NTRK gene fusion detection over more than 100 paediatric undifferentiated round cell sarcomas and tumours of uncertain differentiation: diagnostic opportunities and pitfalls

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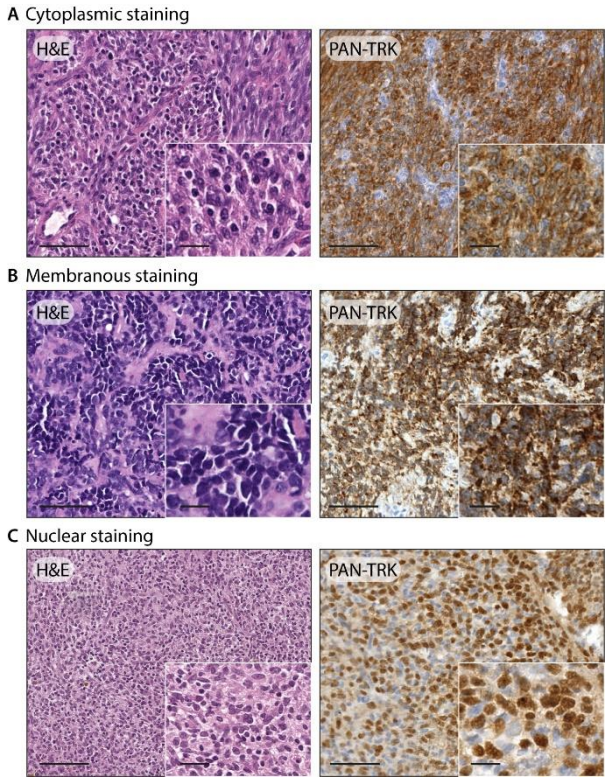
Undifferentiated round cell sarcomas (URCS) of soft tissue and bone are commonly ascribed to the category of neoplasms with low frequency of NTRK gene fusions. However, more recently NTRK-rearranged round cell tumours have been noted in case reports and in limited or heterogeneous cohorts.

The aim of our study is to investigate the presence of NTRK gene fusions in a large retrospective cohort of paediatric URCS after a systematic review of the diagnosis, according to the new WHO classification scheme. One-hundred and five patients with diagnosis of a round cell sarcoma or tumour with uncertain differentiation, involving the bone or soft tissue, were retrospectively evaluated. After the case selection and the histopathological review of the case cohort, pan-Trk immuno-histochemistry testing was performed on formalin-fixed paraffin-embedded tissues. Tumour RNA was extracted from FFPE tissue and subjected to next-generation sequencing library preparation, using a 10-gene NGS fusion panel, sequenced on an Illumina MiSeq.

Pts	Age (years)	Sex	Diagnosis	Location	Mass Volume (cm^3)	AJCC Staging	Oncologic Outcome	IHC Pan-Trk Pattern	NTRK Fusion
1	14	M	ES	Lower Leg	12,6	IIB	DOD	Cytoplasmic	-
2	10	F	ES	Sacrum	0,6	IIA	CDFS	Cytoplasmic	-
3	13	M	TUD	Lower Leg	45	IIB	CDFS	Nuclear	ETV6-NTRK3
4	10	F	ES	Iliac Crest	56	IIB	DOD	Membranous	-
5	11	M	TUD	Jaw	10,8	IIA	CDFS	Nuclear	ETV6-NTRK3
6	2	F	TUD	Lower Leg	3	IIA	NED	Cytoplasmic	-
7	13	M	ES	Lower Leg	28,2	IIIA	CDFS	Cytoplasmic	-
8	12	F	ES	Scapula	241,3	IVB	DOD	Cytoplasmic	-
9	11	F	ES	Sacrum	105,6	IVB	CDFS	Cytoplasmic	-
10	10	M	BCOR	Lower Leg	35	IIA	CDFS	Cytoplasmic	-
11	13	F	ES	Pelvis	12	IIA	CDFS	Cytoplasmic	*
12	16	M	ES	Upper Leg	52,5	IIB	CDFS	Cytoplasmic	*

*Summary of demographic, clinical, immunohistochemical, and molecular data of the twelve IHC pan-Trk positive cases. ES = Ewing Sarcoma; TUD = Tumour of Uncertain Differentiation; BCOR = Sarcoma with BCOR genetic alterations; DOD = Dead of disease; CDFS = Continuous disease-free survival; NED = No evidence of disease; * RNA not suitable for testing..*



The NGS-positive cases were further confirmed by real-time PCR. On immunohistochemical screening, the pan-Trk antibody showed reactivity in 12/105 (11.4%) analyzed samples. NGS RNA fusion panel testing was successfully completed for 10 cases, revealing ETV6-NTRK3 fusion in 2 tumor samples, then confirmed by real-time PCR. NTRK-rearranged cases, showed a nuclear staining distribution pattern when the pan-Trk antibody was performed. As it stands, we can confirm that URCS harbour NTRK fusions in in a small fraction (approximately 2%), in contradistinction to other rare paediatric neoplasms, including secretory carcinoma of the breast, congenital mesoblastic nephroma and infantile fibrosarcoma where NTRK fusion is characteristic and often disease defining. However, the NTRK fusion testing emerged in our study as a diagnostic opportunities when the borderline features between round and spindle cell sarcoma of the two positive cases, together with the demonstration of the NTRK rearrangement, led to include these previously not otherwise specified sarcomas with uncertain differentiation into the emerging category of NTRK-rearranged neoplasms. Our present series suggests that Trk immunoreaction can be used to direct further molecular investigation, but detection of expression is not specific to a fusion event. Thus, the use and the interpretation of the IHC must be carried out with some caution, reserving primary role to the molecular testing, preferably RNA-based methods to detect NTRK gene fusions in sarcomas. This appears to be particularly important when cytoplasmic staining is present in the absence of nuclear, peri-nuclear, or membranous staining.

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AJL reports to be in a consulting role for Hoxo / Bayer and Ignyta / Genentech. DM has received honoraria for professional services and consultancy for Novartis, Bayer HealthCare Pharmaceuticals Inc., Pierre-Fabre, Sanofi Genzyme, MSD Italia S.r.l., Roche, Skyline Dx B.V. FDL is founding scientist of FloNext Srl. The remaining authors declare that they have no conflict of interest.

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