PUBLICATION SNAPSHOT #4

Assoc. Prof. Tracy Stockley
Head of Clinical Laboratory Genetics, University Health Network, Toronto; Dept of Laboratory Medicine and Pathobiology, University of Toronto, Canada.
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This content is supported by an independent educational grant from Bayer.

Disclosures: Assoc. Prof. Tracy Stockley has received honoraria from the following:

• Astellas, AstraZeneca, Bristol Myers Squibb, Janssen, Novartis
DIAGNOSIS AND MANAGEMENT OF TRK FUSION SARCOMAS: EXPERT RECOMMENDATIONS FROM THE WORLD SARCOMA NETWORK

BACKGROUND: HIGH UNMET NEED FOR PATIENTS WITH ADVANCED/METASTATIC SARCOMAS

Sarcoma Sites

<table>
<thead>
<tr>
<th>Bone (20%)</th>
<th>Soft tissue (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull</td>
<td>Head/neck</td>
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<tr>
<td>Ribs</td>
<td>Thorax, trunk, abdomen and upper limb</td>
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<tr>
<td>Long bones of upper limb</td>
<td>Uterus, pelvis</td>
</tr>
<tr>
<td>Long bones of lower limb</td>
<td>Bladder</td>
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<tr>
<td></td>
<td>Lower bones, hips</td>
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</tbody>
</table>

Sarcomas:

- 1% of all adult cancers (=rare cancer)
- 20% of all paediatric solid tumours

For localised setting: curative management = Complete resection (with or without radiation and/or chemotherapy)

For locally advanced or metastatic disease, or those with disease recurrence following surgery: treatment options =
  - systemic therapy
  - radiation, isolated limb perfusion, surgery, and ablation techniques

The median overall survival of patients with advanced soft tissue sarcomas = 20 months
To provide practical guidance on how to optimally integrate the *NTRK* gene fusion biomarker into the clinical management of patients with sarcoma

**OBJECTIVE**

*NTRK* gene fusions discovered as pan-tumour oncogenic drivers

New precision medicine-based treatment options for a subset of patients with sarcoma appeared

*NTRK* gene fusions are rare and diagnostic is complex

Clinicians have several questions and challenges

1. Convened two consensus meetings with expert adult oncologists and pathologists
2. Discuss diagnostic challenges and propose a diagnostic strategy in this area

* World Sarcoma Network (WSN) is a cooperative group gathering the main reference centres for sarcomas around the World dedicated to the development and the support of innovative and collaborative clinical trials and to the drug development in sarcomas

NTRK, neurotrophic receptor tyrosine kinase
# FREQUENCY OF \textit{NTRK} GENE FUSIONS BY SARCOMA SUBTYPES: LITERATURE SEARCH (1/2)

