Every cancer is unique. Let’s treat it that way.\textsuperscript{1,2}

Targeted therapies are being studied to advance treatment options for patients with actionable biomarkers—patients with gene fusions need high-quality molecular testing to realise these opportunities.

To help patients with NTRK-fusion–positive cancer, we must first identify them.

#PutCancerToTheTest

Visit www.fusioncancer.com to learn more.

\*This document doesn’t contain any information on ROS1.
NTRK gene fusions are most common in rare tumors, but have also been detected less frequently in more common cancers:

- **Colorectal cancer** (0.2%)
- **Thyroid** (1.5%)
- **Non-small cell lung cancer** (0.23%)
- **Cholangiocarcinoma** (3% to <5%)
- **Melanoma** (0.3%)
- **Glioblastoma** (1.1% to <5%)
- **Head and neck squamous cell cancer** (0.2% to <5%)

**DETECTING NTRK GENE FUSIONS**

NTRK gene fusions drive cancer through aberrant signalling:

- **NTRK** gene fusions create an oncogenic chimeric protein that activates a signalling cascade implicated in cell proliferation, survival, and angiogenesis.
- **NTRK** gene fusions may be mutually exclusive to other oncogenic drivers.
- Each **NTRK** gene can combine with multiple fusion partners; at least 25 distinct **NTRK** gene fusions have been identified to date.

NTRK gene fusion testing is not routinely incorporated into diagnostic workups, but molecular profiling using next-generation sequencing (NGS) can identify **NTRK** gene-fusion patients and support optimal patient care.

To learn more, visit www.fusioncancer.com.
Incorporating NTRK gene-fusion testing into a diagnostic workup will help advance personalised healthcare for cancer patients.\textsuperscript{12-16} Example ESMO NTRK gene-fusion–detection algorithm.\textsuperscript{17}

