NTRK GENE FUSION TESTING GUIDANCE

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1	19/09/2022		NCCP NTRK Testing Working Group
2	29/11/2023	Updated NGS overview Link to MAP included	NCCP Molecular Diagnostics Advisory Group
3	23/09/2024	Clarification on reimbursement approval application to MMP and generation of prescription on high tech hub	NCCP Molecular Diagnostics Advisory Group

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1 Summary

The aim of this document is to provide guidance on neurotrophic tyrosine receptor kinase (NTRK) gene fusion testing. The scope of this document includes testing of both adult and paediatric patients with a diagnosis of any solid tumour who are eligible to have their tumour tested for this genetic variant. The NTRK gene fusion testing algorithm developed considers

- Stage of disease
- NTRK gene fusion prevalence likely in that cancer type
- Availability of treatment options including current standard of care[1]
- Current NGS panel testing available in country or outsourced abroad

It is anticipated that the development of a HSE Test Directory will supersede this guidance in the future.

2 Background

Neurotropic tropomysoin-related kinases (NTRKs) constitute a receptor kinase family of neurotropin receptors involved in neuronal development. The tropomyosin receptor kinase (TRK) family contains 3 members—TRKA, TRKB, and TRKC— and these proteins are encoded by the genes NTRK1, NTRK2, and NTRK3, respectively. Each of the three NTRK genes can combine with any one of numerous fusion partners and lead to the formation of oncogenic proteins**.** These oncogenic proteins result in aberrant cell signalling and thereby drive the formation of malignancies. To date, 25 different gene fusions involving NTRKs have been identified (1).

NTRK gene fusions have been identified in a variety of solid tumours, affecting both adults and children. However, the prevalence of these gene fusions varies considerably. It occurs rarely (less than 1%) in common tumours such as lung, colorectal and breast while some rare tumour types have more than 90%

[1] a satisfactory SoC is defined as a SoC significantly improving patient outcome as determined by efficacy (ORR, OS), duration of response (DOR, PFS), time to response, safety, and QoL.

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NTRK fusion prevalence (e.g. mammary analogue secretory carcinoma and infantile fibrosarcoma) (2). The prognostic effect of NTRK gene fusions across all tumour types is as yet unknown.

3 NTRK inhibitors

Two medicines are currently licensed by the European Medicines Agency for the treatment of NTRK gene fusion tumours as detailed below; larotrectinib and entrectinib

- *Entrectinib as monotherapy for the treatment of **adult and paediatric patients 12 years of age and older** with solid tumours expressing a neurotrophic tyrosine receptor kinase (NTRK) gene fusion, and who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and who have not received a prior NTRK inhibitor and have no satisfactory treatment options*
- *Larotrectinib for the treatment of **adult and paediatric patients** with solid tumours that display NTRK gene fusion, who have a disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, and who have no satisfactory treatment options*

Larotrectinib and entrectinib are positioned as a last-line treatment option where the alternative is best supportive care.

4 NTRK Testing Methods

There are several techniques available to detect NTRK gene fusions including screening by immunohistochemistry (IHC), fluorescence in-situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR) in addition to next generation sequencing (NGS), each with different advantages and disadvantages. IHC is relatively inexpensive but there are differences in specificity depending on tumour type and it has a lower sensitivity for NTRK3 fusions (79.4%) compared to RNA based NGS (3).

4.1 Immunohistochemistry (IHC)

IHC indicates the presence of a TRK protein but does not clarify whether a fusion is actually present. It can therefore be used as an upstream screening tool for common tumour entities that rarely bear NTRK gene fusions, or have high endogenous TRK expression. In addition, immunohistochemistry seems to have variable specificity according to tumour type. While the antibody appears to have 100% specificity in carcinomas of the colon, lung, thyroid, pancreas and biliary tract, decreased specificity is seen in breast and salivary gland carcinomas, as cytoplasmic staining can occasionally be seen. Specificity is lower in sarcomas, particularly those with neural or smooth muscle differentiation as wild-type TRK protein is physiologically expressed in neural and smooth muscle tissue (4). Thus, tumours with neural or smooth muscle differentiation should not be screened via pan-TRK IHC for NTRK fusion (5).

While IHC is relatively inexpensive in terms of reagent cost there is potential for significant training costs associated with establishing and validating this testing across different tumour types and the additional pathologist time involved in interpreting the stains.

