



**Australian Government**

**Department of Health**

# **RATIFIED PICO**

## **Application 1602:**

**Testing for neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion in patients with locally advanced or metastatic solid tumours, to determine eligibility for tropomyosin receptor kinase (TRK) inhibitors**

Summary of PICO/PPICO criteria to define the question(s) to be addressed in an Assessment Report to the Medical Services Advisory Committee (MSAC)

Component	Description
Patients	<p><b>Test population:</b></p> <ol style="list-style-type: none"> <li>1. Paediatric and adult patients with locally advanced or metastatic solid tumours with <b>high frequency</b> of neurotrophic tyrosine receptor kinase (<i>NTRK</i>) fusions.</li> <li>2. Paediatric patients with locally advanced or metastatic solid tumours with <b>low frequency</b> <i>NTRK</i> fusions.</li> <li>3. Adult patients with locally advanced or metastatic solid tumours with <b>low frequency</b> <i>NTRK</i> fusions, who have relapsed/refractory (R/R) to one or more prior treatments for <i>de novo</i> locally advanced or metastatic disease and/or prior treatments for earlier disease in those who progress to locally advanced or metastatic disease.</li> </ol> <p><b>Drug population:</b> Patients with metastatic or locally advanced solid tumours who test positive for <i>NTRK</i> gene fusion(s) will become eligible for TRK inhibitor treatment.</p>
Prior tests (for investigative medical services only)	<p>Histological evaluation of tumour tissue sample for individuals with low and high frequency <i>NTRK</i> fusions.</p> <p>For locally advanced or metastatic solid tumours with <b>low frequency</b> <i>NTRK</i> fusions, prior tests include immunohistochemistry (IHC), funded through MBS items 72846, 72847, 72849 and 72850 to identify <i>NTRK</i> fusions.</p> <p>There are no additional prior tests for locally advanced or metastatic solid tumours with <b>high frequency</b> <i>NTRK</i> fusions.</p>
Intervention	<p><b>Test:</b> Fluorescence in-situ hybridisation (FISH) or ribonucleic acid (RNA)-based next-generation sequencing (RNA-NGS) test for the detection of the presence of <i>NTRK</i> gene fusions in tumour tissue sample.</p> <p><b>Drug:</b> TRK inhibitor treatment for patients with metastatic or locally advanced solid tumours with <i>NTRK</i> gene fusions.</p>
Comparator	<p><b>Test comparator:</b> No genetic testing for <i>NTRK</i> gene fusions.</p> <p><b>Drug comparator:</b> Untargeted chemotherapy and/or immunotherapies, based on tumour histology.</p>
Outcomes	<p><b>Test outcomes:</b></p> <p><b>Safety</b></p> <ul style="list-style-type: none"> <li>• Adverse events from obtaining a tumour tissue sample for testing</li> <li>• Psychological effects of false positives or false negatives</li> <li>• Adverse events from false positives or false negatives</li> </ul> <p><b>Effectiveness</b></p> <ul style="list-style-type: none"> <li>• Impact on clinical management</li> </ul> <p><b>Analytical validity<sup>1</sup></b></p> <ul style="list-style-type: none"> <li>• Analytical sensitivity and specificity</li> <li>• Likelihood ratios</li> <li>• Rate of repeat testing</li> </ul> <p><b>Clinical validity<sup>2</sup></b></p> <ul style="list-style-type: none"> <li>• Clinical specificity and sensitivity</li> </ul>

Component	Description
	<ul style="list-style-type: none"> <li>• Positive and negative predictive values</li> </ul> <p><b><i>Clinical utility</i></b></p> <ul style="list-style-type: none"> <li>• Prognostic effect of <i>NTRK</i> fusion in patients with locally advanced or metastatic solid tumours.</li> <li>• Treatment effect modification of larotrectinib in patients with locally advanced or metastatic confirmed <i>NTRK</i> fusion solid tumours.</li> </ul> <p><b><i>Healthcare resources</i></b></p> <ul style="list-style-type: none"> <li>• Number of, and cost associated with molecular testing (FISH/RNA-NGS)</li> <li>• Number of, and cost associated with obtaining appropriate tissue via biopsy</li> </ul> <p><b><u>Drug outcomes:</u></b></p> <p><b><i>Safety</i></b></p> <ul style="list-style-type: none"> <li>• Adverse events from larotrectinib</li> <li>• Adverse events from drug interactions</li> </ul> <p><b><i>Effectiveness</i></b></p> <ul style="list-style-type: none"> <li>• Disease-free and/or overall survival</li> <li>• Progression-free survival</li> <li>• Disease-related mortality</li> <li>• Incidence of metastases</li> <li>• Tumour recurrence (relapse/refractory)</li> <li>• Tumour control (regression/remission)</li> <li>• Health-related quality of life</li> </ul> <p><b><i>Healthcare resources</i></b></p> <ul style="list-style-type: none"> <li>• Cost of larotrectinib</li> <li>• Cost offset by reducing number of untargeted therapies</li> <li>• Cost offset by reducing number of hospitalisations for chemotherapy and/or radiotherapy</li> <li>• Specialist visits</li> <li>• Patient monitoring during treatment</li> <li>• Cost per quality-adjusted life year</li> <li>• Total Australian Government healthcare costs</li> </ul>

## POPULATION

PASC confirmed the proposed population (i.e. four subpopulations) that would be eligible for the selective TRK inhibitor (larotrectinib) [adult cancers: low and high frequency of NTRK gene fusions; paediatric cancers: low and high frequency of NTRK gene fusions].

PASC noted that another, less specific TRK inhibitor – entrectinib – is a multikinase inhibitor, which the applicant confirmed was used in a slightly different population. As noted under ‘Proposed MBS Item Descriptor/s and MBS Fees’, PASC determined that the population and MBS descriptors should be consistent (i.e. refer generically to TRK inhibitors, rather than being specific to larotrectinib).

PASC advised that **high frequency** and **low frequency** need to be carefully defined for the test population (and included in the MBS item descriptors).

PASC noted that, for adult and paediatric cancers, a recent study by Penault-Llorca et al (2019) defined the following three groups: low frequency = <5%; intermediate frequency = 5-25%; and high frequency = >80%;, but only referred to ‘low’ and ‘high’ in their testing algorithm.

PASC is of the view that use of the Hsiao et al (2019) definitions will add clarity, which grouped tumours with an NTRK gene fusion as follows: low frequency = <5%; intermediate frequency = 5-75%; and high frequency = >75%.

This is a more comprehensive classification scheme that is consistent with Penault-Llorca et al’s three groups and also consistent with the frequency classification in Table 1 of this PICO. The ‘low’ frequency group described in the management algorithms and MBS item descriptors of this PICO, encompasses Hsiao et al’s (2019) ‘intermediate’ frequency group (5-75%).

PASC recommended a disaggregated approach to the paediatric and adult populations as much as possible throughout the assessment report. This will allow MSAC and PBAC to evaluate variable value propositions across the four subpopulations (adult cancers: low and high frequency of NTRK gene fusions; paediatric cancers: low and high frequency of NTRK gene fusions).

