The tropomyosin-related kinases (Trk) are a family of tyrosine kinase receptors comprising at least three known family members: proteins TrkA, TrkB, and TrkC encoded by the NTRK1, NTRK2 and NTRK3 genes, respectively. 1, 2, 3 Like many other receptors, their tyrosine kinase activity links them with multiple intracellular signaling pathways, and although NTRK genes are expressed in a number of different tissue types, their primary role appears to be in neuronal cell growth and differentiation and in mediating the effects of neurotrophins. 1, 2 For example, NTRK2 serves as a receptor for brain-derived neurotrophic factor (BDNF) and neurotrophin 4. 1, 4 With the growing use of molecular profiling and targeted therapies in human cancers, NTRK gene rearrangements have more recently been recognized and implicated in several human cancers, including sarcomas. 4, 5 The clinical development of targeted therapies for Trk also presents an opportunity for individualized treatment for tumors harboring molecular alterations. 4

**CURRENT MOLECULAR ASPECTS OF SARCOMA**

Sant Chawla, MD, director of the Sarcoma Oncology Center and the Cancer Center of Southern California noted in an interview with Targeted Oncology that while molecular testing is now extensively performed in sarcoma, the results, in terms of therapeutic implications, “are still not there.”

There are, however, some exceptions, such as gastrointestinal stromal tumor (GIST) sarcoma, for which targeted therapies are available. In particular, the tyrosine kinase inhibitor (TKI) imatinib (Gleevec) is effective in about 70% to 80% of these tumors. There are specific molecular alterations in GIST sarcoma, such as chromosome 11 alterations, where imatinib is especially effective, with response rates of 90% or higher. Conversely, alterations in chromosomes 13 and 17 appear to be associated with very low response rates to imatinib, and new drugs are being sought for these more challenging sarcoma types. Chawla also cited the efficacy of nintinib, another TKI, which is known to be more active in patients with chromosome 13 mutation GIST sarcoma. Overall, Chawla notes that, while there are about 75 different varieties of sarcomas, only a couple are presently treatable with targeted therapy—one is GIST sarcoma (as mentioned) and a second type is myxoid liposarcoma, which has shown good responses to specific chemotherapies such as trabectedin, and approval for this indication is expected soon.

“Other types of liposarcomas express markers such as Mdm2, and we have new drugs which are inhibiting the Mdm2 pathways—these are antibodies as well as small molecules—and initial clinical trials are promising for inhibiting Mdm2 in those tumors,” Chawla said. Lastly, he cited therapeutic approaches aimed at other molecular markers, such as NY-ESO-1, which is highly expressed in synovial sarcoma, myxoid liposarcoma, and some other sarcoma types, as well as in ovarian cancers and melanoma. “We are taking this marker and creating an antibody, as well as an immunological approach directed against this antigen, NY-ESO-1.” Initial trials will include approaches using dendritic cells and tumor-infiltrating lymphocytes (TILs) directed against this molecular marker, which he sees as extremely promising.

**ONCOGENIC ROLES OF NTRK**

Trk receptors may contribute to oncogenesis in sarcoma through molecular alterations that result in their inappropriate expression or activation. For example, BDNF and neurotrophin 4/5 expression has been found by immunostaining and quantitative real-time RT-PCR in uterine sarcoma cell lines and in primary tumor samples from patients with uterine leiomyosarcoma. 1 Interestingly, the use of a soluble ectodomain of TrkB, or a specific Trk inhibitor, K252a, significantly suppressed proliferation of these cell lines and led to increased apoptosis; whereas, the addition of exogenous BDNF caused an increase in proliferation. The results suggested the importance of this paracrine Trk signaling pathway in uterine sarcoma as well as a potential benefit to Trk inhibition in sarcoma. 5 TrkB has also been previously shown to have the important pro-oncogenic effect of suppressing anoikis, or apoptosis resulting from a loss of cell-matrix interactions; thus its inappropriate expression in tumors may potentially contribute to an aggressive and metastatic behavior. 5

