Intestinal LMNA::NTRK1-fused spindle cell neoplasm with S100 and CD34 coexpression: a new case

Shabina Rahim,1 Saif Sabah Alkhaldi,2 Khaledah Alasousi,3 Rola H Ali

SUMMARY
Recurrent fusions involving neurotrophin tyrosine receptor kinase (NTRK) genes have been increasingly recognised in spindle cell tumours of somatic soft tissues due to the widespread use of RNA-based sequencing techniques. This heterogeneous group of neoplasms is included as an emerging entity in the current WHO Classification of Soft Tissue and Bone Tumors. A subset of these tumours, associated with NTRK1 fusions, displays a distinctive phenotype in the form of monomorphic cyt morphology, patternless arrangement, perivascular and stromal hyalinisation, and CD34+/S100+/SOX10− immunoprofile. Gastrointestinal tract counterparts have been recently described with emphasis on distinction from KIT/PODFRA/BRAF/RAS wild-type gastrointestinal stromal tumours (GIST). Here, we present a recently encountered intestinal spindle cell neoplasm harbouring an LMNA::NTRK1 gene fusion in a woman in her early 20s, which was initially thought to represent a GIST or a solitary fibrous tumour. Awareness of this emerging tumour type in the gastrointestinal tract is important due to treatment implications.

BACKGROUND
Neurotrophin tyrosine receptor kinase (NTRK) genes are a family of genes that encode transmembrane proteins called tropomyosin receptor kinases (TRK), including TRK A, B and C proteins encoded by NTRK1, NTRK2 and NTRK3 genes, respectively. TRK proteins are normally expressed in the nervous system with a role in neuronal development and differentiation through activation of downstream pathways such as the RAS/MAPK, PI3K/ Akt and PLCγ pathways. Chromosomal rearrangements involving the NTRK genes are oncogenic drivers in a wide variety of paediatric and adult tumour types. Fusion of the 3′ region of the NTRK gene (containing the NTRK tyrosine kinase domain) with a 5′ partner encodes a chimeric TRK protein lacking the ligand binding domain, leading to constitutive activation of the TRK kinase and uncontrolled cell proliferation. NTRK fusions are highly enriched in certain rare cancers (eg, secretory breast carcinoma), yet they are low in frequency across common adult cancers (eg, colorectal, non-small lung cancers, melanoma, gliomas), and intermediate in frequency in others (eg, papillary thyroid carcinoma). In the realm of soft tissue neoplasms, ETV6::NTRK3 fusion has long been regarded as the defining feature of infantile fibrosarcoma until its recent detection in a subset of ALK-negative inflammatory myofibroblastic tumours and a few ‘wild-type’ gastrointestinal stromal tumours (GIST). Additionally, variant NTRK rearrangements have been increasingly appreciated in novel tumour subtypes that, until recently, were deemed unclassifiable based on conventional morphology and immunohistochemistry alone. These include lipofibromatosis-like neural tumours, spindle cell sarcomas resembling malignant peripheral nerve sheath tumours (MPNST), spindle cell sarcomas with myopericytic pattern and a subtype of uterine sarcomas resembling ‘fibrosarcoma’.

In 2016, Agaram et al described an NTRK-rearranged ‘neural’ tumour occurring in somatic soft tissues of young patients with histological resemblance to lipofibromatosis, coexpression of CD34 and S100, and exclusive NTRK1 fusion variants; TPR::NTRK1, TPM3::NTRK1 and LMNA::NTRK1. The tumours were found to span a morphological spectrum from hypocellular monomorphic (including lipofibromatosis-like and solid) to overtly malignant. Suurmeijer et al then described seven spindle cell tumours harbouring NTRK3 rearrangements, a rare finding in non-infantile fibrosarcoma, with a morphology that was at the high-grade end of the spectrum. Despite the resemblance of these tumours to MPNST, they lacked SOX10 expression, retained H3K27me3 expression and lacked association with neurofibromatosis type I and prior irradiation. It was not until 2021 when the first report of gastrointestinal NTRK-rearranged spindle cell neoplasms (NTRK-SCN) was published by Aiqiq et al, providing insights into the morphological spectrum in this specific location and emphasising distinction from GIST.

