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FIND EVERY PATIENT

Establishing a consensus on the testing of *NTRK* gene fusions via the NHS Genomic Medicine Service and Genomic Laboratory Hubs

Agreed aims/objectives

- Clarify the process of *NTRK* testing, from testing request to delivery of results, via the Genomic Laboratory Hubs (GLHs)
- Identify and discuss the unmet needs associated with *NTRK* testing in England
- Provide expert guidance on:
  - How the testing process can be streamlined
  - Which patients should be eligible for *NTRK* testing
  - When in the treatment pathway *NTRK* testing should be conducted for different tumour types

Introduction

NTRK gene fusions are drivers of oncogenesis found with varying frequency across multiple tumour types.[1,2] High frequencies of *NTRK* gene fusions (≥75%) are predominantly found in rare adult and paediatric tumour types. Examples of these tumours include secretory carcinoma of the breast, mammary analogue secretory carcinoma of salivary glands (MASC), congenital mesoblastic nephroma (CMN) and infantile fibrosarcoma (IFS),[3] in which the *ETV6-NTRK3* fusion is considered pathognomonic.[4] Conversely, common tumour types such as non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) display *NTRK* fusions at low frequencies of <5%.[3] *NTRK* gene fusions typically occur when the 5´ region of an unrelated fusion partner gene joins the 3´ region of an *NTRK1, NTRK2* or *NTRK3* gene.[5] NTRK gene fusions are typically mutually exclusive of other driver mutations or rearrangements.[6-10]

The first precision medicine, imatinib for Philadelphia chromosome positive chronic myeloid leukaemia, was approved for use 20 years ago, in 2001.[11-13] Since then, precision medicine has revolutionised the way in which cancer is treated and has resulted in molecular and genetic testing becoming an essential step in the treatment pathway of many tumour types.[12] Vitrakvi® ▼ (larotrectinib) and Rozlytrek® ▼ (entrectinib), tyrosine kinase inhibitors against tropomyosin receptor kinase (TRK) A, B and C, represent a new wave in precision medicine as they are among the first tumour-agnostic targeted treatments. Unlike most previously developed precision-medicine agents aimed at specific disease/histological subtypes, TRK inhibitors are approved based on the presence of specific genetic aberrations, irrespective of tumour type.[4,14,15]

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Declaration of interests

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Larotrectinib is a TRK-specific inhibitor whereas entrectinib has additional activity against c-ros oncogene 1 (ROS1) and anaplastic lymphoma kinase (ALK).[4] The efficacy and safety of larotrectinib and entrectinib in *NTRK* fusion-positive solid tumours has been demonstrated in pooled analyses of phase I/II single arm clinical trials.[16,17] Larotrectinib and entrectinib were granted conditional marketing authorisation in Europe in July 2019 and May 2020, respectively.[18,19] Subsequently, both treatments were recommended for reimbursement in the UK as part of the Cancer Drugs Fund, as follows:

- Larotrectinib is recommended for use within the Cancer Drugs Fund as an option for treating *NTRK* fusion-positive solid tumours in adults and children if the disease is locally advanced or metastatic or surgery could cause severe health problems and they have no satisfactory treatment options[20]
- Entrectinib is recommended for use within the Cancer Drugs Fund as an option for *NTRK* fusion-positive solid tumours in adults and children 12 years and older if the disease is locally advanced or metastatic or surgery could cause severe health problems and they have not had an TRK inhibitor before and they have no satisfactory treatment options[21]

Tumour-agnostic therapies and companion diagnostics pose new challenges for healthcare systems that are designed with services set up in an organ of origin/tumour-orientated manner. The use of TRK inhibitors is contingent on the presence of an *NTRK* gene fusion.[20,21] Given that *NTRK* gene fusions are found across tumour types, there is a need to prioritise testing for patients who would most benefit from treatment in order to maximise cost-effectiveness and use limited resources optimally. Additionally, the implementation strategy of biomarker testing may differ across tumour type, depending on what other testing is already in place.[12]

The most appropriate testing method used to identify *NTRK* gene fusions is under debate. There are several different methods available, each with their own benefits and limitations. The main techniques used for *NTRK* fusion detection include immunohistochemistry (IHC), fluorescent in situ hybridisation (FISH), reverse transcription-polymerase chain reaction (RT-PCR), and multiplex testing via RNA-based and DNA-based next-generation sequencing (NGS).[22-24]