Neurotrophic tyrosine receptor kinase (NTRK) genes *NTRK1*, *NTRK2* and *NTRK3* code for tropomyosin receptor kinase (TRK) proteins TRKA, TRKB and TRKC, respectively. These proteins are primarily involved in the nervous system, where they regulate pain, proprioception, appetite, and memory<sup>3</sup>. Oncogenic gene fusions occur by chromosomal rearrangements of *NTRK1*, *NTRK2* and *NTRK3* genes. These gene fusions cause tissue-agnostic overexpression of TRK proteins that affect downstream signalling, promoting cell proliferation and tumour cell survival.

Approximately 1% of solid tumours in children and adults have somatic chromosomal gene fusions involving *NTRK* genes<sup>4</sup>. Although found at low frequency across all solid tumours as a whole, *NTRK* gene fusions are found at high frequencies (≥80%) in rare solid tumours (e.g. mammary analogue secretory carcinoma, secretory breast carcinoma and some paediatric cancers such as infantile fibrosarcoma). Common solid tumours, such as lung cancer and colorectal cancer, also harbour *NTRK* gene fusions at lower frequencies<sup>5</sup> (the lower end [i.e. 5%-25%] of the intermediate frequency category; and the low frequency category [<5%], as defined in Penault-Llorca et al 2019).

It is estimated that the annual incidence of *NTRK* gene fusions is between 1,500-5,000 people in the United States<sup>6</sup>. Table 1 presents a list of high, intermediate and low frequency cancers, and their respective gene fusions.

**Table 1: Frequency and types of *NTRK* gene fusion cancers in adult and paediatric patients**

Type of cancer	Gene with <i>NTRK</i> fusion	Frequency	Frequency classification
Breast secretory carcinoma	<i>NTRK3</i>	96.0%	High
Infantile fibrosarcoma	<i>NTRK3</i>	95.5%	High
Mammary analogue secretory carcinoma	<i>NTRK3</i>	89.1%	High
Congenital mesoblastic nephroma	<i>NTRK3</i>	83.0%	High
Spitz tumours and spitzoid melanoma	<i>NTRK1</i>	16.4%	Intermediate*
Papillary thyroid carcinoma	<i>NTRK1,3</i>	8.8%	Intermediate*
Intrahepatic cholangiocarcinoma	<i>NTRK1</i>	3.6%	Low
Astrocytoma	<i>NTRK2</i>	3.1%	Low
High-grade glioma	<i>NTRK1,2,3</i>	2.1%	Low
Uterine sarcoma	<i>NTRK1,3</i>	2.1%	Low
Gastrointestinal stromal tumours	<i>NTRK3</i>	1.9%	Low
Lung cancer	<i>NTRK1,2</i>	1.7%	Low
Thyroid carcinoma	<i>NTRK1,3</i>	1.2%	Low
Glioblastoma	<i>NTRK1,2</i>	1.2%	Low
Sarcoma	<i>NTRK1</i>	1.0%	Low
Philadelphia chromosome-like acute lymphoblastic leukaemia	<i>NTRK3</i>	0.7%	Low
Colorectal cancer	<i>NTRK1,3</i>	0.61%	Low
Melanoma	<i>NTRK3</i>	0.3%	Low
Head and neck cancer	<i>NTRK2,3</i>	0.24%	Low
Invasive breast cancer	<i>NTRK3</i>	<0.1%	Low

Source: ESMO (2019)<sup>7</sup> and Penault-Llorca, et al. (2019)<sup>8</sup> \*consistent with Hsiao et al (2019) classification scheme

The proposed populations affected by the proposed intervention (*NTRK* testing) outlined in this application are:

1. Paediatric and adult patients with locally advanced or metastatic solid tumours with **high frequency** *NTRK* gene fusions. This represents a relatively small population of patients to be tested.
2. Paediatric patients with locally advanced or metastatic solid tumours with **low frequency** *NTRK* gene fusions. This represents a relatively small population of patients to be tested.
3. Adult patients with locally advanced or metastatic solid tumours with **low frequency** *NTRK* gene fusions, who have relapsed/refractory (R/R) to one or more prior treatments for *de novo* locally advanced or metastatic disease and/or prior treatments for earlier disease in those who progress to locally advanced or metastatic disease. This represents a relatively large population of patients who will require testing to determine the small proportion with *NTRK* mutation(s).

Figure 1 describes the estimated eligible population for the proposed *NTRK* gene fusion diagnostic test (FISH or RNA-NGS). The proposed *NTRK* gene fusion diagnostic test will only be undertaken on patients with locally advanced or metastatic solid tumours. Due to the lack of data on the incidence of patients with *NTRK*-fusion solid cancers, the number of patients who may be eligible for treatment with selective inhibitors of TRK proteins (e.g. larotrectinib) has been approximated using yearly cancer deaths (for all ages) as a proxy<sup>9</sup>. Estimated mortality for all solid and blood-related cancers is reported to be 49,896 in 2019<sup>10</sup>. Since the proposed intervention only considers solid tumours, deaths due to blood cancers like leukaemia (2,039 people) and multiple myeloma (1,062 people) are removed from the population. The resulting number is 46,795 deaths due to solid tumours.

Based on the number of deaths due to solid tumours (46,795) and the rate of *NTRK* gene fusions in solid tumours (1%), approximately REDACTED people would test positive for *NTRK* gene fusions and consequently be eligible for TRK inhibitor treatment. Since IHC, FISH or RNA-NGS analysis do not



**Figure 1: Process for determining the estimated population size for FISH or RNA-NGS diagnostic testing for NTRK gene fusions in solid tumours**

**REDACTED**

**Prior test**

Histological evaluation of tumour tissue sample for all people with low and high frequency *NTRK* fusions.

Adult and paediatric populations with **low frequency** *NTRK* gene fusion tumours will undergo IHC analysis. IHC detects gene fusions in tumours by identifying abnormal up-regulation of fusion gene expression, and is currently subsidised on the MBS<sup>14</sup> (refer to

## CONSULTATION FEEDBACK

*PASC noted the letters of strong support received as part of consultation feedback.*

## NEXT STEPS

*Upon ratification of PICO 1602, the application can PROCEED to the pre-Evaluation Sub-Committee (ESC) stage.*

*The applicant has elected to prepare its own ADAR (applicant-developed assessment report).*

Appendix A for details).

Only those with a positive IHC test will be referred for FISH or RNA-NGS to confirm the presence of *NTRK* gene fusions.

Adult and paediatric populations with histologically-defined **high frequency** *NTRK* gene fusion tumours require no prior tests.

## **INTERVENTION**

*PASC confirmed the proposed intervention for the test and the drug (as detailed in this PICO).*

*PASC noted IHC enables detection of TRK overexpression as a surrogate for the presence of an *NTRK* gene fusion. However, PASC advised that IHC results must be followed by confirmatory testing (using a molecular method) to verify the presence of a fusion. This is because overexpression of wild-type TRK proteins may also be detected.*

*PASC advised that education and training for pathologists would be required for them to use IHC as a triage test (as it is not commonly used in Australia).*

*PASC noted RNA-NGS has several advantages over DNA-NGS. However, access to RNA-NGS could be an issue, because few laboratories are currently performing this technique in Australia (compared with more widely diffused DNA-NGS methods).*

*PASC noted fresh frozen tissue is preferable for FISH, as it is more reliable than when used on paraffin-embedded tissue. Fresh frozen tissue is also preferable for RNA-NGS, and specimens must be handled in a way that prevents degradation of RNA.*

The proposed intervention includes two types of molecular testing methods: FISH or RNA-NGS. These diagnostic tests are frequently used to detect *NTRK* gene fusions using tumour tissue. The choice of test may depend on the frequency and type of *NTRK* gene fusion in a particular tumour, cost of the test, turnaround time, and availability of expertise and resources.