NTRK-SCN officially appeared in the WHO books as an ‘emerging’ entity in the 5th edition of the WHO Classification of Soft Tissue and Bone Tumors (2020), categorised under ‘tumours of uncertain differentiation’. Soon after, it appeared in the inaugural WHO Classification of Pediatric Tumors as ‘paediatric NTRK-rearranged spindle cell neoplasm’ under myofibroblastic tumours. Gastrointestinal involvement was mentioned in the latter book based on the single study published to date. With the advent and FDA approval of TRK inhibitors, it is important for the pathologist to be able to recognise these tumours particularly at unusual sites. Here, we report an additional NTRK-SCN of the gastrointestinal tract that has posed a diagnostic challenge due to the intestinal localisation of this emerging entity.

CASE PRESENTATION
A previously healthy woman in her 20s presented acutely to the surgical emergency department with a 1 day history of severe abdominal pain. The pain had started at the periumbilical area, gradually intensifying into colicky pain that radiated to the...
right iliac fossa and suprapubic area. This was accompanied by nausea, vomiting and diarrhoea. There was no history of constitutional symptoms or recent weight loss. Medical and surgical history was unremarkable. On admission, her vital signs were stable; temperature 37.2°C, blood pressure 110/60 mm Hg, pulse 80 beats per minute and respiratory rate 40 breaths per minute. On deep palpation, the patient was found to have abdominal tenderness localised to the right iliac fossa with a positive rebound sign, negative Rovsing’s sign and no muscular rigidity. The Alvarado score for acute appendicitis was 5 out of 10. The patient was afebrile and had no leucocytosis on total blood cell count. The overall clinical picture was atypical for acute appendicitis; therefore, an urgent CT scan of abdomen and pelvis was requested mainly to exclude gynaecological emergencies.

The CT scan (figure 1A,B) revealed a relatively defined mass located in the terminal ileum, measuring 2.7 × 2.6 × 1.8 cm, that was isodense on plain images and hyperenhanced postcontrast. This was associated with partial ileal dilation in proximal loops along with fluid levels, suggestive of incomplete intestinal obstruction. There was circumferential mural thickening with mucosal hyperenhancement and submucosal oedema, in addition to prominent adjacent fat stranding indicative of inflammation. Prominent surrounding mesenteric vessels were noted. There was no evidence of intussusception or bowel perforation. The radiological impression was that of a neuroendocrine neoplasm (carcinoid) of the ileum versus GIST. The patient underwent urgent surgery, and the tumour was identified at 30 cm proximal to the ileocecal valve. Segmental small bowel resection was performed.

Gross pathological examination revealed a circumscribed firm grey-white mass in the bowel wall measuring 2.0 × 1.9 × 1.0 cm (figure 2A,B). Microscopically, however, the tumour borders were highly irregular traversing the muscularis propria and the muscularis mucosae and splaying the muscle fibres apart (figure 3A,B). The deep edge of the tumour showed infiltration through mesenteric fat resembling the honeycomb infiltration of dermatofibrosarcoma protuberans (figure 3C). Prominent vascularity was noted with ectatic thin-walled haemangiopericytoma (HPC)-like vessels as well as thick-walled blood vessels. A distinctive feature was the presence of perivascular rings of keloid-like hyalinised collagen as well as band-like stromal collagen (figure 3D). The tumour was composed of haphazardly arranged monomorphic spindle cells in a variably collagenous stroma with a storiform pattern in some foci (figure 3E). The cells were ovoid to spindle showing bland nuclei, inconspicuous nucleoli and scant pale eosinophilic cytoplasm with indistinct cytoplasmic borders. Despite the overall bland appearance, interspersed pleomorphic multinucleated cells were identified on closer inspection (figure 3F). Mitotic count was low with a maximum of 2 mitoses per 10 high power field. Scattered lymphocytes and occasional lymphoid aggregates were seen in the background. Necrosis was absent. The morphological impression was that of a low-grade spindle cell lesion, with GIST being at top of the differential diagnosis in view of the intestinal location. Other than GIST, the differential diagnosis was broad including in no specific order: solitary fibrous tumour (especially in the presence of the HPC-like vascular pattern, perivascular hyalinisation and haphazard arrangement), inflammatory myofibroblastic tumour (especially in view of the young age and inflammatory lymphocytic infiltrate), peripheral nerve sheath tumour, smooth muscle tumour, desmoid fibromatosis and, to a lesser extent, an unusual monophasic synovial sarcoma.