IHC relies on the expression of the precise antigen detected by the immunohistochemical antibody.[25] It is also dependent on tissue fixation and processing techniques, which if not optimised and carefully controlled may adversely affect reactivity of these proteins and lead to a false negative result.[25] IHC has demonstrated high sensitivity and specificity for *NTRK* gene fusions.[26,22] In terms of predicting drug response, IHC has the benefit of directly detecting the protein target of the drug therapy. IHC using a pan-TRK immunohistochemical antibody is therefore a useful screening tool for *NTRK* fusions which can be easily performed in conjunction with other diagnostic IHC tests on a sample, but due to the risk of false negative results should not be used to rule patients out of FISH or NGS-based testing.[27,23]

Similarly to IHC, FISH has proven high sensitivity and high specificity for *NTRK* gene fusions.[22] FISH is an established method for the identification of the *ETV6-NTRK3* fusion in tumours with high *NTRK* gene fusion frequency; for example, the aforementioned secretory carcinoma of the breast, MASC, CMN and IFS.[22,23] However, although FISH has high specificity for *NTRK* gene fusions and can discriminate rearrangements from polysomy and amplification, it cannot determine that a functional fusion gene has been created. This can lead to false positives and tumours that do not respond to treatment.[23] Additionally, FISH is unable to detect the expression of TRK proteins or the partner gene. FISH also requires a separate probe per fusion gene making in unsuitable for screening purposes.[22]

RT-PCR is also an effective method of identifying the *ETV6-NTRK3* fusion in tumours with high *NTRK* gene fusion frequency. RT-PCR detects *NTRK* gene fusions using primers in the coding sequence of the 5' fusion partner and the NTRK kinase domain. However, the target sequence must be known for RT-PCR and novel fusion partners are not detected.[22,23]

Multiplex testing via NGS has the benefit of testing multiple biomarkers in one sample. Both DNA- and RNA-based NGS may be used for *NTRK* gene fusion analysis, though RNA-based NGS has notable advantages; while both methods are highly specific, sensitivity is higher with the RNA-based method. RNA-based NGS detects gene fusions and rearrangement events regardless of where the breakpoints are within the two genes. Provided the probes for the target gene are included in the hybridisation-capture NGS method, the partner gene will also be sequenced. Therefore, both genes involved in the gene fusion will be characterised and the breakpoint also identified. Additionally, as only transcriptionally active fusions are detected by RNA-based NGS, there is additional certainty that the identified fusion is oncogenic. DNA-based NGS only detects gene fusions and rearrangement events if the entire gene sequence of the *NTRK1*, *2* and *3* gene likely to be involved at the breakpoint is included as probes. A hybridisation capture NGS method will need to be used to pull through the sequence of the fusion partner gene for its characterisation and the identification of the breakpoint. In regard to practical considerations, the fixation process of samples can affect DNA and RNA quality and thus limit the utility of NGS.[28,22]

Obtaining a tumour biopsy with sufficient tumour content for molecular analysis can be challenging, in particular from deep, inaccessible anatomical locations such as the liver, pancreas and lung. In these tumours, tissue acquisition may require an invasive procedure.[29] Additionally, biomarkers do not always present uniformly in tumour cells and therefore tumour biopsies need to be of sufficient size to be representative.[29]

Lack of awareness of tumour-agnostic treatments and biomarker testing processes may also present a barrier to testing, in particular within common tumours where the likelihood of ever identifying a patient with *NTRK* gene fusion-positive cancer is rare.[12]

Despite the obstacles of tumour-agnostic treatments and precision medicines in general, the benefit to the patient has been recognised by health services globally, and strategies to ensure optimum and equitable accessibility are underway.[30]

In October 2018, following on from the 100,000 Genomes Project, NHS England launched the NHS Genomic Medicine Service (GMS), a national reorganisation of services with the overarching aim of implementing genomic medicine including whole genome sequencing analysis into routine NHS care by 2025.[31,32] The GMS has a single

national laboratory network consisting of 7 genomic laboratory hubs (GLHs) and a stipulated test list within national genomic test directories for cancer (somatic) and rare (germline) disease.[31,32] The key objectives of this systematic approach are to enable quicker and more accurate diagnoses, ensure patients are matched with the most effective treatments and increase the number of patients surviving cancer.[31,32] The national genomic test directory specifies which genomic tests are commissioned by NHS England and the most appropriate test for each indication.[33,32]