Technologies available for NTRK 1, 2, 3 gene-fusion detection

<table>
<thead>
<tr>
<th></th>
<th>NGS</th>
<th>IHC</th>
<th>FISH</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to detect different fusions</td>
<td>Can detect fusions in all 3 NTRK genes, as well as fusion partner and position.\textsuperscript{1,16}</td>
<td>Pan-TRK antibodies may detect presence of protein encoded by any of the 3 NTRK genes.\textsuperscript{18,22}</td>
<td>Different probes needed for each gene.\textsuperscript{24}</td>
<td>Requires multiple reactions with specific primers and can miss unknown variants.\textsuperscript{30}</td>
</tr>
<tr>
<td>Reliability</td>
<td>Varies based on the assay, depth of coverage, tumour content, and design of the assay (ie, regions of the sequence targeted by the NGS panel).\textsuperscript{19}</td>
<td>Pan-TRK antibodies have been reported to have 95% to 100% sensitivity and up to 100% specificity.\textsuperscript{18,21}</td>
<td>Depends on the probes used; can be used to confirm other results.\textsuperscript{19,26}</td>
<td>Reliable for known fusion variants.\textsuperscript{30}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May miss variant NTRK rearrangements.\textsuperscript{18,25,27}</td>
<td></td>
<td>Requires multiple reactions with specific primers for known variants.\textsuperscript{30}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Can miss detection of unknown/untested variants.\textsuperscript{30}</td>
</tr>
<tr>
<td>Tissue processing</td>
<td>Routinely processed, FFPE samples.\textsuperscript{20}</td>
<td>Routinely processed, FFPE samples.\textsuperscript{22}</td>
<td>Routinely processed, FFPE samples.\textsuperscript{28}</td>
<td>RNA can be successfully extracted from nearly all types and ages of fixed tissues.\textsuperscript{30,31}</td>
</tr>
<tr>
<td></td>
<td>Avoid DNA-damaging fixatives or acidic decalcifying agents.\textsuperscript{21}</td>
<td>Avoid DNA-damaging fixatives or acidic decalcifying agents.\textsuperscript{29}</td>
<td></td>
<td>Yield and quality will vary with sample quality and age.\textsuperscript{30,31}</td>
</tr>
<tr>
<td></td>
<td>RNA samples are more susceptible to degradation over time.\textsuperscript{21}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen requirements</td>
<td>NGS</td>
<td>IHC</td>
<td>FISH</td>
<td>RT-PCR</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----</td>
<td>-----</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>- Amount required varies, depending on the NGS platform&lt;sup&gt;21&lt;/sup&gt;</td>
<td>- Requires dedicated tissue and limits multiplexing&lt;sup&gt;37,39&lt;/sup&gt;</td>
<td>- Requires dedicated tissue&lt;sup&gt;37&lt;/sup&gt;</td>
<td>- Requires dedicated tissue&lt;sup&gt;37&lt;/sup&gt;</td>
<td>- Requires dedicated tissue&lt;sup&gt;30,31&lt;/sup&gt;</td>
</tr>
<tr>
<td>- Samples with greater viable tumour content will return more reliable results&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>- Poor quality and quantity of RNA risks false-negative results&lt;sup&gt;30,31&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of FFPE sections</td>
<td>&gt;5-10 (4-5 μm)&lt;sup&gt;32,33&lt;/sup&gt;</td>
<td>≥2-3 (4-5 μm)&lt;sup&gt;40-44&lt;/sup&gt;</td>
<td>≥4 (4-5 μm)&lt;sup&gt;44,46,67&lt;/sup&gt;</td>
<td>&gt;8-10 (7-30 μm)&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cells/DNA/RNA</td>
<td>≥20% tumour cells&lt;sup&gt;32&lt;/sup&gt;</td>
<td>&gt;50 tumour cells&lt;sup&gt;43,45&lt;/sup&gt;</td>
<td>&gt;50 tumour cells&lt;sup&gt;48&lt;/sup&gt;</td>
<td>0.1-0.5 μg RNA&lt;sup&gt;37,50&lt;/sup&gt;</td>
</tr>
<tr>
<td>Turnaround time</td>
<td>2-21 days&lt;sup&gt;36-37&lt;/sup&gt;</td>
<td>0.5-2 days&lt;sup&gt;37,42&lt;/sup&gt;</td>
<td>2-10 days&lt;sup&gt;37,49&lt;/sup&gt;</td>
<td>1 day&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td>Multigene testing beyond NTRK gene fusions</td>
<td>YES&lt;sup&gt;37&lt;/sup&gt;</td>
<td>NO&lt;sup&gt;37&lt;/sup&gt;</td>
<td>NO&lt;sup&gt;37&lt;/sup&gt;</td>
<td>NO&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td>Advantages</td>
<td>May detect novel fusion partners (depending on the assay used)&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Low cost&lt;sup&gt;38&lt;/sup&gt;</td>
<td>The location of the target within the cell is visible&lt;sup&gt;38&lt;/sup&gt;</td>
<td>High sensitivity and specificity&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Can be used to evaluate multiple actionable targets simultaneously while preserving limited tissue&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Readily available&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Several targets can be detected in one sample using several fluorophores&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Low cost per assay&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Currently used for NTRK testing&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Detects TRKA, B, and C&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Requires knowledge of only one of the two fusion partners when using break-apart probes&lt;sup&gt;38&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RNA-based testing is focused on coding sequences, not introns&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Turnaround time of 1-2 days&lt;sup&gt;38&lt;/sup&gt;</td>
<td>NTRK gene fusions with unknown partners can be detected using break-apart FISH&lt;sup&gt;38&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FISH; is readily available in most laboratories and institutes&lt;sup&gt;38&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Commercially available DNA-based NGS platforms may not be capable of identifying all NTRK gene fusions, especially those involving NTRK2 and NTRK3, which have large intronic regions&lt;sup&gt;38&lt;/sup&gt;</td>
<td>May not be specific for NTRK gene fusion, as it detects both wild-type and fusion proteins&lt;sup&gt;38&lt;/sup&gt;</td>
<td>The target sequence must be known for conventional FISH; otherwise, 3 separate tests are required for NTRK1, NTRK2, and NTRK3&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Target sequences must be known (ie, cannot readily detect novel fusion partners)&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>DNA-NGS is limited by intron size&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Possible false negatives for fusions involving TRKC&lt;sup&gt;38&lt;/sup&gt;</td>
<td>Complex chromosomal translocations can result in false-positive signals&lt;sup&gt;38&lt;/sup&gt;</td>
<td>A comprehensive multiplex RT-PCR assay might be challenging because of the potentially large number of possible 5’ fusion partners&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RNA-NGS is limited by RNA quality&lt;sup&gt;38&lt;/sup&gt;</td>
<td>There is no standardisation of scoring algorithms&lt;sup&gt;38&lt;/sup&gt;</td>
<td>False-negative results may be above 30%&lt;sup&gt;38&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

