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NTRK Gene Fusion Testing Clinical Guidance

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Guideline version	Date	Summary of amendments
V0.1	11/07/20	Addition of pharmacy sections
V0.2	01/09/20	Edits to NTRK testing pathway sections
V0.3	25/09/20	Edits to histopathological sample preparation and interpreting NTRK results sections
V0.4	01/09/20	Edits to phased implementation section
V1.0	01/07/22	Dummy reports added to appendix 5
V2.0	01/07/22	Update to test request process

Contents

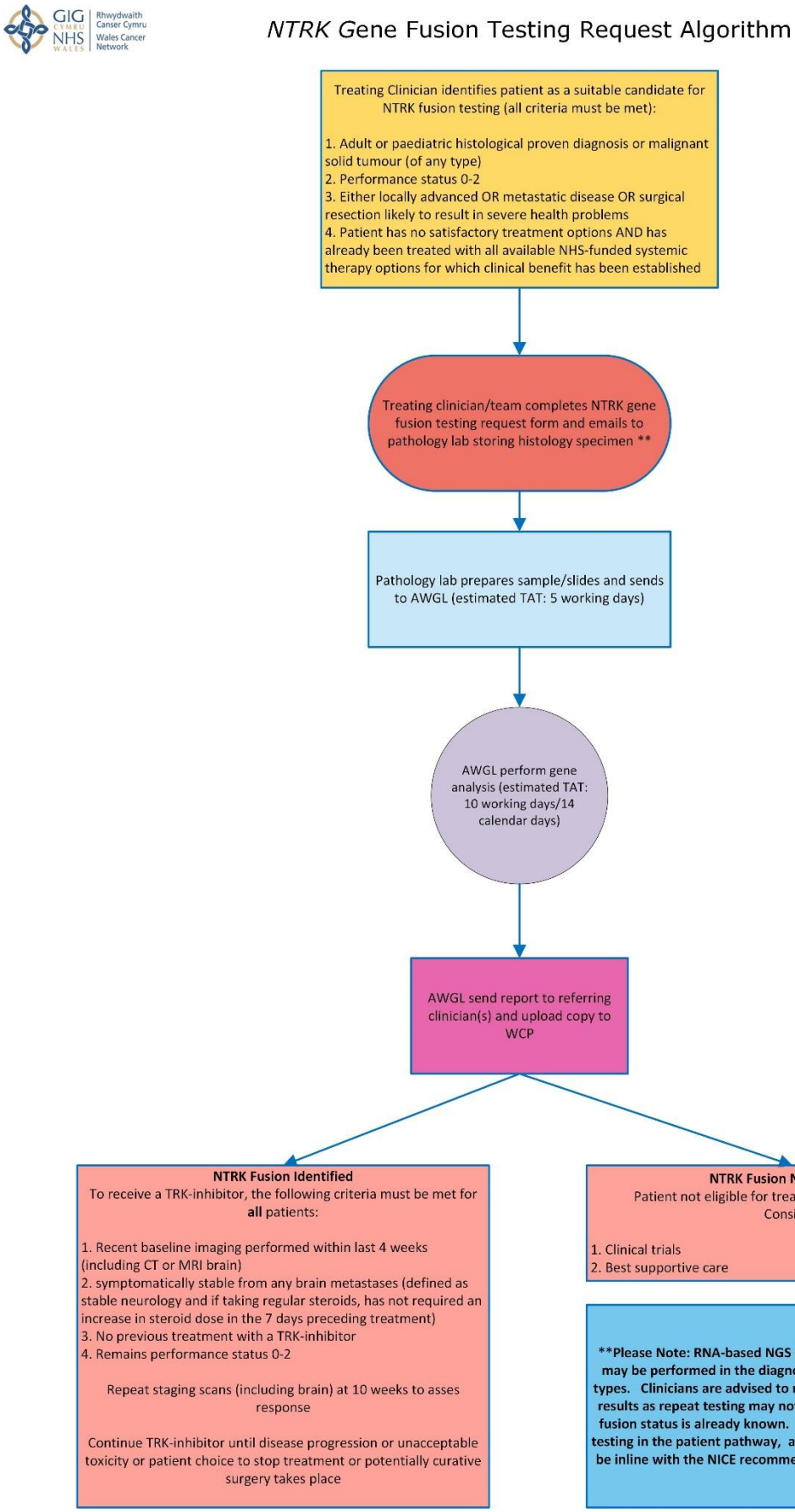
Purpose and Summary of Document

The aim of this document is to provide clinical staff with guidance on the neurotrophic tyrosine receptor kinase (NTRK) gene fusion testing pathway.

The guidance is relevant to all staff involved with the management of adult and paediatric patients (with a diagnosis of any solid tumour) who are eligible to have their tumour tested for this genetic variant.

For those patients whose tumour is subsequently identified to have an NTRK gene fusion and are eligible to receive tropomyosin receptor kinase (TRK)- inhibitors, this guideline summarises the prescribing information and recommended baseline investigations and on-treatment monitoring requirements for these therapies.