Currently, the national test directory includes *NTRK* testing indications for all solid tumour types.[33] However, the full implementation of these tests has been delayed during the COVID-19 pandemic. A phased approach to the NHS implementation of *NTRK* testing in England was initiated in early 2020 to gradually build testing capacity. Guidance on phase 1 of the implementation plan was released in April 2020 detailing eligibility criteria and which of the 7 GLHs would be offering the testing.[34] Patients eligible for testing in phase 1 included those with tumours with very high *NTRK* gene fusion incidence (>90%; secretory carcinoma of the breast, MASC, CMN and IFS), those with tumours with an *NTRK* gene fusion incidence between 5% and 25% (gastro-intestinal stromal tumours [GISTs], thyroid cancers and spitzoid melanocytic neoplasms), and patients ≤25 years of age with solid tumours.[34] Testing was assigned to the following 4 GLHs: North East and Yorkshire; North West; Central and South; and North Thames. Central and South GLH was also assigned referrals from the South West GLH, and the North Thames GLH was assigned referrals from both South East GLH and East GLH.[34] In November 2020, phase 2 of the implementation plan was initiated. In phase 2, patients eligible for *NTRK* testing included all those specified in phase 1 and additionally patients with metastatic or locally-advanced solid tumours, or solid tumours where surgical resection is likely to result in severe morbidity, for whom standard therapies have failed or are unavailable and the patient is fit for further treatment. The South West GLH became a testing laboratory in phase 2 and so no longer refers samples to the Central and South GLH.[34]

Roundtable consensus meeting

Given the novelty of the tumour-agnostic treatments larotrectinib and entrectinib, the ongoing reorganisation of NHS genomic testing services and the added complexity of the COVID-19 pandemic, there is a need to clarify the NTRK testing process in England. Therefore, 9 UK-based oncology, pathology and clinical scientist experts involved in the GMS and GLHs joined a virtual roundtable meeting in July 2020 to establish a consensus on the process of *NTRK* testing. The meeting and subsequent consensus document were sponsored by Bayer UK. Information in this section is based on the experience and expertise of the scientists involved in this roundtable meeting.

The following article consolidates the key discussions of the meeting and provides:

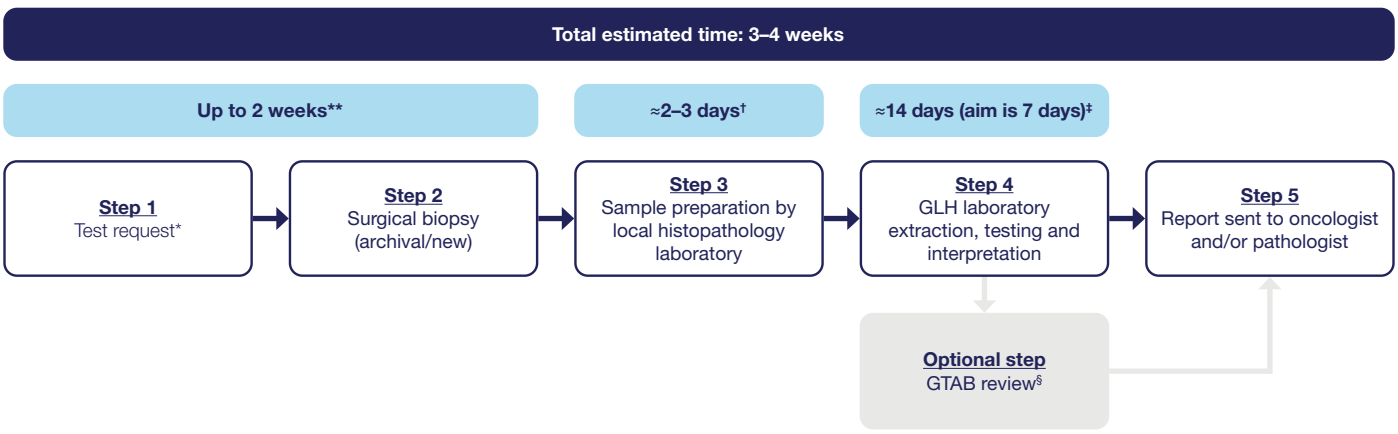
1. Clarity on the *NTRK* testing process
2. Recommendations on how to streamline the *NTRK* testing process
3. Recommendations on how to prioritise tumour types for *NTRK* testing
4. Recommendations on how to integrate *NTRK* testing into current algorithms

Discussion and recommendations

1. Clarity on the NTRK testing process

A general overview of the *NTRK* testing process is outlined in **Figure 1**. The initial steps of requesting a test and obtaining a tissue sample may take up to 2 weeks, although this is dependent on the tumour type. Testing may be ad hoc at oncologist request, a reflex test requested by the pathologist, or part of the diagnostic work-up. Testing may also be carried out on stored samples following tumour progression or exhaustion of other treatment options. Reflex testing is the most efficient process. Samples are prepared by the referring histopathology laboratory as slide mounted unstained formalin-fixed paraffin-embedded (FFPE) tissue sections, accompanied by a