DNA=deoxyribonucleic acid; FFPE=formalin-fixed paraffin-embedded; FISH=fluorescence in situ hybridization; IHC=immunohistochemistry; RT-PCR=reverse transcriptase polymerase chain reaction.
### Archer and Pan-Trk IHC Characteristics with NTRK Rearrangements

**Archer and pan-TRK IHC characteristics of solid cancers with NTRK rearrangements detected on MSK-IMPACT**

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>NTRK Gene</th>
<th>Partner Gene</th>
<th>Novel</th>
<th>Archer</th>
<th>Cytoplasmic</th>
<th>Perinuclear</th>
<th>Membranous</th>
<th>Nuclear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendiceal ADC</td>
<td>NTRK1 exon 12</td>
<td>LMNA exon 4</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>NTRK3 exon 15</td>
<td>ETV6 exon 6</td>
<td>No</td>
<td>Positive</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>NTRK1 exon 12</td>
<td>LMNA exon 12</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>NTRK1 exon 12</td>
<td>LMNA exon 8</td>
<td>No</td>
<td>NT</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>NTRK1 exon 9</td>
<td>TPM3 exon 10</td>
<td>No</td>
<td>NT</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>–</td>
</tr>
<tr>
<td>Colorectal carcinoma</td>
<td>NTRK1 exon 10</td>
<td>TPM3 exon 8</td>
<td>No</td>
<td>Positive</td>
<td>–</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gallbladder ADC</td>
<td>NTRK1 exon 12</td>
<td>LMNA exon 2</td>
<td>No</td>
<td>NT</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>NTRK1 exon 9</td>
<td>AFAP1 exon 4</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>NTRK2 exon 17</td>
<td>BCR exon 1</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>NTRK3 exon 14</td>
<td>EML4 exon 2</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>NTRK3 exon 14</td>
<td>ZNF710 exon 1</td>
<td>Yes</td>
<td>Negative</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung ADC</td>
<td>NTRK1 exon 10</td>
<td>IRF2BP2 exon 1</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung ADC</td>
<td>NTRK1 exon 5</td>
<td>P2RY8 exon 2</td>
<td>Yes</td>
<td>Negative</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung ADC</td>
<td>NTRK1 exon 12</td>
<td>TPM3 exon 8</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>–</td>
</tr>
<tr>
<td>SC of breast</td>
<td>NTRK3 exon 15</td>
<td>ETV6 exon 5</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>SC of salivary gland</td>
<td>NTRK3</td>
<td>ETV6</td>
<td>No</td>
<td>NT</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SC of salivary gland</td>
<td>NTRK3</td>
<td>ETV6</td>
<td>No</td>
<td>NT</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SC of salivary gland</td>
<td>NTRK3 exon 15</td>
<td>ETV6 exon 5</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>SC of salivary gland</td>
<td>NTRK3 exon 15</td>
<td>ETV6 exon 5</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>x</td>
</tr>
<tr>
<td>Melanoma</td>
<td>NTRK1 exon 10</td>
<td>TRIM63 exon 8</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melanoma</td>
<td>NTRK2 exon 15</td>
<td>TRAF2 exon 9</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>–</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>NTRK1 exon 11</td>
<td>LMNA exon 2</td>
<td>No</td>
<td>Positive</td>
<td>x</td>
<td>x</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>NTRK3 exon 11</td>
<td>TPM4 exon 6</td>
<td>Yes</td>
<td>Positive</td>
<td>x</td>
<td>–</td>
<td>x</td>
<td>–</td>
</tr>
</tbody>
</table>