NTRK gene fusion testing request algorithm



Background

It is recognised that malignant tumours can arise due to changes within the DNA of cells. 'Fusion genes' are a particular type of genetic alteration in which two unrelated, separate genes join together to form a new hybrid gene with abnormal cellular functions.

The neurotrophic tyrosine kinase (NTRK) gene family is responsible for the normal development and function of both the central and peripheral nervous system (Amatu et al., 2019). The genes NTRK1, NTRK2 and NTRK3, encode for the tropomyosin receptor kinase (TRK) proteins TRKA, TRKB, and TRKC, respectively. These proteins regulate the proliferation, growth and survival of neurons when specific ligands bind to docking sites on their surface. The fusion of the 3' region of an NTRK gene with a 5' region of its fusion partner can cause the TRK fusion protein to become expressed and activated, even in the absence of ligand binding; over 80 different partner genes have been identified to date. These fusion proteins can drive the growth of a tumour via unregulated cell proliferation and enhanced cell survival via the TRK pathway.

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. They occur very frequently in some rare cancers, for example cases of infantile sarcoma, mammary analogue secretory carcinoma and secretory breast carcinoma, are reported to have a prevalence of >90%, with the ETV6-NTRK3 fusion occurring most frequently in this group (Vaishnavi et al., 2015; Chen & Chi, 2018). Conversely, they are less frequently detected in more common tumour types such as lung or colorectal cancer (see appendix 1; NICE, 2020 a). The rarity of NTRK fusions, means that we currently do not have a complete understanding of their role in the formation of cancer, which particular fusion types or tumour types are more likely to respond to treatment with inhibition of the TRK pathway, or what the impact of a particular gene fusion may have on prognosis (NICE, 2020[b]).

The TRK-inhibitors, larotrectinib and entrectinib, are available as treatment options for adult and paediatric patients with NTRK fusion-positive solid tumours via the Cancer Drugs Fund. These drugs are classed as histology-independent or tumour-agnostic therapies as they target this specific genetic abnormality, regardless of where the cancer originally started within the body. Appendix 2 summaries the current clinical evidence for the efficacy of these drugs from the NICE final appraisal documents (NICE, 2020[b, c]).

There are several techniques available to detect NTRK gene fusions including immunohistochemistry (IHC), fluorescence in-situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR) and next generation sequencing (NGS). NICE recommends the use of nuclei-based assays for NTRK gene fusion testing which must be organised and validated by a recognised genomic laboratory (NICE, 2020[a]).

An initial screening IHC pan-TRK assay followed by a confirmatory FISH or NGS test for suspected cases is not supported given the impact on capacity for IHC in histopathology laboratories associated with this approach and in light of the transition of expanded cancer profiling to genomic laboratories (NICE, 2020[d]). In addition, the advantage of implementing an RNA-based NGS testing service is the ability to interrogate all clinically actionable genomic variants, and it is a tissuesparing approach for broad genomic analysis (Hsiao et al., 2019). This is a particularly important consideration given that the number of genetic markers required to guide treatment decisions for many tumour types is increasing and the NHS is committed to implementing genomic testing for cancer patients at the point of diagnosis (NHS, 2019). Furthermore, RNA-based NGS testing is able to determine the fusion partner gene, (which is likely to become increasingly clinically relevant as evidence emerges as to the prognostic importance of NTRK gene fusions and characterisation by tumour site) as well as being able to identify expressed fusion proteins, (which FISH cannot inform on) and, very importantly, can detect any secondary mutations (with implications for drug response and resistance).

Current status of NTRK gene fusion testing service (Sept 2022)

NTRK gene fusion testing is provided as a Welsh Health Specialised Services Committee (WHSSC) commissioned service for all patients in Wales, provided by the All Wales Medical Genomics Service (AWMGS; https://medicalgenomicswales.co.uk/). Testing was initially introduced in June 2020 using FISH. The validation and implementation of an RNA-based NGS panel in October 2020 provided an increased capacity at AWMGS, which allowed the service to be expanded to deliver testing to a broader cohort of patients using a phased implementation approach for RNA NGS.

AWMGS is now able to offer RNA-based NGS routinely as the first-line testing strategy for NTRK gene fusion testing, regardless of tumour type. FISH analysis is still available for any patient sample that is unsuitable for RNA-based NGS (estimated at 39% of samples) as part of the FISH salvage pathway within the laboratory. Notably, an ongoing service improvement initiative within AWMGS is focusing on improving the success rate of RNA-based NGS to maximise the number of patients who receive NTRK testing using the NGS panel, to reduce the FISH workload in the laboratory and improve turnaround times.

RNA-based NGS testing is the recommended first-line testing strategy for the detection of NTRK gene fusion patients with any solid tumour and includes:

- Adults
- Young adults 18-25 years
- Teenagers aged 16-18 years
- Children ages 0-16 years

FISH testing will only be initiated if there is insufficient tissue for RNA-based NGS or if RNA NGS fails.