Figure 1. Overview of NTRK testing via GLHs



**As ad hoc,* reflex testing, as part of diagnostic work-up request or a testing request of stored samples following tumour progression or exhaustion of other treatment options; **Time dependent on tumour type, reflex testing requests are the most efficient; †May take longer in practice; ‡Dependent on number of testing runs; §Ambiguous cases only.
Abbreviations: GLH, genomic laboratory hub; GTAB, genomic tumour advisory board.

haematoxylin and eosin (H&E)-stained section with the area of greatest tumour cellularity outlined and any areas of necrosis excluded. A copy of the test request form and pathology report containing information on the tissue type, histological diagnosis and tumour (percentage neoplastic nuclei) content of the representative tumour block should also be provided. If tumour content assessment or tissue preparation optimised for subsequent molecular analysis is not available in the referring histopathology department, then it may be preferable for paraffin blocks or tissue sections to be sent to another referral histopathology centre for sample preparation before being forwarded to the GLH laboratory. Alternatively, samples may be sent directly to the GLH laboratory for preparation, depending on local processes established within the GLH. Sample preparation (Figure 1, step 3) is estimated to take 2–3 days, but in practice may take much longer. The extraction and testing of samples by NGS and interpretation of results take place in the GLH laboratory and take on average 2 weeks depending on the number and frequency of NGS runs. The aim of GLHs is to reduce the duration of step 4 to 7 days.

Results are then sent preferably electronically to the requesting oncologist and/or pathologist at the referring centre. Results may be reviewed at a regional genomic tumour advisory board if further clinical interpretation or integration with other genomic analysis is advised, although this rarely occurs. The total estimated time for the process, from steps 1 to 5, is 3–4 weeks.

Prepared samples sent to the GLH laboratory may be unsuitable for NGS due to low tumour quantity and/or sample quality. Samples may be screened by histopathologists working in conjunction with the GLH and redirected for alternative testing. A potential GLH salvage pathway for any samples insufficient for NGS analysis is outlined in **Figure 2**. In the GLH salvage pathway, samples are tested for *NTRK* fusions with IHC and confirmatory FISH in place of NGS (steps 4A and 4B).

2. Recommendations on how to streamline the NTRK testing process

The current *NTRK* testing process is estimated to take 3–4 weeks, although delays can occur at any stage of the pathway. Recommendations to streamline the process are described below.

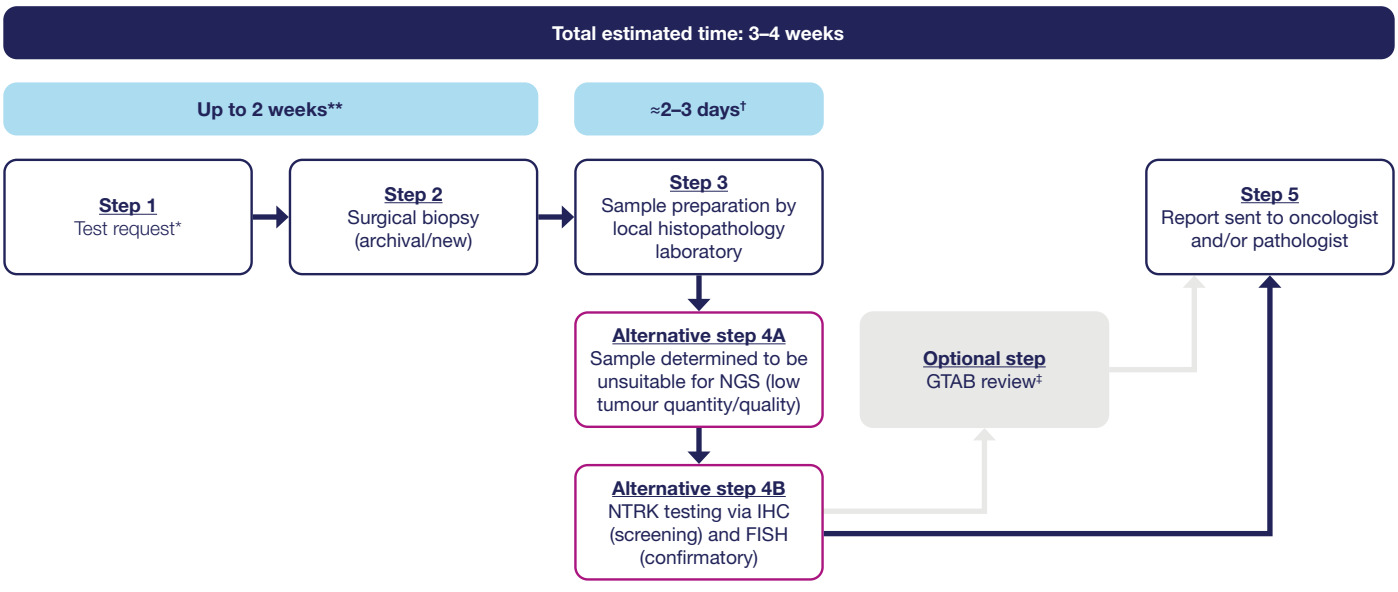
Samples must be prepared by local histopathology laboratories as cut FFPE tissue sections with marked-up H&E before sending to the GLH laboratory. GLH laboratories are genetic laboratories and the majority do not have on-site access to histopathology services. As a result, unprocessed paraffin blocks sent to a GLH laboratory will incur a delay. The authors recommend that protocols for the preparation of samples are clearly stipulated and adopted by local histopathology laboratories.