IHC, FISH and RNA-NGS diagnostic tests used for *NTRK* gene fusion cancers. According to Penault-Llorca et al (2019) and Hsiao et al (2019), IHC, FISH, reverse transcription polymerase chain reaction (RT-PCR) and NGS are commonly used (either standalone or in combination) as diagnostic tests for *NTRK*-fusion cancers.

The applicant proposed a clinical algorithm which includes IHC, FISH or RNA-NGS. Since the tests stated in the literature do not have 100% specificity and sensitivity, they cannot be used as a reference standard for the proposed intervention (FISH or RNA-NGS). No reference standard was identified.

### **Fluorescent in-situ hybridisation (FISH)**

Chromosomal alterations can be detected via FISH analysis by using fusion or break-apart probes. The fusion FISH method is preferred if the specific genes involved in the oncogenic fusion are known. If there is a range of possible gene fusion partners or if the fusion partners are unknown, a break-apart FISH assay is used and it is based on the proto-oncogene partner.

The *ETV6* break-apart probe is known to be very effective in detecting *ETV6-NTRK3* rearrangements in secretory carcinoma and infantile fibrosarcoma. FISH assays are limited to a single gene; hence

three separate assays would be required to include *NTRK1*, *NTRK2* and *NTRK3* which makes the process expensive and time consuming. Also, FISH does not provide information on whether the gene fusion has a productive transcript. Fresh frozen tissue is preferable for FISH, as it is more reliable than when used on paraffin-embedded tissue.

#### **RNA based next generation sequencing (RNA-NGS)**

NGS offers a range of parallel sequencing from specific gene panels to entire genomes, using limited tissue. It is a useful method to assess fusions in multiple genes and exons simultaneously. NGS can also provide information on the genes involved in the fusions and whether those fusions result in a productive transcript.

The NGS method is well established and can use DNA or RNA from FFPE, fresh frozen (FF) or stabilised tumour tissue. Fresh frozen tissue is preferable for RNA-NGS however specimens must be handled in a way that prevents degradation of RNA. RNA-NGS involves RNA extraction from FFPE tumour tissue which is used to prepare cDNA for amplicon or PCR-based sequencing. Since RNA is labile, there is a high risk of RNA degradation in FFPE tissue over time and quality control measures are required to assess the quantity and quality of RNA obtained.

#### **Testing sequence**

Marked differences in the prevalence of NTRK gene fusions across tumour types mean that clinical diagnostic strategies will vary accordingly but will rely on IHC, FISH and NGS assays<sup>6</sup>.

Patients with **high frequency** *NTRK-fusion* tumours that are locally advanced or metastatic will first undergo IHC testing followed by either FISH or RNA-NGS to identify the presence of oncogenic NTRK gene fusions. Although these tests (FISH/RNA-NGS) may be done sequentially in some cases, the applicant has proposed that either FISH or RNA-NGS testing should be specified in the PICO Confirmation. Patients with a positive result will commence TRK inhibitor treatment, and those with a negative result will undergo other investigations and be treated with usual standard of care (i.e. chemotherapy, radiotherapy, palliation, etc).

Patients with **low frequency *NTRK*-fusion** tumours that are locally advanced or metastatic will (in most cases) first undergo IHC analysis, while in some cases [e.g. thyroid or NSCLC], NGS may be routinely performed<sup>6</sup>. Patients with a positive IHC test will then undergo FISH or RNA-NGS. IHC results must be followed by confirmatory testing (using a molecular method) to verify the presence of a fusion. This is because overexpression of wild-type TRK proteins may also be detected. If found to be positive for *NTRK* gene fusions, they will commence on TRK inhibitor treatment. Patients with a negative result will undergo other investigations and be treated with usual standard of care.

Patients with *NTRK* gene fusions are currently treated according to the tumour-specific treatment guidelines which can involve several types of chemotherapy and/or radiotherapy. Untargeted chemotherapy induces tumour resistance which reduces the effect of subsequent lines of chemotherapy. Also, there is a greater risk of toxicity, increased costs from trialling the various lines of chemotherapy and an increased risk of developing secondary cancers. Larotrectinib offers targeted treatment for *NTRK* gene fusion tumours, which reduces the risks involved with untargeted chemotherapy, improves overall survival, and has minimal adverse effects (95% of adverse events were classified as grade 1 or 2). It is reported to shrink solid tumours which facilitates surgical tumour resection.

FISH or RNA-NGS will be required to assess eligibility for Pharmaceutical Benefits Scheme (PBS) funded *NTRK* inhibitor treatment on detecting gene fusions in solid tumour samples.

FISH (to detect *NTRK* gene fusions) and RNA-NGS tests are not currently funded on the MBS, but are accessed on a user-pay basis.

The proposed molecular tests are once-off diagnostic tests that would be accessible via referral from a paediatric specialist or consultant physician. The patient's tumour sample would be delivered to a National Association of Testing Authorities (NATA)-accredited pathology laboratory, for analysis and interpretation by accredited pathologists or medical scientists.

### ***TRK inhibitor treatment***

Selective inhibition of TRK proteins offer a precision medicine approach to the treatment of a range of tumour types. Tyrosine kinase inhibitors (TKIs), like larotrectinib and entrectinib, are being developed and marketed for the treatment of *NTRK* gene fusion tumours. Larotrectinib, a TKI specific for *NTRK*-fusion cancers, demonstrates anti-tumour activity in cells with TRK protein overexpression. The three multi-centre, open-label, single-arm clinical trials, LOXO-TRK-14001, SCOUT and NAVIGATE, involved 55 adult and paediatric patients with *NTRK*-fusion positive, locally advanced, or metastatic solid tumours. The overall response rate (ORR) was 75% (95% CI); 13% of the patients (7 out of 55) had a complete response, 62% (34 out of 55) had a partial response, 13% (7 out of 55) had stable disease and 9% (5 out of 55) had progressive disease. Larotrectinib is US Food and Drug Administration (FDA)-approved for the treatment of adult and paediatric patients with solid tumours who have an *NTRK* gene fusion. Applications/submissions are currently underway for Therapeutic Goods Administration (TGA) and PBS listing. Non-fusion *NTRK* gene alterations, like mutation or amplification, do not respond to larotrectinib. Therefore, *NTRK* gene fusion identification via diagnostic tests is necessary prior to commencing treatment.

## **Table 2: Clinical laboratory techniques used to identify tumours harbouring *NTRK* gene fusions**

(below) compares IHC, FISH and RNA-NGS diagnostic tests used for *NTRK* gene fusion cancers. According to Penault-Llorca et al (2019) and Hsiao et al (2019), IHC, FISH, reverse transcription polymerase chain reaction (RT-PCR) and NGS are commonly used (either standalone or in combination) as diagnostic tests for *NTRK*-fusion cancers.

The applicant proposed a clinical algorithm which includes IHC, FISH or RNA-NGS. Since the tests stated in the literature do not have 100% specificity and sensitivity, they cannot be used as a reference standard for the proposed intervention (FISH or RNA-NGS). No reference standard was identified.