<table>
<thead>
<tr>
<th>Study</th>
<th>Testing method</th>
<th>Proportion of \textit{NTRK} fusions identified, % (n/N)</th>
<th>\textit{NTRK} fusion-positive sarcoma subtypes</th>
<th>\textit{NTRK} genes and fusion partners involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaram NP, et al. 2016</td>
<td>FISH, RNA MPS</td>
<td>71 (10/14)</td>
<td>Lipofibromatosis-like neural tumour</td>
<td>1x TPR-NTRK1, 1x TPM3-NTRK1, 4x LMNA-NTRK1</td>
</tr>
<tr>
<td>Bourgeois JM, et al. 2000</td>
<td>RT-PCR</td>
<td>91 (10/11)</td>
<td>IFS</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>Bui NQ, et al. 2019</td>
<td>Targeted DNA MPS</td>
<td>0.7 (1/152)</td>
<td>Myopericytoma</td>
<td>NR</td>
</tr>
<tr>
<td>Chmielecki J, et al. 2017</td>
<td>Targeted RNA MPS</td>
<td>1 (4/324)</td>
<td>2x IFS, 1x assorted soft tissue sarcoma, 1x hemangioma, 1x bone sarcoma</td>
<td>1x SQSTM1-NTRK1, other fusion partners NR</td>
</tr>
<tr>
<td>Church AJ, et al. 2018</td>
<td>FISH</td>
<td>96% (25/26)</td>
<td>IFS</td>
<td>NTRK3</td>
</tr>
<tr>
<td>Croce S, et al. 2019</td>
<td>Targeted RNA MPS</td>
<td>54 (7/13)</td>
<td>Uterine and vaginal sarcomas resembling fibrosarcoma</td>
<td>6x TPM3-NTRK1, 1x EML4-NTRK3</td>
</tr>
<tr>
<td>Gatalica Z, et al. 2018</td>
<td>Targeted RNA MPS</td>
<td>0.4 (2/478)</td>
<td>1x STS, 1x uterine sarcoma</td>
<td>1x TPM3-NTRK1, 1x SPECC1L-NTRK3</td>
</tr>
<tr>
<td>Shi E, et al. 2016</td>
<td>Targeted DNA MPS</td>
<td>0.5 (1/186) overall (4 [1/24] in quadnegative tumours)</td>
<td>GIST</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>Stranksy N, et al. 2014</td>
<td>TCGA RNAseq dataset</td>
<td>1 (1/103)</td>
<td>Sarcoma</td>
<td>TPM3-NTRK1</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridisation; GIST, gastrointestinal stromal tumour; IFS, infantile fibrosarcoma; IMT, inflammatory myofibroblastic tumour; MPS, massive parallel sequencing; NOS, not otherwise specified; NR, not reported; NTRK, neurotrophic receptor tyrosine kinase; RT-PCR, reverse transcription polymerase chain reaction; STS, soft tissue sarcoma; TCGA, The Cancer Genome Atlas
**FREQUENCY OF NTRK GENE FUSIONS BY SARCOMA SUBTYPES: LITERATURE SEARCH (2/2)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Testing method</th>
<th>Proportion of NTRK fusions identified, % (n/N)</th>
<th>NTRK fusion-positive sarcoma subtypes</th>
<th>NTRK genes and fusion partners involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solomon JP, et al. 2020</td>
<td>Targeted DNA and/or RNA MPS</td>
<td>0.7% (13/1,915)</td>
<td>2x IFS, 2x lipofibromatosis-like neural tumour, 2x uterine sarcoma, 1x uterine high grade pleomorphic sarcoma, 1x high grade spindle cell sarcoma, 1x malignant spindle cell sarcoma, 1x spindle cell sarcoma, 1x angiosarcoma, 1x S-100 positive malignant spindle cell neoplasm, 1x low grade sarcoma</td>
<td>4x LMNA-NTRK1, 3x TPM3-NTRK1, 2x ETV6-NTRK3, 1x TPM4- NTRK3, 1x EEF1A1-NTRK3, 1x PEAR1-NTRK1</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>18% (3/17) IMT ETV6-NTRK3</td>
</tr>
<tr>
<td>Surrey LF, et al. 2019</td>
<td>Targeted RNA MPS</td>
<td>4% (2/45)</td>
<td>Malignant peripheral nerve sheath tumour-like</td>
<td>1x TFG-NTRK3, 1x RBPMS-NTRK3</td>
</tr>
<tr>
<td>Suurmeijer AJH, et al. 2018</td>
<td>FISH, targeted RNA MPS</td>
<td>60% (15/25)</td>
<td>Malignant peripheral nerve sheath tumour-like</td>
<td>8x LMNA-NTRK1, 3x TPM3-NTRK1, 1x SPECC1L-NTRK2, 1x TPR-NTRK1, 2x NTRK1 with unknown fusion partners</td>
</tr>
<tr>
<td>Yamamoto H, et al. 2020</td>
<td>MPS (TBC), IHC</td>
<td>5% (2/40)</td>
<td>IMT</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>Zhu G, et al. 2019</td>
<td>Targeted RNA MPS</td>
<td>3% (5/184)</td>
<td>2x lipofibromatosis-like neural tumour, 1x IFS, 1x IMT, 1x sarcoma NOS</td>
<td>2x ETV6-NTRK3, 2x TPM3-NTRK1, 1x LMNA-NTRK1</td>
</tr>
</tbody>
</table>