On immunohistochemistry, tumour cells were unexpectedly found to be negative for expression of GIST markers CD117 and DOG1 but were positive for CD34 (figure 4A). This prompted a repeat of the CD117/DOG1 stains which again came back negative. Solitary fibrous tumor was second in line due to the ectatic vascularity and CD34 positivity; however, STAT6 was negative excluding this possibility. In addition to CD34, tumour cells only expressed S100 (figure 4B), so at this point, the differential
diagnosis of a nerve sheath tumour was rekindled and SOX10 was requested for confirmation but came back negative. On the other hand, all the following markers were negative: pan-cytokeratin/EMA (arguing against epithelial differentiation and synovial sarcoma), desmin/SMA (arguing against smooth muscle differentiation), HMB45/Melan-A (arguing against melanocytic lesions), ALK1 protein (arguing against inflammatory myofibroblastic tumour), beta-catenin (excluding desmoid fibromatosis) and synaptophysin/chromogranin (excluding neuroendocrine tumours). Ki-67 showed a low proliferation index corresponding to the low mitotic count. Immunostaining for H3K27me3 showed intact nuclear expression further excluding a low-grade malignant nerve sheath tumour.

Molecular testing was performed using a targeted DNA-based next generation sequencing (NGS) panel, namely the OncoMine Comprehensive Assay v3, which showed no mutations in the KIT, PDGFRA, RAS, BRAF or NF1 genes excluding the diagnosis of GIST. The RNA-based gene fusion assay, on the other hand, revealed the presence of a gain-of-function fusion between LMNA (exon2) and NTRK1 (exon11) on chromosome 1. In view of the molecular results, the tumour was sent for pan-TRK immunostaining (clone EPR17341, Biocare) at an external laboratory which was found to be positive in the form of strong cytoplasmic staining (figure 4C).

OUTCOME AND FOLLOW-UP

Three months postoperatively the patient was free of symptoms and was referred to medical oncology for further management and follow-up. Follow-up CT scan showed no recurrent disease in the abdomen and no evidence of distant metastasis. The patient was not offered TRK inhibitor therapy or any other adjuvant therapy due to the low histological grade and localised picture of her disease since such treatment is generally reserved for metastatic, unresectable or progressive disease.

DISCUSSION

NTRK-SCNs, mainly described in somatic soft tissues, constitute a heterogeneous group of tumours encompassing a spectrum of morphological patterns with varying biological behaviour. The current case fits perfectly with the entity described by Suurmeijer et al., characterised by monomorphic spindle cell proliferation with haphazard growth, CD34+ and S100+ coexpression and distinctive stromal and perivascular hyalinisation, with fusions involving RAF1, BRAF or NTRK1/2/3. Gastrointestinal counterparts have recently been reported by Ariq et al., and at the time of writing this case, two similar reports existed on PubMed (gastric and colonic tumours). As is the case in somatic soft tissues, gastrointestinal tract-based NTRK-SCNs can be divided into three categories: (a) infantile fibrosarcoma with NTRK3 fusions, (b) low-grade CD34+/S100+ tumours with NTRK1 fusions and (c) unclassified high-grade spindle cell sarcomas with NTRK1 fusions. Although rare, gastrointestinal infantile fibrosarcoma with canonical ETV6::NTRK3 fusion is well
documented in the literature, classically presenting in infants. Outside of the classic infantile fibrosarcoma, NTRK-rearranged mesenchymal gastrointestinal (GI) tumours are often associated with NTRK1 fusions, spanning a morphological spectrum from low-grade to high-grade (Table 1). Interestingly, at least five documented wild-type GISTs were found to harbour ETV6::NTRK3 fusion, three of which were reported to express CD117 and/or DOG1 expression status not known.† CD117/DOG1 expression status not known.‡ CD34/S100 coexpressed. §CD117 and/or DOG1 expressed. At present, any nuclear, cytoplasmic or membranous staining is considered positive with no clear-cut association between variant fusions and subcellular distribution of the staining.13

It should be noted, however, that the aforementioned morphological features along with the CD34+/S100+/SOX10− immunophenotype do not seem to be unique to NTRK-rearranged tumours. Overlapping features have been observed in tumours harbouring alternative protein kinase fusions particularly those involving RET, RAF1 and BRAF. The actionable NTRK fusions appear to predict response to TRK inhibitors, such as larotrectinib and entrectinib, across multiple tumour types and histologies, necessitating their detection.2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Recent reports describing similar tumours include the following variant fusions: NCOA4::RET (deep dermal tumour on forearm, 35-year-old man),22 PDZRN3::RAF1 (intramuscular thigh tumour, 4-year-old boy),23 CDC42SE2::BRAF (deep soft tissues of forearm, 32-year-old woman),24 TFG::RET (cutaneous scalp tumour, 24-year-old woman).25 This highlights the importance of NGS-based techniques to accurately identify the fusion partners and gain more insights into this novel group of SCNs.