### **Fluorescent in-situ hybridisation (FISH)**

Chromosomal alterations can be detected via FISH analysis by using fusion or break-apart probes<sup>15</sup>. The fusion FISH method is preferred if the specific genes involved in the oncogenic fusion are known. If there is a range of possible gene fusion partners or if the fusion partners are unknown, a break-apart FISH assay is used and it is based on the proto-oncogene partner<sup>16</sup>.

The *ETV6* break-apart probe is known to be very effective in detecting *ETV6-NTRK3* rearrangements in secretory carcinoma and infantile fibrosarcoma<sup>17</sup>. FISH assays are limited to a single gene; hence three separate assays would be required to include *NTRK1*, *NTRK2* and *NTRK3* which makes the process expensive and time consuming. Also, FISH does not provide information on whether the gene fusion has a productive transcript<sup>18</sup>. Fresh frozen tissue is preferable for FISH, as it is more reliable than when used on paraffin-embedded tissue.

### **RNA based next generation sequencing (RNA-NGS)**

NGS offers a range of parallel sequencing from specific gene panels to entire genomes, using limited tissue. It is a useful method to assess fusions in multiple genes and exons simultaneously. NGS can also provide information on the genes involved in the fusions and whether those fusions result in a productive transcript.

The NGS method is well established and can use DNA or RNA from FFPE, fresh frozen (FF) or stabilised tumour tissue. Fresh frozen tissue is preferable for RNA-NGS however specimens must be handled in a way that prevents degradation of RNA. RNA-NGS involves RNA extraction from FFPE tumour tissue which is used to prepare cDNA for amplicon or PCR-based sequencing. Since RNA is labile, there is a high risk of RNA degradation in FFPE tissue over time and quality control measures are required to assess the quantity and quality of RNA obtained<sup>19</sup>.

### **Testing sequence**

Marked differences in the prevalence of *NTRK* gene fusions across tumour types mean that clinical diagnostic strategies will vary accordingly but will rely on IHC, FISH and NGS assays<sup>6</sup>.

Patients with **high frequency** *NTRK*-fusion tumours that are locally advanced or metastatic will first undergo IHC testing followed by either FISH or RNA-NGS to identify the presence of oncogenic *NTRK* gene fusions. Although these tests (FISH/RNA-NGS) may be done sequentially in some cases, the applicant has proposed that either FISH or RNA-NGS testing should be specified in the PICO Confirmation<sup>20</sup>. Patients with a positive result will commence TRK inhibitor treatment, and those with a negative result will undergo other investigations and be treated with usual standard of care (i.e. chemotherapy, radiotherapy, palliation, etc).



Patients with **low frequency** *NTRK*-fusion tumours that are locally advanced or metastatic will (in most cases) first undergo IHC analysis, while in some cases [e.g. thyroid or NSCLC], NGS may be routinely performed<sup>6</sup>. Patients with a positive IHC test will then undergo FISH or RNA-NGS. IHC results must be followed by confirmatory testing (using a molecular method) to verify the presence of a fusion. This is because overexpression of wild-type TRK proteins may also be detected. If found to be positive for *NTRK* gene fusions, they will commence on TRK inhibitor treatment. Patients with a negative result will undergo other investigations and be treated with usual standard of care.

Patients with *NTRK* gene fusions are currently treated according to the tumour-specific treatment guidelines which can involve several types of chemotherapy and/or radiotherapy. Untargeted chemotherapy induces tumour resistance which reduces the effect of subsequent lines of chemotherapy<sup>21</sup>. Also, there is a greater risk of toxicity, increased costs from trialling the various lines of chemotherapy and an increased risk of developing secondary cancers<sup>22</sup>. Larotrectinib offers targeted treatment for *NTRK* gene fusion tumours, which reduces the risks involved with untargeted chemotherapy, improves overall survival, and has minimal adverse effects (95% of adverse events were classified as grade 1 or 2). It is reported to shrink solid tumours which facilitates surgical tumour resection<sup>23</sup>.

FISH or RNA-NGS will be required to assess eligibility for Pharmaceutical Benefits Scheme (PBS) funded *NTRK* inhibitor treatment on detecting gene fusions in solid tumour samples.

FISH (to detect *NTRK* gene fusions) and RNA-NGS tests are not currently funded on the MBS, but are accessed on a user-pay basis.

The proposed molecular tests are once-off diagnostic tests that would be accessible via referral from a paediatric specialist or consultant physician. The patient's tumour sample would be delivered to a National Association of Testing Authorities (NATA)-accredited pathology laboratory, for analysis and interpretation by accredited pathologists or medical scientists.

### ***TRK inhibitor treatment***

Selective inhibition of TRK proteins offer a precision medicine approach to the treatment of a range of tumour types<sup>24</sup>. Tyrosine kinase inhibitors (TKIs), like larotrectinib and entrectinib, are being developed and marketed for the treatment of *NTRK* gene fusion tumours. Larotrectinib, a TKI specific for *NTRK*-fusion cancers, demonstrates anti-tumour activity in cells with TRK protein overexpression<sup>25</sup>. The three multi-centre, open-label, single-arm clinical trials, LOXO-TRK-14001, SCOUT and NAVIGATE, involved 55 adult and paediatric patients with *NTRK*-fusion positive, locally advanced, or metastatic solid tumours. The overall response rate (ORR) was 75% (95% CI); 13% of the patients (7 out of 55) had a complete response, 62% (34 out of 55) had a partial response, 13% (7 out of 55) had stable disease and 9% (5 out of 55) had progressive disease<sup>26</sup>. Larotrectinib is US Food and Drug Administration (FDA)-approved for the treatment of adult and paediatric patients with solid tumours who have an *NTRK* gene fusion. Applications/submissions are currently underway for Therapeutic Goods Administration (TGA) and PBS listing. Non-fusion *NTRK* gene alterations, like mutation or amplification, do not respond to larotrectinib. Therefore, *NTRK* gene fusion identification via diagnostic tests is necessary prior to commencing treatment<sup>27</sup>.

**Table 2: Clinical laboratory techniques used to identify tumours harbouring *NTRK* gene fusions**

Diagnostic test		Sample requirements	Pre-analytical consideration	Turnaround time	Advantages	Disadvantages
IHC		FFPE tissue	<ul style="list-style-type: none"> <li>Variability in fixation processes may impact the quality of staining</li> </ul>	1-2 days	<ul style="list-style-type: none"> <li>Rapid and inexpensive process</li> <li>Established approach, widely available within clinical laboratories</li> </ul>	<ul style="list-style-type: none"> <li>Indication-specific specificity for <i>NTRK</i> gene fusion prediction not well characterised</li> <li>Sensitivity of TRKC proteins may be low</li> <li>Assay not easily multiplexed for other biomarkers</li> </ul>
FISH	Fusion	FFPE tissue, fresh frozen tissue	n/a	1-2 days	<ul style="list-style-type: none"> <li>High specificity</li> <li>Can detect alterations present in small subsets of cells</li> </ul>	<ul style="list-style-type: none"> <li>Individual assay limited to detection of specific 5' partner and <i>NTRK</i> gene pair</li> </ul>
	<i>NTRK</i> break apart	FFPE tissue, fresh frozen tissue	n/a	1-2 days	<ul style="list-style-type: none"> <li>Detects <i>NTRK</i> rearrangements without knowledge of 5' partner</li> </ul>	<ul style="list-style-type: none"> <li>Sensitivity and specificity variable, depending on assay design and parameters</li> <li>Multiple or complex FISH assays may be required for complete coverage</li> </ul>
RNA-NGS		Snap frozen, fresh or FFPE tissue	<ul style="list-style-type: none"> <li>Data acquisition may be affected by tumour heterogeneity</li> <li>Sensitivity for fusions varies according to enrichment method</li> <li>RNA is labile</li> </ul>	5-7 days	<ul style="list-style-type: none"> <li>Ability to interrogate all clinically actionable genomic content</li> <li>Most tissue-sparing approach for broad genomic analysis</li> <li>Only transcriptionally active fusions detected</li> <li>Commercially available kits cover all potentially oncogenic actionable fusions, without knowledge of 5' partners or breakpoints</li> <li>Allows in-frame vs out of frame confirmation for all fusions</li> </ul>	<ul style="list-style-type: none"> <li>May require high level of infrastructure investment</li> <li>Requires high-level bioinformatics capability</li> <li>Does not confirm that protein is generated</li> <li>Detection of transcripts expressed at low levels may be challenging</li> </ul>