4.2 Fluorescence in-situ hybridization (FISH)

Fluorescence in-situ hybridization is a highly sensitive DNA-based assay that identifies oncogenic fusions using either break-apart probes or fusion probes. This testing method is relatively inexpensive and widely available in clinical laboratories, with a short turnaround time of typically 1–3 days.

Each FISH assay evaluates a single NTRK gene, so three separate slides are required to assess NTRK1, NTRK2, and NTRK3 fusions. Development of FISH multiprobes that can simultaneously target all 3 NTRK genes will likely reduce the time and resources needed for testing.

4.3 Reverse transcription polymerase chain reaction (RT-PCR)

Reverse-transcription polymerase chain reaction detects gene fusion RNA transcripts and can be qualitative and potentially quantitative. This technique is relatively inexpensive and the results take approximately one week. An important limitation of RT-PCR in NTRK fusion testing is that it is a targeted assay, designed to detect specific fusion partners and involving specific breakpoints within these, thus similarly to FISH, additional rounds of testing may be needed and detection of novel/alternative fusions may fail. This approach can be considered for tumour histologies with known fusion partners, such as detection of ETV6–

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NTRK3 fusions in infantile fibrosarcoma or secretory breast cancers, where the majority of cases will bear the usual diagnosis-associated fusions.

4.4 Next Generation Sequencing (NGS)

With an NGS method NTRK gene fusion detection occurs at the nucleic acid level and not at the protein level as with IHC.

Depending on the type of sample (DNA or RNA), and the library preparation technology, NGS based testing can have distinct advantages in terms of analytical sensitivity and specificity. As an example RNA-based amplicon library preparation can have very high analytical specificity but does require that fusion partners are known in advance, this might mean that novel fusions are less likely to be detected with this approach. Where NGS methodology is employed for screening for NTRK fusions it is important that the limitations of the assay are clearly stated on the report. NGS is associated with higher cost compared with IHC and FISH testing and is not as widely available. The results generally take 2–4 weeks.

5 NTRK gene fusion testing algorithm proposal

The aim of NTRK gene fusion testing is to identify patients who may benefit from treatment with NTRK inhibitors. It is the responsibility of:

- the treating Consultant Medical Oncologist to ensure the patient's tumour is suitable for NTRK gene fusion testing and that NTRK-inhibitors do not displace any effective therapies for their solid tumour
    - The licensed indications for both entrectinib and larotrectinib are for treatment of disease that is locally advanced, metastatic or where surgical resection is likely to result in severe morbidity, **where patients have no alternative satisfactory treatment options.**

- clinical laboratories in Irish public hospitals to ensure that there is a pathway for NTRK testing locally or externally that allows for **timely** testing of patients should an NTRK inhibitor be approved for reimbursement by the HSE for the above indication

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Histology based triaging should first be carried out to separate the rare cancer subtypes that have high NTRK gene fusion prevalence from those that have a low prevalence of NTRK gene fusions. Table 1 below summarises the proposed algorithm for **NTRK gene fusion testing** for given cancer types. It details the recommended time of testing and testing method considering

- Stage of disease
- NTRK gene fusion prevalence likely in that cancer type
- Availability of treatment options including current standard of care[2]
- Current NGS panel testing available in- country or outsourced abroad

It is anticipated that the ongoing work in the development of a national HSE test directory will supersede this guidance in the future.

[2] a satisfactory SoC is defined as a SoC significantly improving patient outcome as determined by efficacy (ORR, OS), duration of response (DOR, PFS), time to response, safety, and QoL.

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Table 1 NTRK gene fusion testing algorithm