Kinas that become inappropriately activated through gene rearrangements have also been recognized for some time as oncogenic drivers for both hematopoietic and solid tumor types, and their inhibition has been an attractive target for drug development. 4 Trk kinase fusions, in particular, have been described in multiple cancers including colorectal cancer, lung adenocarcinoma, salivary gland cancer, head and neck squamous cell cancer, glioblastoma multiforme, and thyroid cancer, and this has further fueled the development of pan-Trk inhibitors for use in oncology. 3, 4 In accordance with the potential for Trk fusions to be used as molecular targets in cancer, Trk inhibition has been shown in vitro to inhibit the proliferation of cell lines expressing Trk fusions. In a human colorectal cancer cell line (KM-12) driven by constitutively active TrkA fusion TPM3-NTRK1, entrectinib (a pan-Trk/ROS1/ALK inhibitor also known as RXDX-101) exhibited in vitro antiproliferative activity with an IC50 of 17 nM, accompanied by inhibition of TrkA phosphorylation and concomitant inactivation of downstream effectors, PLCg1, AKT, and ERK, as well as cell cycle arrest and apoptosis. In mice bearing KM-12 xenografts, treatment with entrectinib resulted in tumor regression and durable stasis under either intermittent or continuous dosing regimens, accompanied by sustained intratumoral inhibition of phospho-TrkA and PLCg1. 7 In a second preclinical study, in vivo effects of entrectinib as a single agent and in combination with the chemotherapeutic agents irinotecan and temozolomide (irino-TMZ) was tested in TrkA-expressing neuroblastoma (NB) xenografts. Significant tumor growth inhibition was observed compared with controls when entrectinib was used in combination with irino-TMZ and when used as a single agent therapy. 8

A recent clinical study details strong clinical response to a Trk inhibitor by a sarcoma patient. An oncogenic Trk fusion was found in a patient with soft tissue sarcoma, consisting of exons 1-2 of the lamin A/C gene, and exons 11-17 of the NTRK1 gene (LMNA-NTRK1). 4 Further, an examination of the patient’s tumor sample using a proximity ligation assay approach to detect functional signaling complexes demonstrated a robust signaling activity for the LMNA-TrkA fusion protein, but only weak signaling for the normal TrkA protein, as expressed in blood vessel. The findings thus suggested that the LMNA-TrkA fusion protein acted as a key oncogenic driver for this patient’s tumor, and thus the patient could be rationally treated with a pan-Trk inhibitor drug (LOXO-101), as such, the patient was ultimately enrolled in a phase I trial of this agent (100 mg twice daily). 9 The results showed that the patient exhibited improvement in the tumor-related symptom of exer- cise dyspnea and reduction in CA125 marker levels by Cycle 1 of treatment, with no observed treatment-related adverse events (AEs). By Cycle 2, computed tomography (CT) scan showed a partial response, with marked improvement in multiple pulmonary metastases as assessed by RECIST 1.1 criteria, and by Cycle 5 (after 4 months of dosing), there was almost complete regression of the largest tumor. Collectively, although further follow-up is needed to assess the durability of patient’s response, the results showed that Trk fusions could constitute potentially actionable molecular targets for the subset of soft tissue sarcoma patients harboring NTRK rearrangements. 9

**MOLECULAR ANALYSIS OF NTRK IN SARCOMA: GROWING INTEREST**

Herbert H. F. Loong, MBBS, MRCP, FHKCP, FHKAM, is a specialist in medical oncol-
ogy and assistant clinical professor in the Department of Clinical Oncology at Prince of Wales Hospital in The Chinese University of Hong Kong. In an interview with Targeted Oncology, Loong noted that no large published series are currently exploring overall frequency of NTRK gene rearrangements in sarcoma.

“Previously, Trk fusion fusions were known for a rare sarcoma known as infantile congenital fibrosarcoma. However, with the emergence of large-scale screening through next-generation sequencing (NGS) and other platforms, recent data from FoundationOne have detected NTRK1 and NTRK3 fusions in 8 of their 1272 soft-tissue sarcoma samples. This is believed to be an underestimate, and within the community, we believe that NTRK fusions occur more frequently,” Loong said. However, “It is important to note that sarcomas are extremely heterogenous in their own right, and as yet, there have not been conclusive studies that have identified which histological sarcoma subtype (aside from congenital fibrosarcoma) has a higher incidence.”

Loong believes that molecular testing for sarcoma is becoming more common but is now mainly used as a confirmatory test in addition to traditional hematokin-sisin (H&E) and immunohistochemical (IHC) methods. “Given the heterogeneity of sarcomas with over 90+ subtypes in the WHO guidelines, and the relative low incidence of sarcomas in general, molecular testing in this disease is taking a much more prominent role. In particular, about 10% to 15% of all sarcomas bear a recurrent chromosomal translocation, and recent technological advances have led to changes in diagnosis and classification of these tumors,” he said.