Abbreviations: FFPE, formalin-fixed paraffin-embedded

Source: Hsiao et al. (2019)<sup>28</sup>

## COMPARATOR/S

PASC confirmed the proposed comparators, as detailed in this PICO.

PASC noted entrectinib could become a near-market comparator for larotrectinib.

### **Comparator for NTRK gene fusion testing:**

The comparator for the proposed intervention is 'no NTRK gene fusion testing'. MBS subsidised FISH tests are available for assessing eligibility to crizotinib, ceritinib or alectinib in patients with locally advanced or metastatic non-small cell lung cancer. These FISH tests are not suitable comparators since they are not specific for NTRK gene fusions.

### **Comparator for treatment:**

Usual standard of care (SoC) is the comparator for TRK inhibitor treatment. SoC may involve untargeted chemotherapy and/or immunotherapy (with or without radiotherapy) that is chosen on the basis of tumour histology, prior treatments and treatment tolerance. SoC may also include supportive care.

## OUTCOMES

### Patient-relevant outcomes

From a patient perspective, FISH or RNA-NGS analysis offers the opportunity to commence on targeted therapy instead of several lines of untargeted chemotherapy that may result in toxicity, tumour resistance and disease progression.

From a clinical perspective, an accurate diagnosis of the cause of a tumour is important because of its prognostic and therapeutic implications for the patient. Detecting the presence of NTRK gene fusions in tumours may reduce the number of patients with tumours that are over treated and thus exposed to toxic effects from untargeted chemotherapy without deriving benefit. FISH or RNA-NGS would provide clinicians with additional information that would inform whether they recommend a patient for targeted chemotherapy such as TRK inhibitor treatment.

IHC, FISH or RNA-NGS testing have a high sensitivity and specificity profile. However, there is still a risk that patients are missed or misdiagnosed. Incorrectly diagnosed patients may then go on to receive inappropriate treatment, based on false-negative or false-positive results (i.e. not receive TRK inhibitor treatment, or alternatively receive unnecessary TRK inhibitor treatment), exposing them to possible side effects or a missed treatment opportunity, and incurring healthcare costs.

The listed outcomes (under 'Test' and 'Drug' outcomes) below are considered relevant to the assessment of comparative effectiveness and safety of larotrectinib and FISH or RNA-NGS testing for people with locally advanced or metastatic solid tumours.

### Healthcare system outcomes

The availability of FISH or RNA-NGS to detect *NTRK* gene fusions in people with locally advanced or metastatic solid tumour will have implications for the Australian healthcare system.

Depending on the molecular test results, patients may be recommended to receive TRK inhibitor treatment (if listed on the PBS), which might have cost-saving implications for the PBS, MBS, and other healthcare resource use (e.g. public/private hospitalisation and/or day procedure admission, private healthcare



#### Safety:

- Adverse events from larotrectinib
- Adverse events from drug interactions

#### Healthcare resource use:

- Cost of larotrectinib
- Cost offset by reducing number of untargeted therapies
- Cost offset by reducing number of hospitalisations for chemotherapy and/or radiotherapy
- Specialist visits
- Patient monitoring during treatment
- Cost per quality-adjusted life year
- Total Australian Government healthcare costs

### CLINICAL MANAGEMENT ALGORITHMS

*PASC noted the proposed algorithm appeared to be based on cost, rather than test performance (i.e. additional molecular testing is not proposed to be performed if a negative result is achieved by a single method).*

*PASC advised that including IHC testing for all proposed subpopulations would be beneficial, as it reduces complexity of the algorithm and provides a time-efficient and tissue-efficient technique that may be used for routine screening. As high frequency NTRK gene fusions occur in certain rare adult and paediatric cancers, the addition of IHC testing to the proposed testing algorithm in these subpopulations would only lead to a small increase in patient numbers eligible for IHC testing.*

*PASC noted a potential issue will be the substantial overall increase in volume of IHC testing that will occur if the application is recommended. PASC advised that this should be accounted for in the economic evaluation and financial estimates.*

*Since the PASC meeting, the applicant raised the following concerns about the algorithms:*

- 1. The applicant expressed concern about PASC's comment that the proposed algorithm appears to be based on cost, rather than test performance. The applicant stated that its original proposed algorithm was based on the opinions of Australian clinical experts (pathologists and oncologists, from two different advisory boards), and was based on categorisation of tumours (into two groups) using the incidence of NTRK gene fusion, according to international guidelines (ESMO and Penault-Llorca F, et al. 2019).*

*The applicant stated that its original test algorithm (for the different sub-populations) was based on the aetiology of tumours, and availability and performance characteristics of each of the tests, to facilitate an optimal testing strategy.*

*The applicant was informed that this first issue need not hinder progression of the assessment, using the PASC-agreed PICO.*

- 2. The applicant disagreed with PASC's advice: "including IHC testing for all proposed subpopulations would be beneficial, as it reduces complexity of the algorithm and provides a time-efficient and tissue-efficient technique that may be used for routine screening".*

*The applicant stated that, for the “adult and paediatric population with histologically-defined high frequency NTRK gene fusion tumours”, it had suggested ‘no prior screening tests’ and undergoing either FISH or NGS to identify the presence of oncogenic NTRK gene fusions.*

*The applicant claimed that clinical experts support the original algorithm strategy for rare cancers with high frequency NTRK gene fusion tumours, because of the high likelihood of NTRK fusion, the benefit of avoiding IHC false negative results, and better stewardship of tissue scarcity.*

*The applicant provided expert clinical advice from the Peter MacCallum Cancer Centre stating that an IHC test is not appropriate in this sub-population, and there is no clinical utility in conducting the extra test.*