**Additional outcomes from literature search**

- A significant number of NTRK fusion-positive sarcomas show co-expression of S100 protein and CD34
- However the rest have a non-specific immunophenotype

**The published literature on NTRK gene fusion frequency in sarcomas is limited and more data are needed**

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FISH, fluorescence in situ hybridisation; IFS, infantile fibrosarcoma; IHC, immunohistochemistry; IMT, inflammatory myofibroblastic tumour; MPS, massive parallel sequencing; NOS, not otherwise specified; NTRK, neurotrophic receptor tyrosine kinase; TBC, to be confirmed
TARGETED THERAPY FOR NTRK GENE FUSION POSITIVE SARCOMAS IS EFFECTIVE

Clinical development programme with larotrectinib

Adult in Phase 1
Advanced solid tumours
NCT02122913

Paediatric in phase 1/2
Advanced solid tumours
SCOUT: NCT02637687

Adult/adolescent in Phase 2
Advanced solid tumours
NAVIGATE: NCT02576431

Patients: 12
Data cutoff: 19 February 2019

Total of 159 patients with TRK fusion solid tumour

71 (~45%) patients with TRK fusion sarcomas

Parameters

TRK fusion sarcomas treated with larotrectinib (n=71)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ORR, % (95% CI)</th>
<th>Median DoR, months</th>
<th>Median PFS, months</th>
<th>Median OS, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, %</td>
<td>87 (95% CI 77-94)</td>
<td>NE (range 1.6+ to 44.2+)</td>
<td>28.3 (95% CI 16.8-NE)</td>
<td>44.4 (95% CI 44.4-NE)</td>
</tr>
</tbody>
</table>

Clinical development programme with entrectinib

ALKA-372-001: Phase 1
Solid tumours
EudraCT 2012-000148-88

STARTRK-1: phase 1
Solid tumours
NCT02097810

STARTRK-2: Phase 2
Solid tumours
NCT02568267

Patients: 50
Data cutoff: 31 May 2018

Total of 54 adults with advanced or metastatic TRK fusion solid tumour

13 (24%) patients with TRK fusion sarcomas

Parameters

TRK fusion sarcomas treated with entrectinib (n=13)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ORR, % (95% CI)</th>
<th>Median DoR, months</th>
<th>Median PFS, months</th>
<th>Median OS, months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR, %</td>
<td>46 (95% CI 19-75)</td>
<td>10.3 (95% CI 4.6-15.0)</td>
<td>11.0 (95% CI 6.5-15.7)</td>
<td>16.8 (95% CI 10.6-20.9)</td>
</tr>
</tbody>
</table>

CI, confidence interval; DoR, duration of response; NE, not estimable; NTRK, neurotrophic receptor tyrosine kinase; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; TRK, tropomyosin receptor kinase