Jaclyn F Hechtman, Ryma Benayed, David M Hyman, et al, Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions, The American Journal of Surgical Pathology, volume 41, issue 11, p1547-1551.

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ADC=adenocarcinoma; NT=not tested; SC=secretory carcinoma.
Protein expression in head and neck squamous cell carcinoma with wild-type TRK (20x)^51

When you order testing, be certain to test for all 3 NTRK gene fusions: NTRK1, NTRK2, and NTRK3^1,3

Test results will help identify which samples have NTRK gene-fusion–positive solid tumours. The specific content in your samples report will depend on the testing method and the laboratory.

Fusion status based upon NGS reported from an external laboratory-developed test using Oncomine™ Focus Assay^51

Sample NGS report

When you order testing, be certain to test for all 3 NTRK gene fusions: NTRK1, NTRK2, and NTRK3^1,3

Test results will help identify which samples have NTRK gene-fusion–positive solid tumours. The specific content in your samples report will depend on the testing method and the laboratory.

PATHOLOGY REPORT

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>Patient</th>
<th>Tumour Type</th>
<th>TRF#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jane Sample</td>
<td>Lung adenocarcinoma</td>
<td>Lung adenocarcinoma</td>
<td>TRXXXXXXX</td>
</tr>
</tbody>
</table>

PHYSICIAN

<table>
<thead>
<tr>
<th>Ordering Physician</th>
<th>Medical Facility</th>
<th>Additional Recipient</th>
<th>Medical Facility ID</th>
<th>Pathologist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
</tr>
</tbody>
</table>

SPECIMEN

<table>
<thead>
<tr>
<th>Specimen Site</th>
<th>Specimen ID</th>
<th>Specimen Type</th>
<th>Date of Collection</th>
<th>Specimen Received</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
<td>Not Given</td>
</tr>
</tbody>
</table>

TEST FINDINGS

Markers Detected

NTRK1

OTHER ALTERATIONS & BIOMARKERS IDENTIFIED

Results reported in this section are not prescriptive or conclusive for labeled use of any specific therapeutic product. See professional services section for additional information.

Microsatellite Status: MS-Stable^a

Tumour Mutation Burden: 11 Muts/Mb^a

PTCH1: T4 16S

TP53: R267P

CDKN2A/B loss^a

EGFR amplification^a

SAMPLE REPORT

^a Refer to the appendix for limitation statements related to the detection of any copy number alterations, gene rearrangements, and microsatellite instability or tumour mutation burden results in this section.

If FDA-approved therapeutic options are not listed on the first/cover page of the report, subsequent pages may still identify NTRK gene-fusion positivity.

NTRK=neurotrophic tyrosine receptor kinase; TRK=tropomyosin receptor kinase.
Let testing be your guide

For patients with solid tumors,

To learn more about molecular testing, visit www.fusioncancer.com.
Frequently Asked Questions About NTRK Testing

**General**

1. **What kinds of testing methods can be used to detect NTRK gene fusions?**

   NTRK gene fusions can be detected using various testing methods, including NGS, immunohistochemistry (IHC), which detects protein expression of both fusion and wild-type; fluorescence in situ hybridization (FISH), and reverse transcriptase polymerase chain reaction (RT-PCR).[^1-^3]

2. **How does each testing method work and what are the advantages and limitations of each?**

   - **NGS** relies on DNA or ribonucleic acid (RNA) sequencing technologies that are capable of processing multiple DNA or RNA sequences in parallel, and provides the most comprehensive view of several biomarkers. NGS can detect fusions in all 3 NTRK genes (must have NTRK 1, 2, 3 gene-fusion coverage), as well as the NTRK gene fusion partner and position. However, turnaround time for results with NGS may be longer than with IHC or FISH[^1-^2,^4-^5].