*The applicant requested retention of its original diagnostic algorithm, where:*

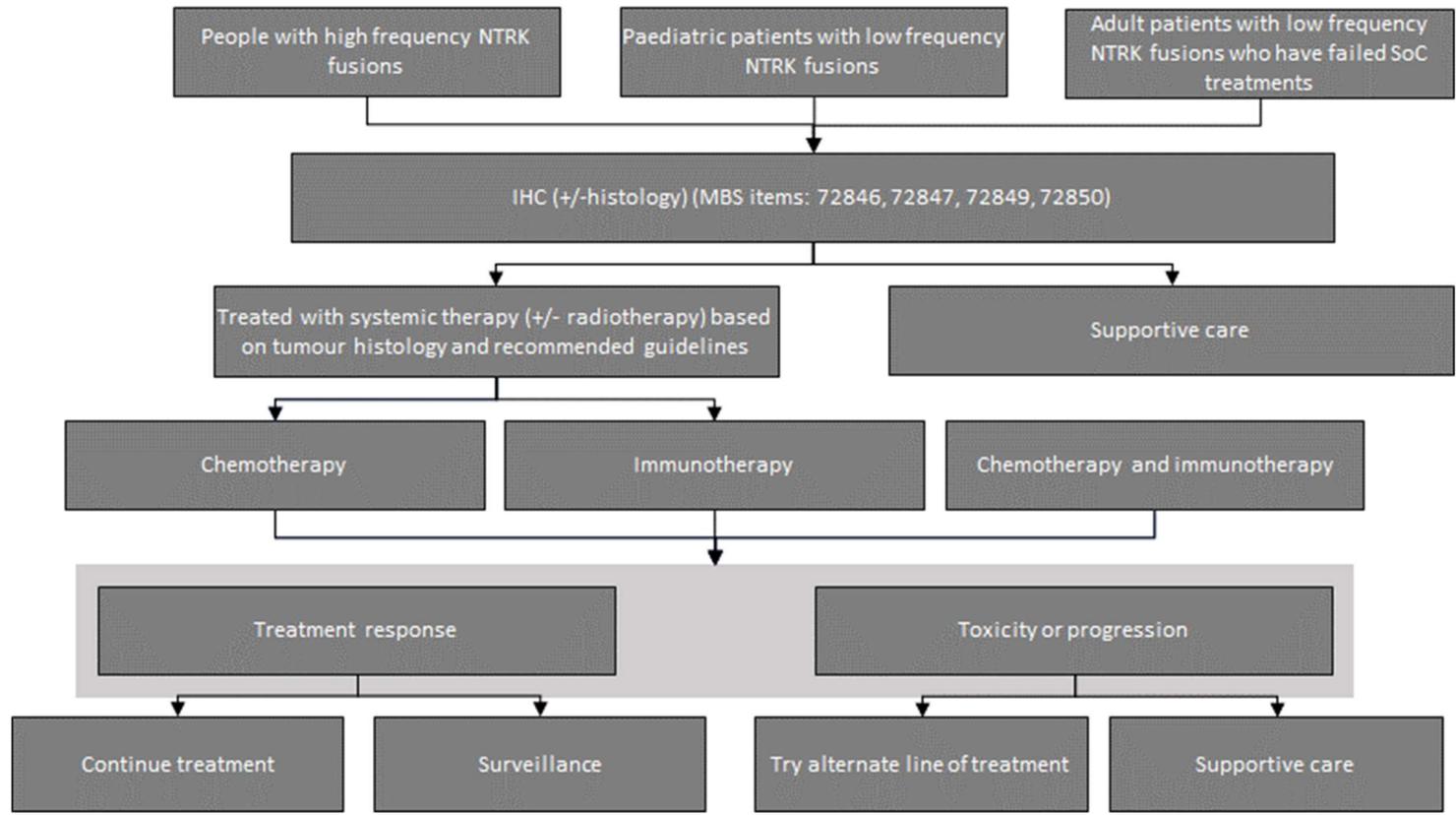
- *tumours with high frequency NTRK fusion require no prior tests, and immediately undergo the “confirmatory test” (FISH/ NGS);*
- *tumours with low frequency NTRK fusion undergo IHC analysis, and only those with a positive IHC test are referred for FISH or NGS to confirm the presence of NTRK gene fusions.*

*This second issue, while representing a separation of views between the applicant and PASC, should not delay progression of the application. The post-PASC (updated) PICO accurately reflects PASC’s advice. In order to avoid a delay in the application’s progression, and noting the applicant (and its clinical experts) still support the original algorithm, the Department advised the applicant to raise and address these issues in its assessment report, thereby submitting them to MSAC for consideration.*

### **Current clinical management algorithm for identified population**

Under the current clinical management pathway, patients diagnosed with metastatic or locally advanced solid tumours are treated with chemotherapy or immunotherapy (with or without radiotherapy) or supportive care. Figure 2 presents the current clinical management algorithm for patients with metastatic or locally advanced solid tumours.

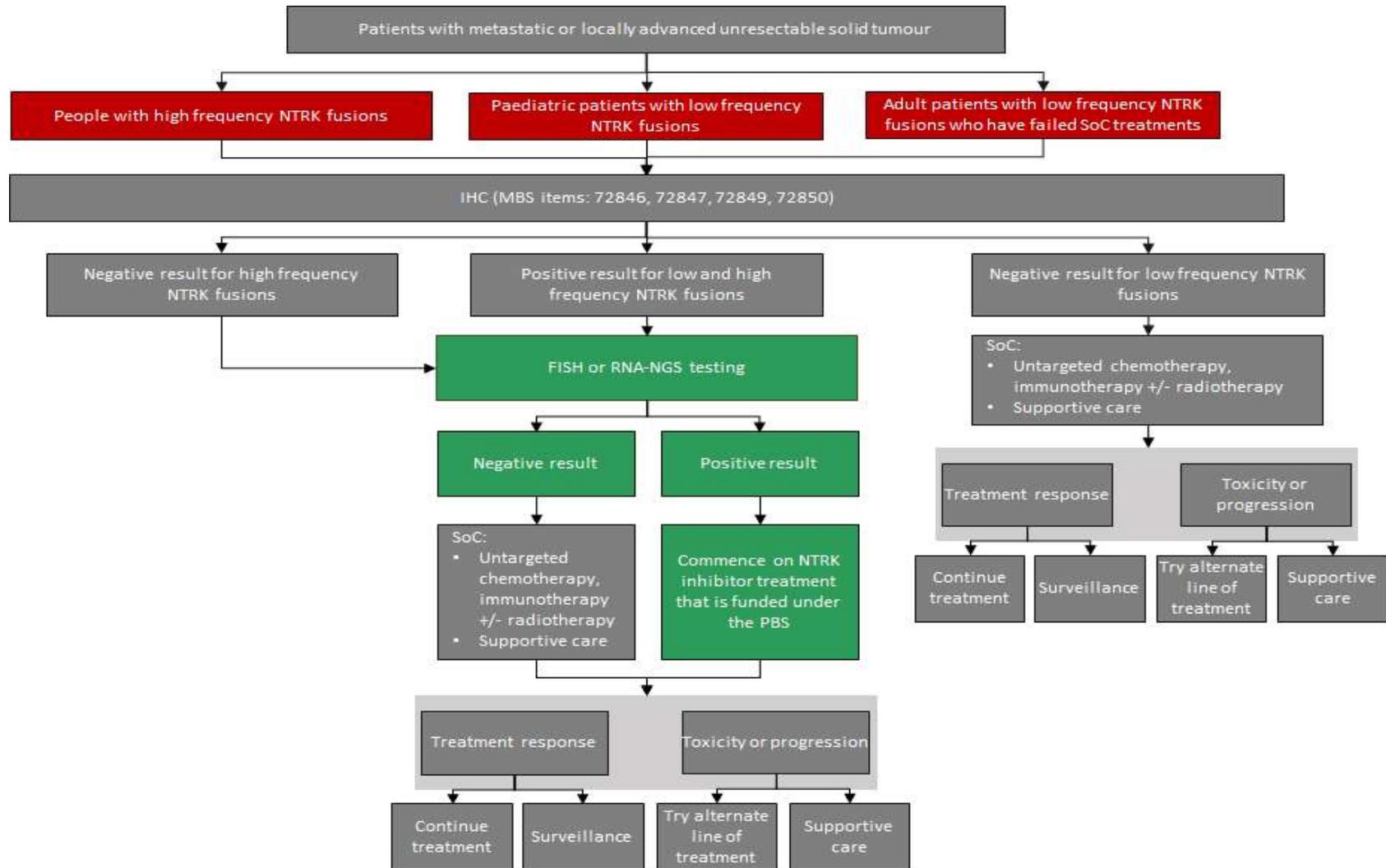
Figure 2: Current clinical management algorithm