The actionable NTRK fusions appear to predict response to TRK inhibitors, such as larotrectinib and entrectinib, across multiple tumour types and histologies, necessitating their detection.2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Recent reports describing similar tumours include the following variant fusions: NCOA4::RET (deep dermal tumour on forearm, 35-year-old man),22 PDZRN3::RAF1 (intramuscular thigh tumour, 4-year-old boy),23 CDC42SE2::BRAF (deep soft tissues of forearm, 32-year-old woman),24 TFG::RET (cutaneous scalp tumour, 24-year-old woman).25 This highlights the importance of NGS-based techniques to accurately identify the fusion partners and gain more insights into this novel group of SCNs.

The actionable NTRK fusions appear to predict response to TRK inhibitors, such as larotrectinib and entrectinib, across multiple tumour types and histologies, necessitating their detection.2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 Recent reports describing similar tumours include the following variant fusions: NCOA4::RET (deep dermal tumour on forearm, 35-year-old man),22 PDZRN3::RAF1 (intramuscular thigh tumour, 4-year-old boy),23 CDC42SE2::BRAF (deep soft tissues of forearm, 32-year-old woman),24 TFG::RET (cutaneous scalp tumour, 24-year-old woman).25 This highlights the importance of NGS-based techniques to accurately identify the fusion partners and gain more insights into this novel group of SCNs.

Learning points

- Neurotrophin tyrosine receptor kinase (NTRK)- rearrangements should be considered in any KIT(−)/DOG1(−) gastrointestinal stromal tumour-like lesion particularly in young individuals.
- The CD34+/S100+/SOX10− immunophenotype is a distinctive feature.
- Molecular confirmation is often needed to verify TRK immunohistochemistry results.
- NTRK fusions may be predictive of response to TRK inhibitors particularly in rare aggressive cases.
- Low-grade examples are satisfactorily treated by complete resection.
REFERENCES

1. Valtiavni A, Le AT, Doebelde CR. TRKKing down an old oncogene in a new era of targeted therapy. Cancer Discov 2015;5:25–34.
2. Cocco E, Scallitri M, Drilon A. NTRK fusion-positive cancers and Trk inhibitor therapy. Nat Rev Clin Oncol 2018;15:731–47.
3. Hsiao S, Zehir A, Sinec AN, et al. Detection of tumor NTRK gene fusions to identify patients who may benefit from tyrosine kinase (trk) inhibitor therapy. J Mol Diagn 2019;21:553–71.
4. Alassiri AH, Ali RH, Shen Y, et al. Etv6-Ntrk3 is expressed in a subset of ALK-negative inflammatory myofibroblastic tumors. Am J Surg Pathol 2016;40:1051–61.
5. Shi E, Chmielecki J, Tang C-M, et al. FGFR1 and NTRK3 actionable alterations in ‘Wild-Type’ gastrointestinal stromal tumors. J Transl Med 2016;14:339.
6. Brenca M, Rossi S, Polan M, et al. Transcriptome sequencing identifies ETV6-NTRK3 as a gene fusion involved in GIST. J Pathol 2016;238:543–9.
7. Agaram NP, Zhang L, Sung Y-S, et al. Recurrent NTRK1 gene fusions define a novel subset of locally aggressive lipofibromatosis-like neural tumors. Am J Surg Pathol 2016;40:1407–16.
8. Suurmeijer AJ, Dickson BC, Swanson D, et al. The histologic spectrum of soft tissue spindle cell tumors with NTRK3 gene rearrangements. Genes Chromosomes Cancer 2019;58:739–46.
9. Suurmeijer AJH, Dickson BC, Swanson D, et al. A novel group of spindle cell tumors defined by S100 and CD34 co-expression shows recurrent fusions involving RAF1, BRAF, and NTRK1/2 genes. Genes Chromosomes Cancer 2018;57:611–21.
10. Haller F, Kropf J, Ackermann A, et al. Paediatric and adult soft tissue sarcomas with NTRK1 gene fusions: a subset of spindle cell sarcomas unifed by a prominent myoepithelial/hemangiopericytiform pattern. J Pathol 2016;238:700–10.
11. Chiang S, Cotzia P, Hyman DM, et al. NTRK fusions define a novel uterine sarcoma subtype with features of fibrosarcoma. Am J Surg Pathol 2018;42:791–8.
12. Davis JL, Lockwood CM, Storl B, et al. Expanding the spectrum of pediatric NTRK-rearranged mesenchymal tumors. Am J Surg Pathol 2019;43:435–45.