   - **IHC** is a protein biomarker test that utilizes specific antibodies to detect expression of the TRK component of the fusion protein. An antibody directed against a conserved region may detect fusion or wild-type proteins derived from TRKA, TRKB, or TRKC proteins[^2,^3].

   - **FISH** probes cannot distinguish fusion variants. FISH assays can often be labor-intensive and more expensive than IHC when performing multiple assays[^2,^6].

   - **RT-PCR** requires multiple primer sets for each gene, since the location of the gene rearrangement is not known. It is very reliable for known fusion variants but requires multiple reactions with specific primers for known variants and can miss detection of unknown/untested variants[^7].

3. **Is there a companion diagnostic assay for selecting NTRK fusion-positive eligible patients?**

   To date, NGS tests are in development for this use, but only one is currently approved as a companion diagnostic for the detection of NTRK rearrangements[^8]. Japan’s Ministry of Health, Labour, and Welfare has granted additional approval of Foundation Medicine’s FoundationOne® CDx Cancer Genomic Profile as a companion diagnostic for entrectinib (ROZLYTREK®), marketed in Japan by Chugai.[^9]

---

NGS=next-generation sequencing; NTRK=neurotrophic tyrosine receptor kinase.

[^1]: These methods are similar in detecting NTRK fusion variants.
[^2]: These methods are similar in detecting NTRK fusion variants.
[^3]: These methods are similar in detecting NTRK fusion variants.
[^4]: These methods are similar in detecting NTRK fusion variants.
[^5]: These methods are similar in detecting NTRK fusion variants.
[^6]: These methods are similar in detecting NTRK fusion variants.
[^7]: These methods are similar in detecting NTRK fusion variants.
[^8]: These methods are similar in detecting NTRK fusion variants.
[^9]: These methods are similar in detecting NTRK fusion variants.
1. What is the role of NGS vs IHC in \textit{NTRK} testing strategy?

Per ESMO, in solid tumours where gene fusions are common but the frequency of \textit{NTRK} gene fusions is lower, an NGS panel that includes \textit{NTRK} fusions is recommended. For tumours with a very low frequency of \textit{NTRK} gene fusions but where molecular screening is common, inclusion of \textit{NTRK} genes in routine NGS analysis is recommended. For tumours with a low frequency of \textit{NTRK} fusions, where NGS is not available or is not routinely performed for a histotype, pan-TRK IHC should be performed for screening with NGS confirmation of positive IHC results.\(^\text{10}\)

2. How is IHC different from NGS?

IHC is a protein biomarker test that utilizes specific antibodies to detect expression of the TRK component of the fusion protein.\(^2\) NGS relies on DNA or RNA sequencing technologies that are capable of processing multiple DNA or RNA sequences in parallel, and provides the most comprehensive view of several biomarkers, including rare and common alterations. NGS can detect fusions in all 3 \textit{NTRK} genes, as well as the \textit{NTRK} gene fusion partner and position. However, turnaround time for results with NGS may be longer than with IHC. IHC mainly detects gene expression at the protein level. It doesn’t detect the fusion itself.\(^1,2,4-6,11\)

3. Does IHC miss any \textit{NTRK} fusions?

In order to detect all TRK proteins, the antibody must detect TRKA, TRKB, and TRKC proteins. Some clones are less sensitive than others and there are reports that the sensitivity of IHC for some \textit{NTRK3} fusions is lower than for \textit{NTRK1} and \textit{NTRK2}.\(^3\)