### [Proposed clinical management algorithm for identified population](#)

Figure 3 presents the proposed clinical management algorithm for FISH or RNA-NGS diagnostic testing of gene fusions in patients with metastatic or locally advanced solid tumours. The main difference between the current and proposed clinical algorithm is that FISH or RNA-NGS analysis is used to detect *NTRK* gene fusions in patients with locally advanced or metastatic solid tumours. Although both tests could be done sequentially, the applicant stated that either RNA-NGS or FISH will be performed, which is reflected in the proposed clinical management algorithm and in the MBS item descriptors. These tests will assess the patient's eligibility for targeted treatment (TRK inhibitor treatment), which might avoid treatment with untargeted chemotherapy, immunotherapy or supportive care.

Figure 3: Proposed clinical management algorithm



## PROPOSED ECONOMIC EVALUATION

*PASC confirmed the economic evaluation should be a cost-effectiveness/cost-utility analysis.*

The applicant's overall clinical claim is that the proposed co-dependent technologies, namely *NTRK*-fusion testing and larotrectinib, are superior in terms of comparative effectiveness, compared with the main comparator, being no testing with SoC treatment in patients with locally advanced or metastatic *NTRK*-fusion solid tumours. Given the claim of clinical superiority, a cost-effectiveness or cost-utility analysis should be presented in the assessment report.

### *Proposed economic evaluation for the test*

The clinical claim is that FISH or RNA-NGS testing for *NTRK* gene fusions in solid tumours is inferior (in terms of safety) and superior (in terms of clinical effectiveness), compared to no *NTRK* gene fusion testing for the proposed populations.

According to the *Technical Guidelines for preparing assessment reports for the Medical Services Advisory Committee: Investigative*, the required economic analysis is therefore a cost-effectiveness and/or cost-utility analysis. This type of analysis will determine the incremental cost per extra unit of health outcome achieved, expressed in quality-adjusted life years (QALYs), because of a claimed reduction in the number of people being treated with untargeted chemotherapy and/or radiotherapy.

For the economic evaluation of FISH and RNA-NGS, QALYs should be calculated for each of the endpoint outcomes. If QALYs cannot be calculated, then the measure of effectiveness can be expressed in life years or other outcomes. PASC considered this application was NOT relevant for a clinical utility card (CUC) approach.

**Note:** It is anticipated that there will be a substantial overall increase in the volume of IHC tests if this application is recommended.

### *Proposed economic evaluation for the drug*

The clinical claim is that larotrectinib treatment is superior in terms of safety and clinical effectiveness, compared to SoC for patients with locally advanced or metastatic solid tumours caused by *NTRK* gene fusions.

## PROPOSED MBS ITEM DESCRIPTOR/S AND MBS FEES

*PASC noted that, if IHC testing is included for both high-frequency and low-frequency subpopulations, the number of proposed MBS items would be reduced to two (one for FISH [Item AAAA] and one for RNA-NGS [Item CCCC]).*

*PASC requested the proposed item descriptors be amended to refer to 'TRK inhibitors' as a generic term, rather than larotrectinib. The applicant agreed.*

*PASC confirmed the proposed MBS fee for FISH, noting it is consistent with similar MBS items.*

*PASC noted the applicant's willingness to provide sufficient evidence to justify a higher MBS fee for RNA-NGS.*

Two separate MBS items are proposed, one for each test.

- One item is for the FISH test that includes prior IHC testing requirements (AAAA) [e.g. for people with **low frequency NTRK** gene fusion solid tumours].
- The second item is for the RNA-NGS test that includes prior IHC testing requirements (CCCC) [e.g. for people with **low frequency NTRK** gene fusion solid tumours].

The current fee for FISH under the MBS is \$400 (i.e. MBS items 73341 and 73344).

The two proposed items are:

Item AAAA	Category 6 (Pathology services) – Group P7 Genetics
Fluorescence in-situ hybridisation (FISH) test of tumour tissue from a patient with a low or high frequency locally advanced or metastatic neurotrophic receptor tyrosine kinase ( <i>NTRK</i> ) fusion cancer, with documented evidence of tropomyosin receptor kinase (TRK) A, TRKB or TRKC immunoreactivity by immunohistochemical (IHC) examination, requested by a specialist or consultant physician to determine if requirements relating to <i>NTRK</i> fusion for access to TRK inhibitors under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.	
This item cannot be claimed if MBS item BBBB has been claimed for the same patient.	
<b>Fee:</b> \$400 <b>Benefit:</b> 75% = \$300 85% = \$340	

No MBS fee (and associated benefit) was included by the applicant for RNA-NGS testing.

A costing study of RNA-NGS testing was identified in a brief literature search, which was a French costing study of NGS testing for cancer diagnosis, using targeted gene panels. The mean total cost of NGS analysis of somatic cells was estimated to be 607€ ± 207€<sup>31</sup>. The conversion rate used for the MBS item estimate is 1€=\$1.614 AUD (as at 4 November 2019).

This suggests an MBS fee for this item could be \$980 (100%), with a 75% (admitted patient) benefit of \$735, and an 85% (non-admitted patient) benefit of \$833. However, this 85% benefit amount would be subject to the MBS Greatest Permissible Gap (GPG) rules, because the suggested MBS fee is more than \$565. From 1 November 2019, the GPG was set at \$84.70, which means any out-of-hospital Medicare service which has an MBS fee of \$565.00 or more, attracts a benefit that is greater than 85% of the MBS fee (in the case of the suggested RNA-NGS test, it would be \$980 minus \$84.70, which equals \$895.30). This will need to be clarified and addressed in the assessment report.