Solid Tumour Type	When to test	Recommended Test Method	*Testing Access
High Frequency NTRK gene fusion tumours			
Congenital mesoblastic nephroma (CMN) Infantile Fibrosarcoma Secretory breast carcinoma Mammary analogue secretory carcinoma (MASC) of salivary gland	At diagnosis	RNA based NGS	[a] Yes - CHI at Crumlin [a] Yes - CHI at Crumlin [a] Yes - SJH/Beaumont /CUH [a] Yes – SJH/Beaumont /CUH
Intermediate Frequency NTRK gene fusion tumours			
Thyroid Cancers - unresectable or metastatic/advanced patients	At diagnosis	Paediatric- RNA based NGS Adult- in absence of panel testing in thyroid cancer screening with IHC and subsequent confirmation with RNA based NGS where TRK expression is detected by IHC or results are inconclusive	Yes - CHI at Crumlin (Paediatric) [a]Adults – yes at SJH/Beaumont/CUH on request
Paediatric glioma (low and high grade)	At diagnosis	NTRK testing using RNA based NGS is recommended	[a] Yes – outsourced to GOSH SJH currently provide NTRK service when requested [a] Expected Beaumont Q2 2023
Spitzoid neoplasms	At diagnosis	NTRK testing using RNA based NGS is recommended	[a]Yes can be included going forward at CHI at Crumlin (Paediatric) [a] [b]Include on existing melanoma panels
Low frequency NTRK gene fusion (<5%) with limited treatment options where knowledge of NTRK status is of clinical benefit / where disease progression or relapse occurs			
Gastrointestinal stromal tumours (GISTs)- where SACT is indicated	At diagnosis	Include NTRK testing on the standard of care GIST panel. Testing using IHC is not recommended due to lack of specificity	[a]Include opportunistically on existing panel
Adult Gliomas (high grade)	At diagnosis .	RNA based detection test IHC not recommended for screening	[a]Yes – outsourced to GOSH [a]SJH currently provide NTRK service when requested CUH Expected Beaumont Q2 2023
Soft tissue Sarcoma	At diagnosis	RNA based detection test IHC not recommended due to reduced specificity and sensitivity for IHC analysis	[a]Yes - CHI at Crumlin (Paediatric) [a]Adult - Include opportunistically on existing panel
Low frequency NTRK gene fusion (<5%)			
Advanced Lung, Colorectal Cancer and Melanoma[b]	At diagnosis	Consideration should be given to the inclusion of NTRK on the existing lung, colorectal and melanoma NGS panels	Include opportunistically on existing panel
Other solid tumours • No previous NGS panel testing	Serial testing after first	IHC screening (except where IHC not indicated due to	[a]Yes can be included going forward at CHI at Crumlin (Paediatric) [a]

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	testing for other more common clinically actionable oncogenic drivers and treatment options remaining for the patient.	reduced specificity and sensitivity) and subsequent confirmation with RNA based NGS where TRK expression is detected by IHC or results are inconclusive	
• Previous NGS panel testing and NTRK not reported	Bioinformatics review for NTRK analysis of original panel test if appropriate should be carried out		

[a]Centralised funding not provided

[b]Centralised panel funding in place in SJH/Beaumont

*Based on information available to NCCP on 08/09/2022. It is acknowledged that hospitals may have existing agreements in place for cancer molecular diagnostics with other external laboratories

6 Eligibility criteria for treatment with NTRK-inhibitors

Patients are eligible for consideration for treatment with an NTRK inhibitor where **all** the following criteria are met;

- Presence of an NTRK gene fusion without a known acquired resistance mutation is identified using a validated test method
- Meets the eligibility criteria of the NCCP national chemotherapy regimen for NTRK inhibitor (s) available here[3]

Applications for reimbursement approval for NTRK gene fusion therapies (i.e. entrectinib or larotrectinib) under the High Tech Arrangement will only be considered from consultant medical oncologists registered with the Irish Medical Council, who have agreed to the terms of this MAP available here and who have been approved by the HSE ('approved consultants').

[3] The list of cancer drugs approved for reimbursement by the HSE are available here

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Approved consultants are responsible for ensuring that the patient or their representative/guardian is aware that the application for reimbursement approval is being made on their behalf.

If a patient is recommended for reimbursement by the MMP, the High Tech prescription should be generated on the High Tech Hub (HTH). The prescribing of NTRK gene fusion therapies for approved patients under the High Tech Arrangement will be confined to the approved consultants and their teams. The governance of the team on the High Tech Hub, including access, rests with the approved consultant

7 References

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4. Solomon et al. Identifying patients with NTRK fusion Cancer. Annals of Oncol 2019;30 (supplement 8): 6-12.
5. Solomon, J. P. & Hechtman, J. F. Detection of NTRK fusions: merits and limitations of current diagnostic platforms. Cancer Res. 2019;79: 3163–3168.

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Appendix 1. Membership of NCCP NTRK Testing Working Group

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Dr Jane Cryan	Consultant Neuropathologist
Dr Mark Doherty	Consultant Medical Oncologist
Dr Brendan Doyle	Consultant Histopathologist , Faculty of Pathology Representative
Professor Stephen Finn	Consultant Histopathologist
Professor Maeve Lowery	Consultant Medical Oncologist
Professor Patrick Morris	Consultant Medical Oncologist
Professor Maureen O'Sullivan	Consultant Paediatric Pathologist

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