## Current Methods to Test NTRK Gene Fusions: Advantages and Disadvantages

<table>
<thead>
<tr>
<th>FISH</th>
<th>IHC</th>
<th>RT-PCR</th>
<th>MPS</th>
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</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td></td>
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<tr>
<td>Available in many clinical laboratories</td>
<td>Widely available</td>
<td>Widely available</td>
<td>Allows <em>simultaneous detection</em> of fusions between NTRK1-3 and any number of known or novel fusion partner genes</td>
</tr>
<tr>
<td>Rapid turnaround time</td>
<td>Rapid turnaround time</td>
<td>Rapid turnaround time</td>
<td></td>
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<tr>
<td>Relatively inexpensive</td>
<td>Inexpensive</td>
<td>Inexpensive</td>
<td></td>
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<tr>
<td>Allows identification of specific cell types harbouring the NTRK fusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Requires specific expertise</td>
<td>Detection of wild-type protein expression, especially in tumours with neural and myogenic differentiation</td>
<td>Requires knowledge of fusion partner gene sequence</td>
<td>Not routinely conducted in all clinical laboratories</td>
</tr>
<tr>
<td>False negativity rate of ≤30%</td>
<td>False negativity rate of ~10% this rate may be higher in tumours harbouring NTRK3 fusions</td>
<td>Challenging to test for fusions involving multiple NTRK and fusion partner genes in parallel</td>
<td>Relatively long turnaround time</td>
</tr>
<tr>
<td>Does not distinguish between in-frame and out-of-frame fusion events</td>
<td></td>
<td></td>
<td>Relatively expensive</td>
</tr>
<tr>
<td><strong>Notes</strong>:</td>
<td></td>
<td></td>
<td>DNA MPS may <em>miss NTRK2 and NTRK3 fusions</em> due to large introns</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>RNA MPS requires high quality RNA</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Variable detection rates of different panels</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>May identify non oncogenic NTRK rearrangements</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; MPS, massive parallel sequencing; NTRK, neurotrophic receptor tyrosine kinase; RT-PCR, reverse transcription polymerase chain reaction
For patients at high risk of relapse, NTRK gene fusion testing might provide clinically actionable information for later in the disease course. If histology is typical then confirmation by MPS is recommended. Treatment may be considered concurrently with confirmatory MPS. Consider parallel validation by MPS or RT-PCR to confirm that fusion is in-frame. Avoid IHC screening in cases with myogenic and neural differentiation due to the high rate of false positivity.

**NTRK fusion Testing**

- **In primary, resectable sarcomas**
  - not necessary

- **For patients at high risk of relapse**
  - might provide clinically actionable information for later in the disease course

- **For patients with locally advanced, unresectable tumours**
  - Should be performed

- **For patients with metastatic disease failing conventional therapies**
  - Should be performed

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*For patients at high risk of relapse, NTRK gene fusion testing might provide clinically actionable information for later in the disease course. If histology is typical then confirmation by MPS is recommended. Treatment may be considered concurrently with confirmatory MPS. Consider parallel validation by MPS or RT-PCR to confirm that fusion is in-frame. Avoid IHC screening in cases with myogenic and neural differentiation due to the high rate of false positivity.*

FISH, fluorescence in situ hybridization; GIST, gastrointestinal stromal tumour; IFS, infantile fibrosarcoma; IHC, immunohistochecmy; IMT, inflammatory myofibroblastic tumour; LPS, liposarcoma; MPS, massive parallel sequencing; NTRK, neurotrophic tyrosine receptor kinase; RT-PCR, reverse transcription polymerase chain reaction; TRK, tropomyosin receptor kinase.
CONCLUSIONS AND DISCUSSION

Conclusions

• **First diagnostic proposal in sarcoma** taking into account disease stage and histologic and molecular subtypes

• Provides algorithms for diagnostic testing, including how massive parallel sequencing (MPS) is best used in sarcomas to ensure cost-effectiveness

• **Immunohistochemistry (IHC)** provides a valuable pre-screening tool prior to MPS panels

Discussion

• **Further research is necessary** to fully establish the sensitivity and specificity of pan-TRK IHC

• **Multinational comparative studies** to increase the reproducibility of MPS assays

• **Prospective studies critical** to determine the frequency of **NTRK gene fusions** in different sarcoma subtypes and correlation with morphological, biological, and clinical features

• The same approaches should be undertaken in all relevant solid advanced tumours where TRK inhibitors are effective in order to build an effective diagnostic strategy

NTRK, neurotrophic receptor tyrosine kinase; TRK, tropomyosin receptor kinase
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Watch us on the Vimeo Channel NTRK CONNECT
Email froukje.sosef @cor2ed.com
NTRK CONNECT
Bodenackerstrasse 17
4103 Bottmingen
SWITZERLAND

Dr. Froukje Sosef MD
+31 6 2324 3636
froukje.sosef@cor2ed.com

Dr. Antoine Lacombe Pharm D, MBA
+41 79 529 42 79
antoine.lacombe@cor2ed.com

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