The increasing demand on histopathology departments for tissue processing requires additional resource. The authors recommend increased dialogue between relevant stakeholders and funders, including NHS England, NHS Improvement, the Royal College of Pathologists and regional cancer alliances, to resolve this issue.

A closer working relationship between histopathology departments and GLH laboratories with clear communication and understanding of each other's roles will be essential for optimal delivery of the GMS and personalised medicine for patient care. Optimised preparation of tissue for molecular analysis will increasingly be required. The authors recommend sample preparation requirements are communicated efficiently and effectively to histopathology laboratories. The authors also recommend collaboration between histopathology and GLH laboratories to optimise protocols, share best practice and identify pathology-related reasons for false positives and sample fails.

During previous biomarker testing implementations by the NHS, self-nominated Local Champions, usually an oncologist, would assume

Figure 2. Overview of salvage pathway for NTRK testing via GLHs



As ad hoc, reflex testing, as part of diagnostic work-up request or a testing request of stored samples following tumour progression or exhaustion of other treatment options;
**Time dependent on tumour type; reflex testing requests are the most efficient; †May take longer in practice; ‡Ambiguous cases only.
Abbreviations: GLH, genomic laboratory hub; GTAB, genomic tumour advisory board; IHC, immunohistochemistry; FISH, fluorescence in situ hybridisation; NGS, next-generation sequencing.

responsibility to oversee adoption and implementation of a new precision medicine biomarker. Similarly, during the 100,000 Genomes Project, Genomic Champions were nominated. The purpose of the Champion was to communicate information regarding the testing process and requirements to their peers within the tumour-specific multidisciplinary team and be a 'point of contact' for the wider clinical network. For a disease agnostic biomarker, the identification of a Local Champion is more challenging because multiple cancer types are involved and oncologists are highly subspecialised. However, a champion for *NTRK*-fusion positive cancer may be useful to help disseminate important information on eligibility, sample preparation requirements and testing processes and to tighten communication between disease-specific testing pathways and the GLH. The authors recommend identification of suitable clinical lead(s) to act as a point of contact for the wider clinical network regarding *NTRK* testing.

Communication between oncologists, local histopathology laboratories and GLH laboratories is essential to ensure the testing process runs smoothly. Currently, the GMS is evolving and NGS schedules regularly change. However, once schedules become stable and fixed, the authors recommend that the GLH laboratories notify histopathology laboratories and hospitals of testing schedules. This may help to coordinate preparation and sending of samples to the GLH laboratory and to ensure samples are included on the next available testing run.

The demand for *NTRK* testing will increase as the implementation plan progresses and an increase in workforce will be needed to meet this demand. The authors identified clinical scientists as a specific group in which resource is lacking. Clinical scientists perform the interpretation and analysis of NGS sequence data to issue the clinical report. The authors highlight the inclusion of the clinical scientists in the multidisciplinary team. The GMS can play a role in raising awareness of the crucial role of the clinical scientist in genomic testing. Additionally, the authors recommend upskilling other roles within the GMS, such as genetic technologists, to assist with the analysis and interpretation of NGS data prior to final approval by the clinical scientist. It was also suggested that clinical scientists would be a valuable participant of multidisciplinary team meetings. However, there was concern around this due to the relative shortage of clinical scientists in the UK.