In a study of 203 adolescent and young adult sarcomas, three NTRK1 fusions were found, suggesting a prevalence of 1.5%. “These patients had multiple NTRK1 fusion partners, and were found in an angiosarcoma, a leimyosarcoma, and an undifferentiated sarcoma. However, given the large size of the NTRK genes, particularly large intronic regions of NTRK2 and NTRK3, as well as the many fusion partners, it is likely that there may be higher incidence of fusions that are missed using currently available testing methodologies. Recent diagnostic technology improvements use hypothesis-free RNA-based testing to eliminate both false-negative issues associated with large intronic repetitive regions and a priori assumptions about gene partner or fusion location. This technology has been developed for use in Ignyta’s phase II NTRK/ROS1/ALK basket trial across solid tumors including sarcomas, of the compound entrectinib.”

**TRK-TARGETED THERAPIES OFFER NEW TREATMENT POSSIBILITIES**

In a late-breaking abstract at this year’s European Society for Medical Oncology (ESMO) meeting, phase I results with entrectinib in patients with solid tumors having molecular rearrangements in the NTRK 1/2/3, ROS1, or ALK genes were presented. The safety, pharmacokinetic and recommended phase II dosing (RP2D) for entrectinib was investigated across two phase 1 clinical trials, using a 3+3 dose escalation, with entrectinib given intermittently or with once daily (QD) dosing at ranges between 100 to 1600 mg/m² and at fixed doses of 600 mg and 800 mg. RECIST v1.1 criteria were used to evaluate antitumor activity of entrectinib in the study. Overall, entrectinib was well tolerated. The most frequent (>10% incidence) treatment-related AEs were fatigue/asthenia, dysgeusia, parasthesia, nausea, and myalgia. In addition, three treatment-related serious AEs were reported, two of which occurred above the RP2D, with the remainder, a case of Grade 2 fatigue and fall, occurring at 600 mg fixed.

Notably, an Overall Response Rate of 72% (13/18 patients) and a disease control rate of 89% (16/18 patients) was observed in the population with NTRK, ROS1 and ALK gene rearrangements. Of special interest was a non-small cell lung cancer patient with NTRK1 rearrangement who had a partial response along with complete resolution of numerous CNS metastatic lesions. The favorable results with entrectinib seen in the two trials, with good tolerability, predictable pharmacokinetics, and antitumor activity in a population of patients with relevant molecular alterations (including NTRK alterations) have prompted the recent initiation of a global phase II basket study of entrectinib (STARTTRK-2 trial).

“A basket trial is a new and evolving form of clinical trial design where the predicted hypothesis is that the presence of a particular molecular marker predicts response to a targeted therapy independent of tumor histology,” Loong explained. “Essentially, patients of all histologies (although the design can be to incorporate cancers, which are known to have more of a particular mutation/ aberrations instead of all-comers) have their tumors screened for particular molecular aberrations, and patients who have that particularly actionable mutation/aberration are permitted participation. The success of a basket trial depends in large part on the strength of the data linking the target and targeted therapy, in line with what he calls Ignyta’s ‘Dx-Rx’ strategy—with molecular diagnosis first, followed by rational targeted treatments. TT

**References**


**Table.** Frequencies of NTRK Gene Fusions

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<th>Gene</th>
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<th>Salivary gland-mammary analog secretory carcinoma</th>
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NOS indicates not otherwise specified.

**T A B L E.** Frequencies of NTRK Gene Fusions

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*These large studies that early signals of efficacy are seen, and if that is the case, the trial can have an expansion cohort of this particular histological type. It is extremely difficult to set up and recruit patients in stand-alone trials of particular histological subtypes in rare tumors, due to the lack of patients that can be accrued and the costs/efficacy ratio involved.*

Chawla noted that his own group will also be involved with the entrectinib basket study, which will involve molecular testing for NTRK gene rearrangements in samples from patients with sarcoma at his institution. He noted the positive data that have been seen with entrectinib in patients with NTRK rearrangements in colon cancer, lung cancer, and salivary gland cancer, but which has not yet been tested in sarcomas. He expects that (as has been the case with other targeted treatments in basket trials) any sarcoma patients identified with NTRK gene rearrangements will be eligible for treatment, in line with what he calls Ignyta’s ‘Dx-Rx’ strategy—with molecular diagnosis first, followed by rational targeted treatments. TT