4. Does IHC detect \textit{NTRK} fusions?

The VENTANA\textsuperscript{\textregistered} pan-TRK (EPR17341) assay is a CE-IVD analytic assay intended for the immunohistochemical detection of the C-terminal region of the TRK proteins A, B, and C, which is known to be conserved across wild-type (WT) and chimeric fusion proteins. As the VENTANA\textsuperscript{\textregistered} pan-TRK (EPR17341) assay can’t differentiate between WT and \textit{NTRK} fusions, it is important to note that WT TRK proteins are not expected in non-neural tissues, and this expression may indicate a fusion that should be confirmed with other methods.\(^6,12,13\)

5. What is the sensitivity of IHC?

Pan-TRK antibodies have been reported to have 95% to 100% sensitivity and up to 100% specificity.\(^2,6\)

6. Is there a scoring algorithm or criteria to determine positivity of IHC?

No, there is no scoring algorithm or criteria to determine IHC positivity. The VENTANA\textsuperscript{\textregistered} pan-TRK (EPR17341) assay is a CE-IVD analytic assay intended for the immunohistochemical detection of the C-terminal region of the TRK proteins A, B, and C, which is known to be conserved across WT and chimeric fusion proteins.\(^12,14\)
NGS

1. When should I use RNA- vs DNA-based NGS?

NGS detects DNA or RNA at nucleotide, exon, and whole-genome levels. RNA-based NGS sequences a small portion of the genome that is transcribed, and, thus, may have a lower cost. In RNA-based NGS, the introns are spliced out in the RNA, which removes the technical limitations of intronic coverage. In addition, detection of RNA-level fusions provides direct evidence that they are functionally transcribed, and analysis of the spliced sequence can determine whether the protein would be translated and in-frame. Fusion transcripts can also be detected with high confidence in the RNA of low tumour purity samples because gene fusions are often highly expressed in the tissue. However, this method relies heavily on the quality and length of the RNA. DNA-based sequencing may provide a more comprehensive characterization of genetic alterations in the genome, but this technique is typically more expensive and has a longer turnaround time.3,10,15-17

2. Can I use liquid biopsy to test for NTRK gene fusions?

Liquid biopsy may serve patients with suboptimal or a lack of available tissue samples.18

3. Does FoundationOne® liquid report NTRK fusions?

No, the current version of FoundationOne® liquid does not detect NTRK1, NTRK2, or NTRK3 fusions.19

4. What is the difference between FoundationOne® Heme and FoundationOne® CDx?

Foundation Medicine Inc’s FoundationOne® CDx test detects genetic abnormalities in 324 genes as well as genomic signatures, including microsatellite instability and TMB. It is FDA-approved as a companion diagnostic as of November 30, 2017, and can assist in the identification of patients who may be eligible for treatment with 19 FDA-approved targeted therapies in accordance with the approved therapeutic product labeling. FoundationOne® Heme is validated to detect the 4 main classes of genomic alterations in more than 400 cancer-related genes. In addition to DNA sequencing, FoundationOne® Heme employs RNA sequencing across more than 250 genes to capture a broad range of gene fusions, common drivers of hematologic malignancies, and sarcomas.8,20,21 We are not promoting or endorsing these products. If more information is needed, we can refer you to the diagnostic manufacturer and their information (eg, websites).

5. Does NGS miss any NTRK fusions?

NGS can detect fusions in all 3 NTRK genes (must have NTRK1, 2, 3 gene-fusion coverage), as well as the NTRK gene-fusion partner and position. One disadvantage of DNA-NGS is that DNA-NGS is limited by intron size.1,3,6

CE-IVD=CE in vitro diagnostic; TRK=tropomyosin receptor kinase.
Validation

1. **The prevalence of NTRK rearrangements and expression is low across most tissues. How should I validate the assays?**

   Laboratory directors should use studies performed by the laboratory or reported in published or other reliable sources to validate new assays. The College of American Pathologists recommends that laboratory medical directors determine that fewer than 20 validation cases are sufficient for a specific marker (eg, rare antigen). When that determination is made, the rationale must be documented.22,23