NTRK gene fusions are rare but found across tumour types.[1,2] Ensuring awareness of *NTRK* fusions, available TRK inhibitors and *NTRK* testing amongst the relevant healthcare professionals and scientists across tumour types is a key challenge. The authors recommend increased education around *NTRK* fusions, the testing process and sample requirements. The authors also recommend that a prospective audit is undertaken to assess for any geographic variations in testing patterns that might arise and require to be addressed by ongoing education.

3. Recommendations on how to prioritise tumour types for NTRK testing

Patients eligible for treatment with a TRK inhibitor include adults and children, in the case of larotrectinib, or adults and children over 12 years of age, in the case of entrectinib, with locally advanced or metastatic solid tumours, or tumours in which surgery could cause severe health problems, that have no satisfactory treatment options and, in the case of entrectinib, have not received a prior *NTRK* inhibitor.[20,21] During phase 2 of the implementation plan, patients eligible for *NTRK* testing include patients with metastatic or locally-advanced solid tumours, or solid tumours where surgical resection is likely to result in severe morbidity, for whom standard therapies have failed or are unavailable and the patient is fit for further treatment (as described above).[34] Given the large number of patients now potentially eligible for treatment, it is essential to devise a plan that prioritises patients for testing according to need and potential benefit.

The co-occurrence of gene fusions with driver mutations is extremely rare, although occasionally reported.[6-10] For example, a retrospective US study using data from 15,971 samples from the Flatiron Health-Foundation Medicine Clinico-Genomic Database found a total of 29 patients with *NTRK* fusion-positive cancer. Of these patients, none had a co-occurrence with ALK rearrangement, *ERBB2* amplification or *ROS1* alteration. One patient had a co-occurrence with a *BRAF* alteration, one with an *EGFR* alteration and 3 with a *KRAS* alteration. 18% (N=17) of patients with *NTRK* gene fusions had a microsatellite instability-high status.(9) A second US study by Foundation Medicine, of over 300 cancer-related genes on 166,067 tumours from 75 solid tumour types, found that *NTRK* and *ROS1* fusions generally did not occur with other clinically actionable or oncogenic genetic alterations. No enrichment or exclusivity was seen with *EGFR, ERBB2, RET, ALK* or *MET*. However, there was significant co-occurrence of *NTRK* fusions with alterations in 15 genes, including *IGF1R, CDKN2B* and *CDK4*.[10] Another analysis of 2,314 advanced CRC samples that identified a total of 21 fusions. Eight of the 21 fusions were *NTRK* fusions and occurred in *BRAF/RAS* wildtype CRC samples.[8] A separate analysis of 11 patients with NSCLC and *NTRK* gene fusions found no co-occurrence with alterations in *KRAS, EGFR, ALK, ROS1* or other known oncogenic drivers.[7] Another study compared characteristics of 27 patients with metastatic CRC and *ALK, ROS1* and *NTRK* rearrangements with a cohort of 319 patients without rearrangements. The frequency of gene fusion and *BRAF* codon600 mutation co-occurrence was low; however, one patient showed a co-occurrence of *SLC34A2-ROS1* fusion and a *BRAF*V600E mutation.[6] Finally, a study of 186 GISTs identified an *NTRK* gene fusion (*ETV6-NTRK3*) in a quadruple wild-type GIST sample.[35] Taking current data into account, the authors recommend prioritising patients without other known driver mutations for *NTRK* testing. This recommendation is in line with international consensus,[5] although it is acknowledged that the data are relatively immature given the rarity of *NTRK* gene fusion. Therefore, more information on the co-occurrence of fusions with other mutations would be valuable to collect in order to refine the testing strategy. Further characterisation of *NTRK* gene fusions, in terms of tissue-specific mechanisms and prognosis, is also recommended by NICE.[20]

4. Recommendations on how to integrate NTRK testing into current algorithms

There is no national guidance for when *NTRK* testing should occur in the treatment pathways of individual tumour types. Different genetic testing approaches and processes across tumour types add to the complexity of implementing *NTRK* testing. The authors recommend *NTRK* testing should be incorporated into existing molecular testing pathways, where possible.

The authors recommend developing separate testing algorithms for the following groups of tumours:

- Tumours that currently receive RNA-based NGS testing

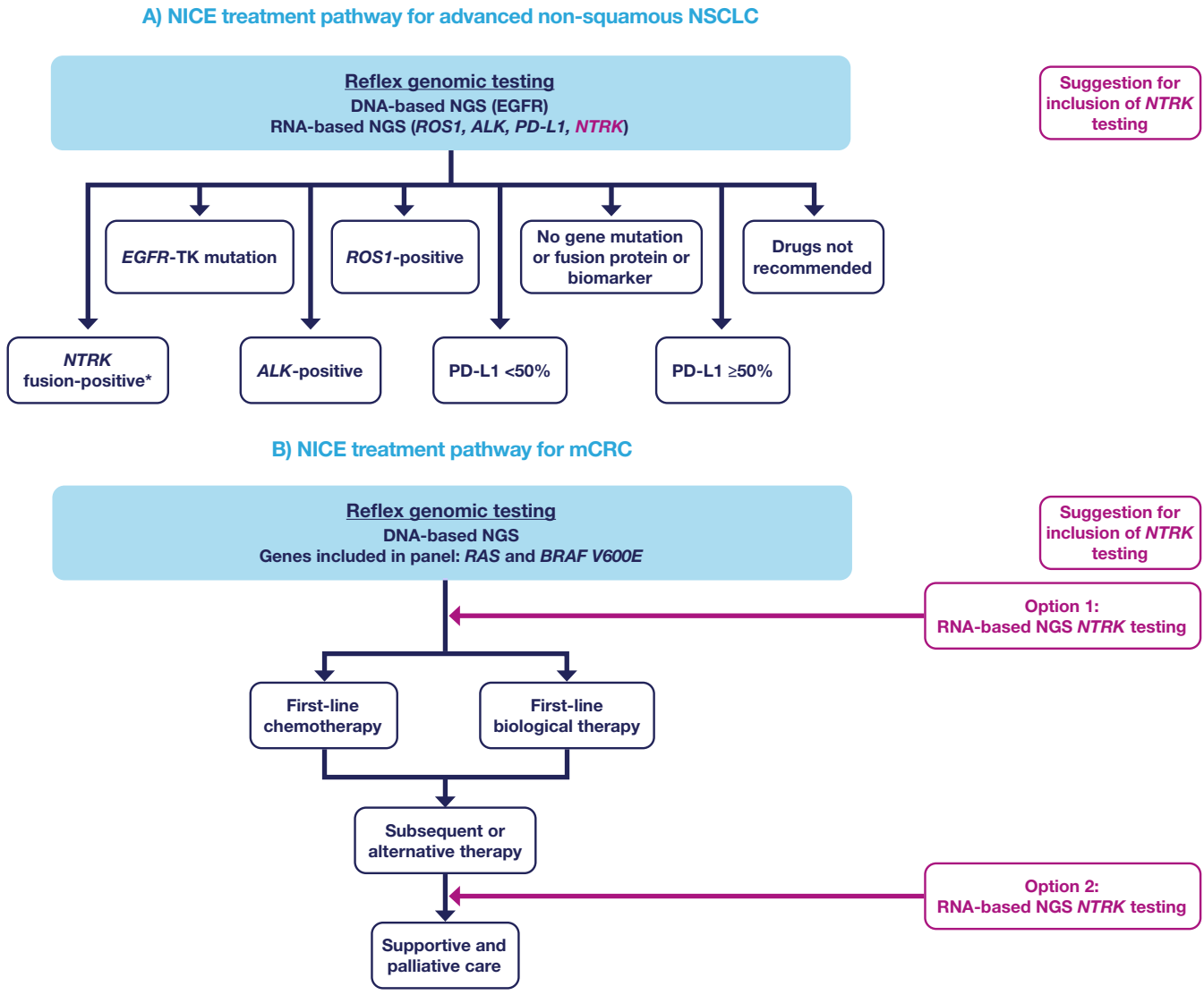
- Tumours that currently do not receive RNA-based NGS testing
- Tumours for which *NTRK* gene fusions are diagnostic

Examples of tumours in which RNA-based NGS testing is evolving include NSCLC and central nervous system tumours.[36,12] In these tumour types, the authors recommend *NTRK* testing be included as part of the RNA-based NGS panel (**Figure 3A**). The rationale behind this is that for the majority of RNA-based NGS panels, *NTRK1, 2* and *3* are already included, so their inclusion in the sequencing analysis occurs no additional cost or sample tissue; it is effectively a free test. The only additional work is the recording of the *NTRK* gene fusion result, should a fusion be detected, in the patient's record for the time when treatment with a TRK inhibitor is suitable.

In tumours such as CRC, testing currently uses DNA-based rather than RNA-based techniques in view of the requirement to detect point mutations to guide therapy.[12] DNA-based NGS panels do not generally include probes to detect *NTRK1, 2* and *3* gene fusions. One potential solution is to include an RNA-based NGS panel in the upfront testing; however, given the high prevalence of CRC this would involve a considerable increase in cost and workload. Additionally, patients identified with *NTRK*-positive cancer would not be eligible for treatment with a TRK inhibitor until later in the pathway.[20,21] Alternatively, *NTRK* testing may be carried out later in the pathway for patients who would be eligible for TRK inhibitor treatment and without other known driver mutations, and this is a recommended approach until phase 3 *NTRK* testing is established (**Figure 3B**). The authors recommend that the timing of *NTRK* fusion testing in tumours without existing RNA-based NGS testing should be based on the frequency of *NTRK* fusions expected and on economic modelling.

The presence of *NTRK* gene fusions is diagnostic for certain rare tumours such as MASC.[37] The authors recommend *NTRK* testing to be included as part of the diagnostic work-up for this group. The type of testing platform most suitable for this group is not clear; however, IHC is commonly used as part of the diagnostic work-up for

Figure 3. Examples of where NTRK testing may sit in the treatment pathways of tumours A) with reflex RNA-based NGS testing and B) without reflex RNA-based NGS testing



*According to NICE guidance, the point at which to use TRK inhibitors in solid tumour treatment pathways is uncertain.
Abbreviations: mCRC, metastatic colorectal cancer; NICE, National Institute for Health and Care Excellence; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer.

characterising morphologically unusual tumours and is likely to have utility as a screening tool in this situation. As NGS becomes more widely used, it may be used for rare tumours in place of IHC.

Summary

The availability of TRK inhibitors and the implementation of *NTRK* testing mark a new wave in precision medicine. In the UK, the organisation of genomic testing across seven regional GLHs provides a platform to progress adoption of *NTRK* into routine diagnostic and care pathways across multiple cancer types. The GLH network creates a forum to standardise and harmonise high quality testing nationally. This article highlights where clarity is needed to fully embed *NTRK* testing and reinforces the need to consider the process according to cancer type and patient pathway to realise the full potential of disease agnostic precision medicines.

Summary of recommendations:

Clarity on the *NTRK* testing process

- **1.1** A general overview of the *NTRK* testing process is outlined in Figure 1
- **1.2** A potential salvage pathway for unsuitable samples is outlined in Figure 2

Recommendations on how to streamline the *NTRK* testing process

- **2.1** Ensure protocols for the preparation of samples are adopted by local histopathology laboratories
- **2.2** Ensure sample preparation requirements are communicated regularly by the GLHs to histopathology laboratories
- **2.3** Collaboration between histopathology and GLH laboratories to optimise protocols, share best practice and identify pathology-related reasons for false positives and sample fails
- **2.3** Consider identification of suitable clinical lead(s) to act as a point of contact for the wider clinical network regarding *NTRK* testing
- **2.4** Maintain coordination across the GLHs to ensure a standardised and equitable approach to *NTRK* testing
- **2.5** Consider upskilling other roles within the GMS, such as genetic technologists, to assist with the analysis and interpretation of NGS data prior to final approval by the clinical scientist
- **2.6** Increase education on *NTRK* fusions, the testing process and sample requirements for healthcare professionals across tumour types and the clinical teams involved
- **2.7** Consider undertaking a prospective audit to assess for any geographic variations in testing patterns that might arise and require to be addressed by ongoing education

Recommendations on how to prioritise tumour types for *NTRK* testing

- **3.1** Prioritise patients according to the TRK inhibitor licences and NICE recommendations
- **3.2** Prioritise patients without any other known driver mutations for *NTRK* testing

Summary of recommendations:

Recommendations on how to integrate *NTRK* testing into current algorithms

- 4.1 Develop separate testing algorithms for the following groups of tumours: tumours that currently receive RNA-based NGS testing; tumours that currently do not receive RNA-based NGS testing; and tumours for which *NTRK* gene fusions are diagnostic
  - **4.1i** Include *NTRK* testing in existing RNA-based NGS panels in tumours for which they already exist
  - **4.1ii** Base the timing of *NTRK* testing on the frequency of *NTRK* fusions and economic modelling in tumours for which RNA-based NGS testing does not occur
  - **4.1iii** Include *NTRK* testing as part of the diagnostic work-up for tumours in which *NTRK* gene fusions are diagnostic

Tumour-agnostic treatments and companion diagnostics present unique challenges to healthcare systems. However, efforts to achieve a streamlined testing process and equitable service are essential to ensure optimum care of patients. In addition, the lessons learned and the progress made with TRK inhibitors will be a paradigm to aid implementation of future tumour-agnostic treatments, moving personalised treatment of cancer forward another significant step.

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