NTRK inhibitors: what we learned and where to go

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Milano, Italy
**TRK receptors mediate neurotrophin signaling**

- Neurotrophins are important growth factors involved in the growth, differentiation, and survival of neurons.
- Neurotrophin signaling occurs through activation of the TRK receptor family.

<table>
<thead>
<tr>
<th>TRK Receptor</th>
<th>Gene (Chromosomal Location)</th>
<th>Functions</th>
<th>Adult</th>
<th>Natural Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRKA</strong></td>
<td>NTRK1 (1q23.1)</td>
<td>Cellular differentiation/sensory neuron subtype specification and development of pain and thermoregulation modalities</td>
<td>Pain signaling, thermoregulation</td>
<td>Nerve growth factor (NGF), neurotrophin-3 (NT-3)</td>
</tr>
<tr>
<td><strong>TRKB</strong></td>
<td>NTRK2 (9q21.33)</td>
<td>Development of sensory neurons in the brain</td>
<td>Regulation of movement, memory, mood, appetite, body weight</td>
<td>Brain-derived neurotrophic factor (BDNF), neurotrophin-3/4/5 (NT-3/4/5)</td>
</tr>
<tr>
<td><strong>TRKC</strong></td>
<td>NTRK3 (15q25.3)</td>
<td>Neuronal differentiation, axon outgrowth/guidance, and synaptic plasticity</td>
<td>Proprioception</td>
<td>NT-3</td>
</tr>
</tbody>
</table>

**Neurotrophin Family of Receptors**

![Diagram of Neurotrophin Signaling](image-url)
Gene fusions: an important class of oncogenes

- Associated with a diverse range of solid tumours and hematologic malignancies
- \(NTRK\) gene fusions are associated with many human cancers
  - Associated with \(\geq 19\) tumour types
  - Implicated in up to 1% of all solid tumours

**TRK fusion proteins are primary oncogenic drivers**

![Diagram showing gene fusions and oncogenic drivers](image)

**Landscape of recurrent kinase fusions in solid tumours**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Known fusion</th>
<th>Known kinase – novel partner</th>
<th>Known kinase – novel indication</th>
<th>New fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>BRAF</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EGFR</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>FGFR1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>FGFR2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>FGFR3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FGR</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MET</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NTRK1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NTRK2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NTRK3</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PDSFRA</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PKN1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PRKCA</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PRKCB</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>RAF1</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>RET</td>
<td>31</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>ROS1</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

![Gene fusions in solid tumours](image)
TRK fusion cancer occurs across a wide range of childhood and adult tumour types

- Rare tumours with high NTRK gene fusion frequency
- Common tumours with low NTRK gene fusion frequency

Paediatric (infants) high-grade glioma
Infantile fibrosarcoma
Congenital mesoblastic nephroma
Sarcoma (multiple)

Thyroid cancer
Spitz nevi

GBM, glioblastoma multiforme; MASC, mammary analogue secretory carcinoma; NTRK, neurotrophic tyrosine receptor kinase; TRK, tropomyosin receptor kinase.
What we are learning: brain tumors

Single-driver tumors particularly suitable for precision medicine

High unmet need both in terms of chance of relapse but also current therapies can lead to significant treatment burden and long term side effects
What we learned: rapid clinical development

- Identification of nerve growth factor (NGF), the first neurotrophin
- Identification of TRKA, TRKB and TRKC as high-affinity neurotrophic receptors
- Severe neuropathies developed by Nrtrk knockout mice
- Loss-of-function NTRK1 mutations identified in patients with congenital insensitivity to pain with anhidrosis (CIPA)
- BDNF–TRKB pathway involvement in neuroblastoma progression
- Data emerge implicating the involvement of TRK signalling in ovulation
- Crystal structure of NGF in complex with TRKA determined
- First activating TRKA alternative variant identified
- TRKB downregulation associated with hyperphagia and hyperdipsia in mice
- Crystal structures of the kinase domains of TRKA and TRKB determined

Timeline:
- 1950s: Identification of NTRK as an oncogene: TPM3–NTRK1 found in a human colorectal carcinoma
- 1982: Identification of neurotrophin 3 (NT-3)
- 1993–1994: Identification of NTRK1 fusions in papillary thyroid carcinoma
- 2000: Identification of the first NTRK3 fusion (ETV6–NTRK3) in infantile fibrosarcoma
- 2010: Identification of NTRK2 fusions in pilocytic astrocytoma
- 2015: First-generation TRK inhibitors enter clinical trial testing
- 2017: Second-generation TRK inhibitors enter clinical trial testing
- 2018: Larotrectinib achieves histology-agnostic and age-agnostic responses in NTRK fusion-positive solid tumours
- 2018: Larotrectinib and entrectinib receive FDA breakthrough designation for the treatment of NTRK fusion-positive solid tumours
Larotrectinib

- Larotrectinib is the first and only selective TRK inhibitor
- Highly potent against TRKA, TRKB, and TRKC
  - $IC_{50}$ 5–11 nM
- Highly selective
  - Limited inhibition of other kinases
  - No inhibition of 229 other kinases at 1000 nM
- Demonstrate activity in CNS disease
Larotrectinib for paediatric solid tumours harbouring NTRK gene fusions: phase 1 results from a multicentre, open-label, phase 1/2 study

Theodore W Loertscher*, Steven G DuBois*, Leo Mascarenhas, Brian Turpin, Noah Federman, Catherine M Albert, Ramamoorthy Nagasubramanian, Jessica L Davis, Erin Rudzinski, Angela M Feraco, Brian B Tuch, Kevin T Ebata, Mark Reynolds, Steven Smith, Scott Cruickshank, Michael C Cox, Alberto S Raposo*, Douglas S Hawkins*
Larotrectinib

Study design: paediatric subset (non-CNS tumours; N=52)

SCOUT phase I/II trial (NCT02637687)
- Age <21 years
- Advanced solid tumours

Adult/adolescent phase II ‘basket’ trial NAVIGATE (NCT02576431)
- Age ≥12 years
- Advanced solid tumours
- TRK fusion cancer

52 children and adolescents (aged <18 years) with non-CNS TRK fusion cancer

- Dosing
  - (Cohort 1; n=3) 9–55 mg/m² BID
  - (Cohort 2; n=6) 17.5–120 mg/m² BID
  - (Cohort 3; n=43) maximum dose 100 mg/m² BID

- TRK fusion status
  - Determined by local CLIA (or similarly accredited) laboratories

- Primary endpoint
  - Objective response rate (RECIST 1.1, investigator-assessed)

- Secondary endpoints
  - Duration of response
  - PFS
  - OS
  - Safety

Data cut-off February 19, 2019
BID, twice daily; CLIA, Clinical Laboratory Improvement Amendments; CNS, central nervous system; OS, overall survival; PFS, progression-free survival; TRK, tropomyosin receptor kinase

No child should die of cancer
Larotrectinib

Best change in target lesions (INV-assessed by RECIST v1.1)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Maximum change in tumour size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infantile fibrosarcoma</td>
<td>-80</td>
</tr>
<tr>
<td>Congenital mesoblastic nephroma</td>
<td>-80</td>
</tr>
<tr>
<td>Other soft tissue sarcoma</td>
<td>-80</td>
</tr>
<tr>
<td>Thyroid</td>
<td>-80</td>
</tr>
<tr>
<td>Melanoma</td>
<td>-80</td>
</tr>
</tbody>
</table>

Data cut-off: Feb 19, 2019. *Patient with 0% change in tumour size (TPM3-NTRK1 melanoma).
Pathological complete response. †One patient (ETV6-NTRK3 IFS) was not evaluable due to lack of first post baseline assessment at data cut-off. Total values for complete and partial responses include 3 and 6 patients, respectively, pending confirmation. RECIST, Response Evaluation Criteria In Solid Tumors

What we learned: efficacy
Entrectinib

**Intracellular signalling pathways**
- e.g. MAPK, PI3K-AKT, PLCγ-PKC

**Tumor cell proliferation**
- Cell survival

**Proposed mechanism of action:** Entrectinib inhibits constitutively activated TRK/ROS1/ALK tyrosine kinases thereby decreasing growth and proliferation signals from MAPK and PI3K pathways.
Entrectinib activity in NTRK fusion-positive solid tumours: individual patient responses by tumour type

<table>
<thead>
<tr>
<th>NTRK+ patients (n=54)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR (95% CI)</td>
<td>57.4% (43.2–70.8)</td>
</tr>
<tr>
<td>SD</td>
<td>9 (16.7)</td>
</tr>
<tr>
<td>PD</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>Non-CR/PD, missing or unrevaluable</td>
<td>10 (18.5)</td>
</tr>
</tbody>
</table>

Results per Blinded Independent Central Review (BICR)

Cut-off date: 31 May 2018

Note: Patients (n=6) without matched pre/post therapy scans were excluded from the plots.

CI: confidence interval; CRC: colorectal cancer; MASC: mammary analogue secretory carcinoma; NSCLC: non-small cell lung cancer
Entrectinib

Measureable and durable responses in CNS tumors
<table>
<thead>
<tr>
<th></th>
<th>Entrectinib</th>
<th>ORR</th>
<th>Larotrectinib</th>
<th>ORR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra-CNS NTRK+</td>
<td>3/3 (3 PR)</td>
<td>100% (29-100%)</td>
<td>32/34 (11 CR, 21 PR)</td>
<td>94% (80-99%)</td>
</tr>
<tr>
<td>CNS NTRK +</td>
<td>3/4 (1 CR, 2 PR, long)</td>
<td>75% (19-100%)</td>
<td>5/11 (2 CR, 3 PR)</td>
<td>45% (17-77%)</td>
</tr>
<tr>
<td>CNS ROS1 +</td>
<td>2/2 (1 PR, 1 uPR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra-CNS ROS1 +</td>
<td>1/1 (1 PR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extra-CNS ALK +</td>
<td>Fusion: 2/2 (2 CR) Mut: 1/1 (1CR)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Difference response rate to extra-CNS and CNS tumor related to CNS penetrance?
- Larotrectinib was designed to have limited CNS penetration to reduce the potential for on-target toxicity due to inhibition of normal TRK receptors in the brain.
- Entrectinib was first developed as an ALK inhibitor.
Larotrectinib

Time to response and treatment duration

Duration of treatment: 1.3+ to 34.0+ months

75% of patients (n=39) continuing treatment at the time of data cut-off


No child should die of cancer.
**Larotrectinib**

**Secondary efficacy endpoints**

- **Duration of response**
  - Median DOR: NE (range 1.6+ – 29.5+)
  - Median follow-up: 11.1 months

- **Progression-free survival**
  - Median PFS: NE (range 0.03+ – 30.4+)
  - Median follow-up: 9.9 months

- **Overall survival**
  - Median OS: NE (range 1.34 – 34.0+)
  - Median follow-up: 12.8 months

Data cut-off: Feb 19, 2019. DOR, duration of response; NE, not estimable.
Larotrectinib

Adverse events in ≥20% of pediatric patients (N=52)

<table>
<thead>
<tr>
<th></th>
<th>Treatment-emergent adverse events (%)</th>
<th>Treatment-related adverse events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1</td>
<td>Grade 2</td>
</tr>
<tr>
<td>Vomiting</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>ALT Increased</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>AST Increased</td>
<td>31</td>
<td>10</td>
</tr>
<tr>
<td>Cough</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>Neutrophil count decreased</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Constipation</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>Fatigue</td>
<td>23</td>
<td>2</td>
</tr>
<tr>
<td>Leukocyte count decreased</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Anaemia</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Blood alkaline phosphatase increased</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Nausea</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

- Discontinuation due to AE related to study drug occurred in 1 (2%) patient

Treatment-emergent adverse events in ≥20% of patients and corresponding values for treatment-related events.
Potential NTRK inhibitors toxicities

Neurological consequences
- Impairments in memory, learning and nociception and development of obesity caused by hyperphagia and hyperdipsia in mice and humans (NTRK2/Ntrk2 mutant)
- Defect in proprioception, impairment of motor neuron afferents and loss of a population of dorsal root ganglia neurons (Ntrk3 null)
- Lack populations of motor neurons as well as dorsal root and trigeminal neurons (Ntrk2 null)
- Severe sensory and sympathetic neuropathies (Ntrk1 null)
- Congenital insensitivity to pain with anhidrosis (CIPA) (NTRK1 mutant)

Non-neurological consequences
- Increased apoptosis of cardiac endothelial cells and decrease in intramyocardial blood vessel density (Ntrk2 null)
- Atrial and ventricular septal defects and valvular defects (Ntrk3 null)
- Inhibition of the ovulation in rats (TRKA inhibition)
- BDNF–TRKB axis has a role in oocyte development into pre-implantation mouse embryos (TRKB inhibition)

No data on late effects

The phase I adult trial of larotrectinib enrolled patient starting in March 2015
Entrectinib

STARTRK-NG summary of safety: Dose discontinuations, reductions, and adverse events

- Discontinuations:
  - 2 patients (6.9%) discontinued drug
    - One treatment-related AE (pulmonary edema)
    - One event not related to treatment (dyspnea)

- Reductions:
  - 11 patients (39.7%) were dose reduced for treatment-related AE – see table

- Notable adverse events:
  - Elevated Creatinine
    - 41% of all patients – all G1/G2
    - May not reflect true renal impairment since Entrectinib inhibits the MATE1 transporter which is involved in creatinine excretion.¹
  - Weight gain
    - Possible on-target effect (hyperphagia, obesity)¹⁻⁴
    - Most common reason for dose reduction
    - More common in patients on the drug for prolonged period (i.e. responders)
    - 2 patients have experienced bilateral femoral neck fractures possibly related to study drug, rapid weight gain, and steroid use.
  - Dysgeusia/Ataxia/Falling
    - Also possible on-target effects¹⁻⁴
    - Sensory impairments from TRK protein inhibition?
    - Dysgeusia 21% total - G1/G2
    - Ataxia and falling < 10% total

<table>
<thead>
<tr>
<th>AE leading to dose reductions by patient</th>
<th>Phase 1 dose escalation (n=5/16)</th>
<th>Phase 1b (n=6/13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased blood creatinine</td>
<td>Weight gain</td>
<td></td>
</tr>
<tr>
<td>Weight gain (2 episodes)</td>
<td>Ataxia</td>
<td></td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>Intermittent falling episodes</td>
<td></td>
</tr>
<tr>
<td>Pulmonary edema (3 episodes)</td>
<td>Weight gain</td>
<td></td>
</tr>
<tr>
<td>Increased blood creatinine</td>
<td>Headache</td>
<td>Prolonged QT interval</td>
</tr>
</tbody>
</table>
# NTRK inhibitors approved in NTRK-fusion positive tumours

## Larotrectinib

**Approved:** FDA 2018; Brazil, Canada, EMA 2019

**Indication:** For the treatment of patients with solid tumours harbouring NTRK fusions in paediatric or adult patients

**Dose:** 25-mg or 100-mg oral capsule or 20-mg/mL oral solution
- Adults and children with BSA ≥1.0m²: 100 mg orally BID
- Children with BSA ≤1.0m²: 100 mg/m² orally BID

## Entrectinib

**Approved:** FDA and Japan 2019

**Indication:** For the treatment of NTRK fusion-positive advanced or recurrent solid tumours in adult patients and paediatric patients aged ≥12 years old

**Dose:** 100-mg and 200-mg oral capsule
- Adults: 600 mg orally once daily
- Children: Recommended dose based on BSA
Where to go?

Identification of NTRK fusions in pediatric patients

Treatment: when? how long?

Toxicity and late effects

Resistances and second generation NTRKi
ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research

1. As a confirmatory technique, use FISH, RT-PCR or targeted RNA NGS assays with specific probes for the fusion involving the known NTRK gene.

2. Is the histologic tumour type known to harbour highly recurrent NTRK fusions?
   - **YES**
   - **NO**

3. Is there a sequencing platform available?
   - **NO**
   - **YES**

4. Use IHC as a screening tool:
   - NO TRK expression
   - Detection of TRK expression

5. Use front line NGS reliably detecting NTRK fusions, preferably including RNA testing when possible.
Japan society of clinical oncology/Japanese society of medical oncology-led clinical recommendations on the diagnosis and use of tropomyosin receptor kinase inhibitors in adult and pediatric patients with neurotrophic receptor tyrosine kinase fusion-positive advanced solid tumors, cooperated by the Japanese society of pediatric hematology/oncology

Algorithm for NTRK testing

No in tumor with activating mutation mutually exclusive to NTRK
TRK Fusion Cancers in Children: A Clinical Review and Recommendations for Screening

Catherine M. Albert, MD; Jessica L. Davis, MD; Noah Federman, MD; Michela Casanova, MD; and Theodore W. Laetsch, MD

(A) Immunohistochemistry
- Detects protein expression, which may be attributable to a fusion event.
- (A) Strong panNTRK antibody expression in NTRK1 fusion-positive IFS.

(B) FISH
- Detects gene rearrangements in DNA that may generate a fusion transcript.
- (B) ETV6 FISH break-apart probe demonstrating an ETV6 rearrangement.

Reverse Transcriptase Polymerase Chain Reaction
- Detects known fusion transcripts in RNA.
- Detects 5′-3′ imbalance as a fusion signature, but cannot determine novel partner.

(C) Next-Generation Sequencing
- Detects known and novel fusions with arbitrary breakpoints in DNA or RNA.
- Exact capabilities depend on enrichment strategy and data analysis pipeline.
- (C) Sequencing read piles aligned to the human genome and visualized using the Integrative Genomics Viewer. The rainbow plot shows discordant mate pairs in the tumor with one mate mapping to intron 1 of TPM3 on chromosome 1 and the other mapping to intron 8 of NTRK1 also on chromosome 1.

Need for specific recommendations for children.
<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Clinical summary</th>
<th>Screening methodology</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infantile fibrosarcoma</td>
<td>• Most common soft tissue sarcoma in children &lt;1 year</td>
<td>IHC/FISH (ETV6 and/or NTRK3) RT-PCR; NGS if negative</td>
<td>High frequency of ETV6-NTRK3 fusion; variant NTRK3 and NTRK1 fusions have been described</td>
</tr>
<tr>
<td></td>
<td>• More than half of infants have unresectable or metastatic disease that requires neoadjuvant treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellular congenital mesoblastic nephroma</td>
<td>• Most common kidney tumour in the first month of life</td>
<td>IHC/FISH (ETV6 and/or NTRK3) RT-PCR; NGS if negative</td>
<td>High frequency of ETV6-NTRK3 fusion; variant NTRK3 and NTRK1 fusions have been described</td>
</tr>
<tr>
<td></td>
<td>• 96% overall survival rate</td>
<td></td>
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<tr>
<td></td>
<td>• Most patients treated with nephrectomy; ~20% treated with adjuvant chemotherapy</td>
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</tr>
<tr>
<td>Secretory breast cancer</td>
<td>• Rare subtype of breast cancer (&lt;0.02% of patients)</td>
<td>IHC/FISH (ETV6 and/or NTRK3) RT-PCR; NGS if negative</td>
<td>High frequency of NTRK3 fusions</td>
</tr>
<tr>
<td></td>
<td>• Prognosis generally good when disease is localised</td>
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<td></td>
<td>• Standard treatment is surgery with/without radiation</td>
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<tr>
<td></td>
<td>• Lymph node metastases can occur, some patients with metastatic recurrence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MASC of the salivary gland</td>
<td>• Histologically resembles secretory breast cancer</td>
<td>IHC/FISH (ETV6 and/or NTRK3) RT-PCR; NGS if negative</td>
<td>High frequency of NTRK3 fusions</td>
</tr>
<tr>
<td></td>
<td>• Primarily occurs in adults; some cases in adolescents</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Almost all tumours harbour ETV6-NTRK3 fusions</td>
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</tr>
</tbody>
</table>
## Tumours with medium-frequency TRK fusions (10% to 40%)

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Clinical summary</th>
<th>Screening methodology</th>
<th>Rationale</th>
</tr>
</thead>
</table>
| **Spitzoid melanomas**                   | • Rare melanocytic lesions (ranging from benign to malignant spitzoid melanoma)  
• 90% of patients diagnosed are <30 years old  
• Children aged 10–18 years are at greater risk of metastatic spread and death vs younger children  
• Recently, recurrent kinase fusions have been described                                                                                                                                                                       | IHC/NGS                | TRK fusions occur in 15–25% of paediatric melanocytic neoplasms                                                                                                                                         |
| **Papillary thyroid cancer**              | • Thyroid cancers account for ~4% of malignancies in children; >90% of these are PTC cases  
• Good prognosis, with >95% overall survival rate at 5 years  
• Treatment consists of thyroidectomy for localised tumours, and radioactive iodine for metastatic tumours  
• TRK fusions may be associated with higher-stage disease                                                                                                                                                                | IHC/NGS                | TRK fusions (NTRK1 and NTRK3) occur in approximately 25% of children, and BRAF activating mutations occur in ~50% of patients                                                                          |
| **High-grade gliomas, especially in young children** | • Gliomas are the most common brain tumours in children  
• Poor survival outcomes in children with high-grade gliomas (<25% 3-year overall survival rate)  
• NTRK fusions shown to occur in ~40% of high-grade gliomas in children <3 years old                                                                                                                                                                                                 | NGS                    | TRK fusions occur, especially in tumours of children <3 years of age; patients have poor prognosis; IHC has not been validated in gliomas, which can have normal physiologic expressions of TRK |

IHC/NGS: Immunohistochemistry/Next-Generation Sequencing
## Tumours with unknown or low-frequency TRK fusions (<5%)

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Clinical summary</th>
<th>Screening methodology</th>
<th>Rationale</th>
</tr>
</thead>
</table>
| **Undifferentiated sarcoma (without known defining fusion)** | • Link with TRK fusions largely anecdotal, so frequency has not been estimated  
• Historically, treatment of undifferentiated sarcoma has been variable, more recently risk-based chemoradiotherapy | NGS                    | Prevalence of TRK fusions unknown but described; in addition to NTRK, other targetable fusions have been described |
| **Inflammatory Myofibroblastic Tumour**            | • Locally aggressive neoplasms  
• Median age at diagnosis: 9 years  
• Metastases are rare (<5%), and complete surgical resection is usually curative  
• Patients with ALK fusions (50–60% of cases) have a good treatment response with crizotinib                                                                                                                                 | NGS                    | TRK fusion tumours may share inflammatory myofibroblastic tumour-like morphology; mutually exclusive fusions of ALK and ROS1 also occur                                                                                                                                 |
ICH: sensitivity and specificity is high in mesenchymal tumors, useless in brain tumors

Pan-Trk Immunohistochemistry Identifies NTRK Rearrangements in Pediatric Mesenchymal Tumors

Erin R. Rudzinski, MD,* Christina M. Lockwood, PhD,† Bradley A. Stohr, MD, PhD,‡ Sara O. Vargas, MD,§ Rachel Sheridan, MD,¶ Jennifer O. Black, MD,‖ Veena Rajaram, MD,¶ Theodore W. Laetsch, MD,** and Jessica L. Davis, MD,‡‡†

Expanding the Spectrum of Pediatric NTRK-rearranged Mesenchymal Tumors

Jessica L. Davis, MD,*‡ Christina M. Lockwood, PhD,‡ Bradley Stohr, MD, PhD,† Carolin Boecking, MD,† Alyaa Al-Ibraheemi, MD,§ Steven G. DuBois, MD,¶ Sara O. Vargas, MD,§ Jennifer O. Black, MD,¶ Michael C. Cox, PharmD,‡ Mark Luquette, MD,** Brian Turpin, DO,‡† Sara Szabo, MD,‡‡ Theodore W. Laetsch, MD,§§ Catherine M. Alberti, MD,¶¶ David M. Parham, MD,¶¶ Douglas S. Hawkins, MD,¶¶ and Erin R. Rudzinski, MD##

SIOPe NTRK testing workshop
Molecular profiling programs at relapse

1. Generate individual molecular information at relapse
   - Molecular Matching Trials
     - MAPPYACTS (France, Spain, Italy, Ireland, Israel)
     - INFORM (Germany, Austria, Sweden, Switzerland, Belgium)
     - iTHER (The Netherlands)
     - SM-PAEDs (UK)

2. Match treatment and tumor profile

3. Evaluate activity of drugs and combinations
   - Phase 1 Trials (Industry and ISTs)
   - Phase 2 Trials (Industry and ISTs)
   - Genentech Roche Matrix Trial
   - ESMART Multiam trial
     European Proof-Of-Concept Therapeutic Stratification Trial of Molecular Anomalies in Relapsed or Refractory Tumors in children (ESMART)

4. Generate new knowledge, new druggable pathways
   - EU Clinico Biological (WES/RNAseq) Data Base
   - Project A, B, C, D, E
   - Pediatric New Drug Development

ACCELERATE
INNOVATION FOR CHILDREN AND ADOLESCENTS WITH CANCER
MYCKID STUDY:
MOLECULAR IDENTITY CARD FOR KIDS, ADOLESCENT AND YOUNG ADULT WITH NON RHABDOMYOSARCOMAS SOFT TISSUE SARCOMA
FFPE material (molecular characterization):
- RNAseq, CGH: CINSARC, GI
- Transcript fusion, clustering: diagnosis

Frozen material (molecular characterization):
- Whole exome sequencing (WES), RNAseq for fusion detection, DNA methylation
- Ancillary studies (organoids, single cell RNA sequencing)
Additional data are coming in the next years in adults and children

<table>
<thead>
<tr>
<th>Study/Identifier</th>
<th>Design</th>
<th>Patients</th>
<th>Objectives</th>
<th>Estimated primary completion</th>
<th>Estimated total enrollment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatric MATCH (phase 2)</td>
<td>Patients receive larotrectinib orally or via nasogastric or gastric tube BID for up to 2 years</td>
<td>• Children and young adults (12 months to 21 years) • Relapsed or refractory advanced solid tumors, NHL or histiocytic disorders with NTRK gene fusions</td>
<td>• ORR • PFS • Tolerability • PK</td>
<td>March 2022</td>
<td>49</td>
</tr>
<tr>
<td>NCT03213704</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pediatric MATCH (phase 2)</td>
<td>Patients are tested for genetic alterations, and receive corresponding targeted therapy for up to 2 years (6 therapies available; patients with NTRK gene fusion receive larotrectinib)</td>
<td>• Children (12 months to 21 years) • Recurrent or refractory solid tumors</td>
<td>• ORR • Toxicity • PK • PFS</td>
<td>December 2021</td>
<td>1500</td>
</tr>
<tr>
<td>NCT03155620</td>
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<td></td>
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</tr>
<tr>
<td>NCI MATCH (phase 2)</td>
<td>Patients are tested for genetic alterations, and given a corresponding targeted therapy for up to 2 years (30 therapies available; patients with NTRK gene fusion receive larotrectinib)</td>
<td>• Adults (≥18 years) • Solid tumor or lymphoma</td>
<td>• ORR • OS • PFS • TTP</td>
<td>June 2022</td>
<td>6452</td>
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<tr>
<td>NCT02465960</td>
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</tbody>
</table>
Larotrectinib in Treating Patients With Previously Untreated TRK Fusion Solid Tumors and TRK Fusion Relapsed Acute Leukemia – COG ADVL1823

• **Primary outcome measure**
  ORR of children with infantile fibrosarcoma (IFS) treated with neoadjuvant larotrectinib prior to local control

• **Secondary outcome measures**
  EFS/OS/DoR in IFS treated with neoadjuvant larotrectinib

  ORR/EFS/OS/DoR in newly diagnosed TRK fusion solid tumors other than IFS treated with neoadjuvant larotrectinib prior to local control

  Incidence of adverse events

  Percentage of patients with TRK fusion solid tumors with detectable ctDNA
### Chemotherapy versus Larotrectinib as first-line therapy in IFS

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>Larotrectinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV (catheter)</td>
<td>Oral</td>
</tr>
<tr>
<td>Effective (75%)</td>
<td>Effective (&gt;90%), rapid response</td>
</tr>
<tr>
<td>Acute toxicity (VOD)</td>
<td>No severe acute toxicity</td>
</tr>
<tr>
<td>Duration of therapy: median 4 months</td>
<td>Duration of therapy?</td>
</tr>
<tr>
<td>Long term toxicity only to second line therapy</td>
<td>Long term effects?</td>
</tr>
<tr>
<td>alkylating agents, anthracycline</td>
<td></td>
</tr>
<tr>
<td>Costs: cheap</td>
<td>Cost: very expensive</td>
</tr>
<tr>
<td>Available</td>
<td>Current availability only within trials</td>
</tr>
</tbody>
</table>

---

**Larotrectinib**
- **Oral**
- Effective (>90%), rapid response
- No severe acute toxicity
- Long term effects?
- Current availability only within trials

**Chemotherapy**
- EV (catheter)
- Effective (75%)
- Acute toxicity (VOD)
- Duration of therapy: median 4 months
- Long term toxicity only to second line therapy (alkylating agents, anthracycline)
- Costs: cheap
- Available

**Duration of therapy**: median 4 months

**Costs**: cheap

**Available**: Current availability only within trials

**Long term toxicity only to second line therapy**
- alkylating agents, anthracycline
EpSSG/EXPeRT guidelines for IFS recommend conservative surgery or neoadjuvant chemotherapy as first-line therapy

- **Tumour assessment**: Resectability without any functional or mutilating consequences with RO intent
  - **Yes**: Surgery first
    - IRS I–II: no further therapy
    - IRS III: chemotherapy
  - **No**: Neoadjuvant chemotherapy until maximum tumour shrinkage
    - Conservative surgery
      - RO, R1 margins, complete necrosis: no further therapy
      - R2: post-operative chemotherapy

- **VA regimen recommended initially**
  - If response to VA is not sufficient to permit conservative surgery, ifosfamide and/or cyclophosphamide and/or doxorubicin should be added
  - If no response to VA, the IFO-DOXO regimen should be adopted

- **New targeted therapies**
  - The use of TRK inhibitor drugs should be certainly considered when conventional chemotherapy fails
Toxicity and late affects

Neurocognitive outcome
- Very young children
- Patients who remain on treatment for years

Discussion with parents on this topic is essential
Multiple initiative

Brussels 5th Feb: Readdressing the need for long-term follow up
Accelerate group: Danielle Horton Taylor/Mark Kieran
Acquired resistance mechanisms

Resistance to NTRK inhibition by larotrectinib and entrectinib is mediated by recurrent mutations to the kinase domain of NTRK gene at three different locations (solvent-front mutations, gatekeeper and xDFG).

The kinase solvent-front mutation is mediated through G595R substitution in the TRKA protein and G623R substitution in the TRKC protein.

The gatekeeper mutation is found at F589L substitution in the TRKA.

The xDFG motif mutation is found at G667C substitution in the TRKA protein and G696S substitution in the TRKC protein.
Second generation NTRK inhibitors are being developed to overcome acquired resistance

<table>
<thead>
<tr>
<th>Drug (company)</th>
<th>Objective</th>
<th>MoA</th>
<th>Development stage (Clinicaltrials.gov identifier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selitrectinib (formerly known as LOXO-195)* (Bayer)</td>
<td>To overcome resistance mediated by solvent-front mutations, and xDFG substitutions</td>
<td>Selectively targets NTRK 1/2/3 gene fusions</td>
<td>Phase 1/2 trial (NCT03215511)</td>
</tr>
<tr>
<td>Repotrectinib** (Turning Point Therapeutics)</td>
<td>To overcome resistance due to solvent-front substitutions</td>
<td>High selectivity for wild-type and mutated TRKA, TRKB, TRKC, ROS1, and ALK proteins</td>
<td>Phase 1/2 trial (NCT03093116)</td>
</tr>
</tbody>
</table>
Thank you

michela.casanova@istitutotumori.mi.it