Item BBBB	Category 6 (Pathology services) – Group P7 Genetics
RNA-based next generation sequencing (RNA-NGS) test of tumour tissue from a patient with a low or high frequency locally advanced or metastatic neurotrophic receptor tyrosine kinase ( <i>NTRK</i> ) fusion cancer, with documented evidence of tropomyosin receptor kinase (TRK) A, TRKB or TRKC immunoreactivity by immunohistochemical (IHC) examination, requested by a specialist or consultant physician to determine if requirements relating to <i>NTRK</i> fusion for access to TRK inhibitors under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.	
This item cannot be claimed if MBS item AAAA has been claimed for the same patient.	
<b>Fee:</b> \$TBA <b>Benefit:</b> 75% = \$ TBA 85% = \$ TBA	

## CONSULTATION FEEDBACK

*PASC noted the letters of strong support received as part of consultation feedback.*

## NEXT STEPS

*Upon ratification of PICO 1602, the application can PROCEED to the pre-Evaluation Sub-Committee (ESC) stage.*

*The applicant has elected to prepare its own ADAR (applicant-developed assessment report).*

## Appendix A

Item 72846	Category 6 (Pathology services) – Group P5 Tissue Pathology
Immunohistochemical examination of biopsy material by immunofluorescence, immunoperoxidase or other labelled antibody techniques with multiple antigenic specificities per specimen - 1 to 3 antibodies except those listed in 72848	
(Item is subject to rule 13)	
<b>Fee:</b> \$59.60 <b>Benefit:</b> 75% = \$44.70 85% = \$50.70	

Item 72847	Category 6 (Pathology services) – Group P5 Tissue Pathology
Immunohistochemical examination of biopsy material by immunofluorescence, immunoperoxidase or other labelled antibody techniques with multiple antigenic specificities per specimen - 4-6 antibodies	
(Item is subject to rule 13)	
<b>Fee:</b> \$89.40 <b>Benefit:</b> 75% = \$67.05 85% = \$76.00	

Item 72849	Category 6 (Pathology services) – Group P5 Tissue Pathology
Immunohistochemical examination of biopsy material by immunofluorescence, immunoperoxidase or other labelled antibody techniques with multiple antigenic specificities per specimen - 7-10 antibodies	
(Item is subject to rule 13)	
<b>Fee:</b> \$104.30 <b>Benefit:</b> 75% = \$78.25 85% = \$88.70	

Item 72850	Category 6 (Pathology services) – Group P5 Tissue Pathology
Immunohistochemical examination of biopsy material by immunofluorescence, immunoperoxidase or other labelled antibody techniques with multiple antigenic specificities per specimen - 11 or more antibodies	
(Item is subject to rule 13)	
<b>Fee:</b> \$119.20 <b>Benefit:</b> 75% = \$89.40 85% = \$101.35	

## References

- <sup>1</sup> Analytical validity: the reproducibility and repeatability of the assay, that is, the ability of the test to measure gene expression accurately and reliably.
- <sup>2</sup> Clinical validity: measures the test's ability to predict the presence or absence of disease, that is, the sensitivity, specificity, and positive and negative predictive values, in this case
- <sup>3</sup> Drilon, A., T. W. Laetsch, S. Kummar, S. G. DuBois, U. N. Lassen et al. (2018). "Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children." *N Engl J Med* 378(8): 731-739.
- <sup>4</sup> Ricciuti, B., C. Genova, L. Crino, M. Libra and G. C. Leonardi (2019). "Antitumor activity of larotrectinib in tumors harboring NTRK gene fusions: a short review on the current evidence." *Onco Targets Ther* 12: 3171-3179.
- <sup>5</sup> Amatu, A., A. Sartore-Bianchi and S. Siena (2016). "NTRK gene fusions as novel targets of cancer therapy across multiple tumour types." *ESMO Open* 1(2): e000023.
- <sup>6</sup> Biooncology. "TRK: A Drive of Oncogenesis." Retrieved 07/10/2019, from <https://www.biooncology.com/pathways/cancer-tumor-targets/trk/trk-oncogenesis.html>.
- <sup>7</sup> ESMO (2019). "Epidemiology of Cancers with NTRK Gene Fusion. *Oncology Pro*. Retrieved 07/10/2019, from <https://oncologypro.esmo.org/Oncology-in-Practice/Anti-Cancer-Agents-and-Biological-Therapy/Targeting-NTRK-Gene-Fusions/Overview-of-Cancers-With-NTRK-Gene-Fusion/NTRK-Gene-Fusions-as-Oncogenic-Drivers/Epidemiology-of-Cancers-With-NTRK-Gene-Fusion>.
- <sup>8</sup> Penault-Llorca, F., E. R. Rudzinski and A. R. Sepulveda (2019). "Testing algorithm for identification of patients with TRK fusion cancer." *J Clin Pathol* 72(7): 460-467.
- <sup>9</sup> Australian Institute of Health and Welfare, *Cancer in Australia 2019*, A. government, Editor. 2019.
- <sup>10</sup> Australian Institute of Health and Welfare, *Cancer in Australia 2019*, A. government, Editor. 2019.
- <sup>11</sup> James P. Solomon and Jaclyn F. Hechtman (2019). "Detection of NTRK Fusions: Merits and Limitations of Current Diagnostic Platforms." *American Association for Cancer Research* 79(13).
- <sup>12</sup> ESMO *Oncology Pro*. (2019). "Algorithm for NTRK Gene Fusion Testing." Retrieved 25/10/2019, from <https://oncologypro.esmo.org/Oncology-in-Practice/Anti-Cancer-Agents-and-Biological-Therapy/Targeting-NTRK-Gene-Fusions/Algorithm-for-NTRK-Gene-Fusion-Testing>.
- <sup>13</sup> Hsiao, S. J., A. Zehir, A. N. Sireci and D. L. Aisner (2019). "Detection of Tumor *NTRK* Gene Fusions to Identify Patients Who May Benefit from Tyrosine Kinase (TRK) Inhibitor Therapy." *The Journal of Molecular Diagnostics* 21(4): 553-571.
- <sup>14</sup> Australian Government Department of Health. "MBS Online: Medicare Benefits Schedule." Retrieved 15/10/2019, from <http://www9.health.gov.au/mbs/search.cfm>.
- <sup>15</sup> Hsiao, S. J., A. Zehir, A. N. Sireci and D. L. Aisner (2019). "Detection of Tumor *NTRK* Gene Fusions to Identify Patients Who May Benefit from Tyrosine Kinase (TRK) Inhibitor Therapy." *The Journal of Molecular Diagnostics* 21(4): 553-571.
- <sup>16</sup> Hsiao, S. J., (2019). *Ibid*.
- <sup>17</sup> James P. Solomon and Jaclyn F. Hechtman (2019). "Detection of NTRK Fusions: Merits and Limitations of Current Diagnostic Platforms." *American Association for Cancer Research* 79(13).
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- <sup>20</sup> Penault-Llorca, F., E. R. Rudzinski and A. R. Sepulveda (2019). "Testing algorithm for identification of patients with TRK fusion cancer." *J Clin Pathol* 72(7): 460-467.
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- <sup>23</sup> Drilon, A. et al. (2018). *op. cit*.
- <sup>24</sup> Amatu, A., A. Sartore-Bianchi and S. Siena (2016). "NTRK gene fusions as novel targets of cancer therapy across multiple tumour types." *ESMO Open* 1(2): e000023.
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<sup>29</sup> Analytical validity: the reproducibility and repeatability of the assay, that is, the ability of the test to measure gene expression accurately and reliably.

<sup>30</sup> Clinical validity: measures the test's ability to predict the presence or absence of disease, that is, the sensitivity, specificity, and positive and negative predictive values, in this case.

<sup>31</sup> Marino, P., R. Touzani, L. Perrier, E. Rouleau, D. S. Kossi, Z. Zhaomin, et al. (2018). "Cost of cancer diagnosis using next-generation sequencing targeted gene panels in routine practice: a nationwide French study." *European Journal of Human Genetics* 26(3): 314-323.