2. **What is the control tissue?**

   For NGS and RT-PCR, commercial sources exist, such as the Seraseq® FFPE Tumour Fusion RNA Reference Material. Seraseq® is manufactured for use as positive reference standards in molecular assay testing (PCR or NGS) of 15 NTRK gene fusions in adult and pediatric cancer patients. For IHC, examples of positive control tissues for the Ventana® pan-TRK (EPR17341) assay include cerebellum and appendix.13,24

Pediatric

1. **Are pediatric patients eligible to be tested using all methodologies?**

   Yes, pediatric patients are eligible to be tested using all methodologies.10

Guideline

1. **Are there disease state guidelines that recommend NTRK gene fusion testing?**

   The National Comprehensive Cancer Network (NCCN) guidelines for treatment by cancer type include NTRK gene-fusion testing.

   - **Non−Small Cell Lung Cancer (NSCLC) v3.2019 / Category 2A:** Recommends NTRK gene-fusion testing as part of broad molecular profiling in patients with advanced or metastatic adenocarcinoma, large-cell, or NSCLC not otherwise specified, based on recent data.11

   As of August 2019, some additional NCCN panel guidelines that consider NTRK gene-fusion testing as an option include the following:

   - **Rectal Cancer v2.2019 / Category 2A:** Recommends NTRK gene-fusion testing in patients with metastatic colorectal cancer.25
   - **Cutaneous Melanoma v2.2019 / Category 2A:** Recommends NTRK gene-fusion testing as an emerging molecular technology for cutaneous melanoma diagnosis and prognostication.26
   - **Occult Primary v2.2019 / Category 2A:** Per physician discretion, TRK protein testing may be considered as part of broad IHC testing (a positive test should then be confirmed with NGS).27
Colon Cancer v2.2019 / Category 2A: Recommends *NTRK* gene-fusion testing in patients with metastatic colorectal cancer.\(^{28}\)

Head and Neck Cancers v2.2019 Salivary Gland Tumors / Category 2A: Recommends *NTRK* gene-fusion status should be checked for secretory carcinoma.\(^{29}\)

2. Are there specific guidelines for specimen acquisition and management for *NTRK* gene-fusion testing?

Although tumour testing has been focused primarily on the use of FFPE tissues, laboratories often accept other specimen types, particularly cytopathology preparations not processed by FFPE methods. Although testing on cell blocks is not included in the FDA approval for multiple companion diagnostic assays, testing on these specimen types is highly recommended when it is the only or best material available.\(^{11}\)

Testing Algorithms

1. What is the ESMO algorithm?

Experts who were recruited by the European Society for Medical Oncology (ESMO) Translational Research and Precision Medicine Working Group have published their recommendations. There are other recommendations published, such as one from Dr Penault-Llorca.\(^{10,30}\)

2. Are there testing algorithms for different tumour types?

From the ESMO working group, it was recommended that in tumours where *NTRK* fusions are highly recurrent, FISH, RT-PCR, or NGS-based sequencing panels can be used as confirmatory techniques, whereas in the scenario of testing an unselected population where *NTRK* 1, 2, 3 fusions are uncommon, either frontline sequencing (preferentially RNA-sequencing) or screening by IHC followed by sequencing of positive cases should be pursued. According to Dr Penault-Llorca’s findings in tumours with a high frequency of *NTRK* gene fusion events, FISH is recommended, with pan-TRK IHC as an alternative if FISH is unavailable. Confirmation by targeted NGS in those cases with positive pan-TRK IHC can be conducted concurrently with treatment considerations. In solid tumours where gene fusions are common, but the frequency of *NTRK* gene fusions is low (5%–25%), an NGS panel that includes *NTRK* fusions is recommended as the preferred test for patients. For tumours with a very low frequency of *NTRK* gene fusions (<5%), but where molecular screening is common, inclusion of *NTRK* genes in routine NGS analysis is recommended. For tumours with a low frequency of *NTRK* fusions, where NGS is not available or is not routinely performed, pan-TRK IHC should be performed for screening with NGS confirmation of positive IHC results.\(^{10,30}\)

FFPE=formalin-fixed paraffin-embedded.
For patients with solid tumours,
Let testing be your guide

References: