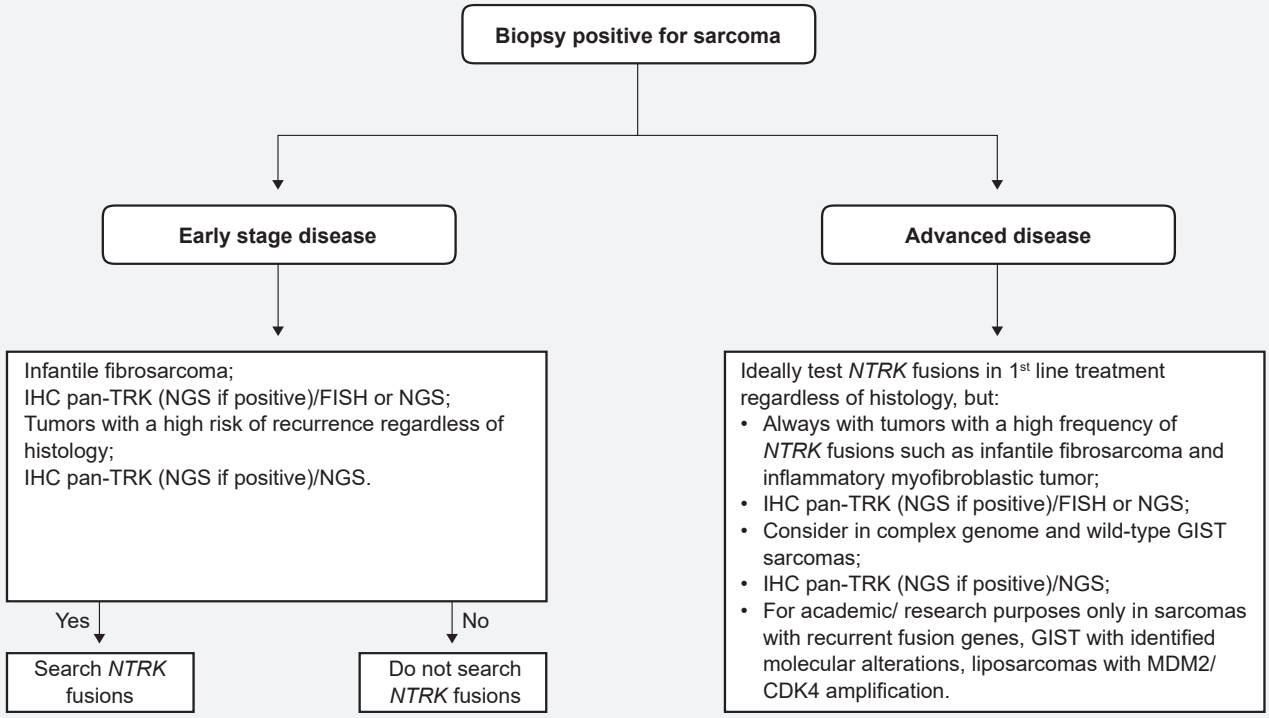

Figure 1 – *NTRK* diagnostic algorithm
FISH: fluorescence *in situ* hybridization; GIST: gastrointestinal stromal tumors; IHC: immunohistochemistry; NGS: next-generation sequencing; *NTRK*: neurotrophic tyrosine receptor kinase; TRK: tropomyosin receptor kinase

benefit of patient's healthcare.

CLINICAL MANAGEMENT OF *NTRK*-FUSED SARCOMAS

Therapies for patients with TRK fusion sarcoma

Several small molecules, grouped into multi-kinase inhibitors or more-selective TRK inhibitors, with different levels of affinity to the TRK domain, are currently in clinical trials (CTs) and some are already approved.[64] Many of them have demonstrated efficacy in *NTRK* fusion-positive solid tumors.[31] The multi-kinase inhibitor group includes entrectinib, crizotinib, cabozantinib, lestaurtinib, ponatinib, nintedanib, merestinib, MGCD516, PLX7486, DS-6051b, and TSR-011.[31] The most specific TRK inhibitor is larotrectinib, the first FDA-approved TRK inhibitor. Larotrectinib and entrectinib are now the first-generation of TRK inhibitors approved for adult and pediatric patients who have a solid tumor with a *NTRK* fusion and no acquired-resistance mutations, which is metastatic or unresectable and a relapse prior to therapy, or without satisfactory alternative treatment options.

FIRST-GENERATION TRK INHIBITORS

Larotrectinib

Larotrectinib is a highly effective and highly selective pan-TRK inhibitor,[2,65] with a binding affinity capacity of more than 100-fold when compared with a panel of several kinases.[66] It has demonstrated a robust tumor-agnostic effect in various sarcomas, including osteosarcoma, dedifferentiated chondrosarcoma, GIST, infantile fibrosarcoma and other soft tissue sarcomas (adult fibrosarcoma, inflammatory myofibroblastic tumor, infantile myofibromatosis, lipofibromatosis, malignant peripheral nerve sheath tumor, myopericytoma, spindle cell sarcoma, high-grade endometrial stromal tumor, and synovial sarcoma).[1,65]

On the clinical setting, larotrectinib has demonstrated a high efficacy profile in a pooled analysis of the first 55 consecutively enrolled patients of three phase I/II clinical trials (CTs) in adult and pediatric TRK fusion-positive cancers, regardless of patient age or tumor type.[57] The overall response rate (ORR) was 75%, the median time of response was 1.8 months and, after one year, 71% of responses were ongoing, with 55% of all patients remaining progression-free.[56] In a recent pooled analysis of the same CTs, including 159 patients with TRK fusion-positive cancer aged from < one month to 84 years and treated with larotrectinib, an objective response of 79% (95% CI 72 - 85) was reported, with 16% having complete responses.[68]

Larotrectinib is available in oral, liquid, or capsule formulations with similar pharmacokinetics, which allows proper administration in infants and children.[69] Additionally,

the treatment is well tolerated, and no grade 4/5 adverse events (AEs) nor related deaths were attributed to the treatment.[69,70] The most common AEs were fatigue, dizziness, anemia, increased liver enzyme levels, hematological toxicity, arthralgia/myalgia, and vomiting.[56,68-71]

More recently, long-term follow-up studies demonstrated that larotrectinib leads to a median OS of > 36 months with an increased survival benefit and a favorable extended safety profile,[72,73] thus contributing to a clinically significant impact in the quality of life (QoL) of 90% of adult and 67% of pediatric patients.[74]

Larotrectinib is also able to cross the blood-brain barrier producing objective and durable responses in subsets of patients with primary CNS tumors or brain metastases from non-CNS solid tumors.[75-78]

The role of larotrectinib may also be extended to a neoadjuvant setting, shifting to a new treatment paradigm for patients with a locally advanced *NTRK* fusion-positive tumor that, without this alternative, would face morbid surgery. Two children with locally advanced infantile fibrosarcoma avoided a possible amputation after larotrectinib treatment substantially reduced the tumor, thus enabling a limb-sparing surgery instead.[56] Four other patients with partial responses underwent surgical resection of the tumor, avoiding morbid surgery. Three of them were classified as a pathological complete response after no viable tumor was detected on the microscopic examination.[69,79]

Entrectinib

Entrectinib is a multi-kinase inhibitor targeting pan-TRK, ROS1, and ALK kinases, with IC50 values in the nanomolar range between 0.1 and 1.7 nM,[80] among other structurally similar off-target kinases.[81] Besides adult and the recent approval for > one month-old pediatric patients with *NTRK* fusion-positive solid tumors, entrectinib is also FDA-approved for patients with *ROS1* fusion-positive metastatic non-small cell lung cancer (NSCLC).[82]

The regulatory and efficacy data for entrectinib approval were based on three phase I/II CTs, comprising a 54-patient analysis. The patients were adults with advanced or metastatic *NTRK* fusion tumors, including with baseline CNS metastases. The ORR was 59%, and the median duration of response was 12.9 months.[83] Long-term follow-up studies showed an increased ORR of 63.5% and response duration of 20 months, supporting that entrectinib is able to induce clinically meaningful improvements with durable systemic and intracranial responses.[84,85]

Entrectinib is available in an oral capsule formulation and is well tolerated, the most common treatment-related AEs being grade 1 or 2 and non-serious, like weight gain, anemia, and fatigue; the most common serious AEs were nervous system disorders, in 4% of patients.[85]

Regarding the pediatric population, entrectinib leads to an objective ORR of 86% in patients with recurrent or refractory solid tumors, including primary CNS tumors.[86] Only 32.4% of patients had to reduce the dosage, and 8.8% discontinued drug-treatment, in both cases, due to AEs.[87] Entrectinib can reduce tumor burden and produce rapid and durable responses, with a progression-free survival of 17.5 months, in children and adolescents.[86,88]

Considering QoL, patients harboring *NTRK* fusion tumors treated with entrectinib reported a stable health status, with a tendency to improve clinical outcomes.[89]

Larotrectinib and entrectinib also provide improved clinical results when compared with prior therapies, and they are progressively being integrated into national and international clinical practice guidelines for the treatment of *NTRK* fusion positive tumors.

Next-generation TRK inhibitor

Tumors treated with first-generation TRK inhibitors can develop resistance to therapy, resulting from resistant mutations. If the resistance is off target (activation of compensatory signaling pathways), patients might benefit from an inhibitor directed to the activated signaling pathway to manage the disease progression.[90]

The resistance mutations can also be on-target when they occur within the TRK kinase domain.[91] These alterations can cause further structural changes on the kinase domain or alter the ATP-binding affinity, reducing the ability of first-generation TRK inhibitors to bind to the TRK kinase domain. Next-generation agents are being developed not only to address on-target resistance but also to maintain the potency against wild-type TRK fusion proteins.[92–94]

Selitrectinib/LOXO-195, a highly effective and sensitive TRK kinase inhibitor, was evaluated in two patients, both with advanced-stage *NTRK* fusion-positive cancers, after acquired resistance to larotrectinib. One patient experienced a rapid clinical response with a reduction of tumor burden and only dizziness as treatment-related AE. The other patient, after an initial partial response to selitrectinib, experienced respiratory distress resorting to hospitalization and her condition worsened afterwards.[93] Considering the CTs results, selitrectinib/LOXO-195 was not moved forward.

Repotrectinib is a highly potent inhibitor against ROS1, ALK, and TRK inhibitors. A proof-of-concept case, harboring an acquired resistance mutation in the *NTRK3* gene, experienced a rapid and robust response within the first few days of treatment with a reduction of tumor burden. After a slow disease progression and, consequently, dose escalation, this patient re-established disease control.[92] More recently, cellular assays showed that repotrectinib is 10-fold more potent against wild-type and mutated TRKA, B, and C proteins than selitrectinib.[94] Repotrectinib has shown

promising results in inhibiting most on-target *NTRK* resistance mutations, and currently, is in phase I/II CTs to establish safety, dosing, and clinical efficacy.[94]

ISSUES AND QUESTIONS ON *NTRK* FUSION SARCOMAS

Testing difficulties

Each molecular diagnosis technique has advantages and disadvantages. Testing decisions should ultimately be made based on the type of tumor and the resources available, including the quality and quantity of biopsy material and equipment accessibility.[16]

Although costly, RNA-based NGS is the gold standard to test *NTRK* gene fusions in sarcomas. IHC is well accepted as a pre-screening tool, but it gives a high rate of false-negative staining in the case of *NTRK3* fusions. RT-PCR and FISH are highly sensitive techniques; however, the former only detects previously known *NTRK* gene fusions, while the latter may not detect some rearrangements derived from small genomic deletions.[16,18,96]

These technologies are optimized to work in formalin-fixed paraffin-embedded sample tissue, and it is important to have an image-guided biopsy to collect the material.[30,51,55]

More recently, some technologies have been developed to take advantage of liquid biopsies, from which circulating tumor cells and circulating cell free tumor DNA/RNA can be harvested.[96,97] Circulating tumor DNA represents a non-invasive approach that allows monitoring tumor recurrence or progression throughout treatment. However, the sensitivity level of this method will vary with the cell shedding capacity of the tumor and, consequently, with the amount of material for detection in circulation.[97]

The FISH and IHC methods have already been optimized to directly detect gene rearrangements in filtration-enriched circulating tumor cells from NSCLC.[96] Still, validation from other groups is needed before clinical implementation.

Genetic variability and mutations

A variety of *NTRK* alterations, other than fusions, have been identified in 14% of several tumor types, including point mutations, amplifications, deletions, and splice variants.[98] Data showing the response of tumors with non-fusion *NTRK* alterations treated with TRK inhibitors is still limited. A case-report presented one patient with an *NTRK* amplification that exhibited a partial response of short duration; however, none of the tumors with *NRTK* point mutations responded to treatment.[69] Another described a patient with a metastatic esophageal squamous cell carcinoma harboring an *NTRK1* amplification treated with larotrectinib. Initially, the patient showed a partial response of the primary and metastatic tumors, but 3.5 months later, the disease progressed.[99]

CONCLUSION

Since *NTRK* fusions are present in 1% to 20% of the more prevalent adult and pediatric sarcomas, and more than 90% of very rare subsets of tumors, patients eligible for TRK inhibitors are a minority within the overall number of cases of patients with sarcoma.[1,18] Nevertheless, the inhibition of TRK activity with first-generation of TRK inhibitors is effective and well tolerated in adult and pediatric patients, independently of the tumor type.[69,70,87]

The therapeutic benefit to those patients compensates for the difficulties of identifying *NTRK* gene fusions. Accordingly, pathologists play a critical role in the diagnosis and assessment of patients with cancer. Several clinical guidelines and *NTRK* gene fusion testing recommendations have been developed to help identify *NTRK* fusion-positive cancers.[63,100] Following these diagnostic algorithms, pathologists should consider the optimal use of tumor tissue and testing prioritization when tumor tissue is limited, such as small biopsies and cytological samples.

In this manuscript, we have reviewed the etiology of *NTRK* cancers and gene fusions in soft tissue sarcomas, namely infantile fibrosarcoma, GIST, and osteosarcoma, and the therapies for patients with TRK fusion sarcoma, including first- and next-generation TRK inhibitors. We reviewed the technologies for testing *NTRK* fusions and discussed the diagnostic challenges. Aiming at optimizing clinical management of these patients we propose a diagnostic algorithm for identifying *NTRK* fusion sarcomas (Fig. 1).

In Portugal, evidence is limited due to regulatory issues. Despite the most recent data and the consensus among the participants in this working group, there is no public coverage in Portugal for these medicines, limiting patients' access to therapeutics. Real-world evidence studies will be essential to demonstrate the improvement in survival with QoL for sarcoma patients with *NTRK* fusion.

AUTHOR CONTRIBUTIONS

All authors participated in the consensus-elaboration meeting and contributed to the diagnostic algorithm elaboration, paper revision and validation.

COMPETING INTERESTS

IF received research funding from PharmaMar and Roche.

AF received honoraria for presentations from Novartis, AstraZeneca and Gilead; support for attending meetings and travel from Pfizer and Gilead; participated on an advisory board from AstraZeneca, Daichi and Lily; has a leadership role in the Portuguese Oncologic Study Group.

JL received honoraria for meeting presentations from Thermofisher; honoraria for manuscript preparation from Roche; research funding from Thermofisher.

PRS received research and travel funding from Bristol-Myers Squibb, Celgene, Gilead, Incyte, Kern, MSD, Novartis, Pfizer, and PharmaMar.

HV received medical writing support from Bayer; has a leadership role in Grupo Português de Estudos em Sarcomas.

All other authors have declared that no competing interests exist.

FUNDING SOURCES

Bayer financially supported the medical writing company Prime Focus by providing writing assistance.

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