It should be noted that in some tumour types, RNA-based NGS is requested as part of the diagnostic work-up (https://medicalgenomicswales.co.uk/index.php/health-professional-information/cysgodi). As NTRK gene fusions are included as standard within the RNA NGS panel, it will not be necessary for a separate NTRK gene fusion test to be requested.

Clinicians should review previous genomic test results before requesting NTRK gene fusion testing. This is relevant to patients with a diagnosis of thyroid malignancy, glioma and non-small cell lung cancer (NSCLC).

Whilst the NTRK gene fusion status will be available at an earlier stage in the treatment pathway for such patients, those individuals with a NTRK gene fusion will still only be eligible to receive a TRK-inhibitor when there are no satisfactory treatment options available to them (see 'Eligibility criteria for NTRK gene fusion testing section').

Eligibility Criteria for NTRK gene fusion testing

Patients are eligible to have their diagnostic histological specimen screened for NTRK gene fusions if all of the following criteria are met:

1. Adult or paediatric* histological proven diagnosis of solid tumour (of any type^)
2. Performance status 0-2
3. Either locally advanced OR metastatic disease OR surgical resection likely to result in severe health problems
4. Patient has no satisfactory treatment options AND has already been treated with all available NHS-funded systemic therapy options for which a clinical benefit has been established.

[*Entrectinib is recommended in children 12 years and older; larotrectinib does not have any age restrictions]

[^This does not include myeloma, leukaemia or lymphoma]

The purpose of NTRK gene fusion testing is to identify patients who may benefit from treatment with TRK-inhibitors. NICE has recognised that the term 'no satisfactory treatment options' may be open to interpretation. **The NICE final appraisal documents state that both larotrectinib and entrectinib are positioned as a last-line treatment option where the alternative is best supportive care.** This is because clinical benefit has only been established in single-arm trials in a relatively small sample of patients and the effect of treatment with TRK-inhibitors may differ depending on tumour type and other possible gene alterations. Entrectinib is yet to receive its marketing authorisation; as such, the indications for treatment mirror those of larotrectinib.

It is the responsibility of the treating clinician to ensure the above criteria are met and that TRK-inhibitors must not displace any effective therapies. It is recommended that clinical groups within each of the cancer centres review and update their systemic anticancer treatment algorithms to clearly identify when treatment with TRK-inhibitors is indicated within the standard treatment pathway.

The patient is not required to sign a consent form to proceed with NTRK gene fusion testing. However, the treating clinician should inform the patient as to the rationale for testing, the likelihood of detecting an NTRK gene fusion based on their solid tumour diagnosis and what treatment with TRK-inhibitors entails prior to requesting the test.

NTRK gene fusion testing request process

NTRK-inhibitors are positioned as last-line treatment options for NTRK fusion-positive solid tumours. As such, the majority of patients will already be known to an oncologist and it is anticipated that in tumour types which do not routinely access RNA-based NGS, requests for NTRK gene fusion testing will be made by the treating clinician rather than via diagnostic MDTs.

NTRK gene fusion testing is performed on the diagnostic histological specimen which requires preparation (slide cutting and tumour assessment) by the local pathology laboratory storing the sample prior to them sending it to the All Wales Medical Genomics Laboratory (AWGL) in Cardiff for analysis. **Requests should therefore not be made directly to the AWGL as samples are not stored here and histopathology services are unavailable in this laboratory.** Due to DNA and RNA degradation over time, the sample should be less than 5 years old; a re-biopsy may be necessary to acquire fresh tissue if the diagnostic sample is older than this.

There are likely to be local considerations across the various regions of Wales in terms of the test requesting pathway. However, all requests should be made using the appropriate AWGL request form which is available at:

https://medicalgenomicswales.co.uk/index.php/download-services

Select the 'Oncology' filter on the 'Specialty' drop down menu and download the 'NTRK (RNA)' form. The requestor should:

1. Complete the patient demographic information section
2. Complete the requestor details and email addresses section
3. Indicate the primary tumour type
4. Select the 'RNA based NGS (replaces FISH testing for ALK and ROS1, and covers NTRK 1/2/3)' option.

In order to reduce turnaround times, it is recommended that the form is then emailed to the local pathology laboratory storing the diagnostic specimen which is to be tested. The majority of laboratories now have generic email addresses, the accounts for which are checked on a daily basis (see table 1). If a generic address is not available, the request should be sent to a named individual at the local pathology laboratory who knows to expect the request and initiate the required sample preparation thus avoiding unnecessary delays.

University Health Board	Generic email address(es)
Aneurin Bevan	Hist.ReferralRGWOLD.ABB@wales.nhs.uk
Betsi Cadwaladr	BCU.CellPathMolecular@wales.nhs.uk
Cwm Taf Morgannwg	Generic email address not yet available. Please email request forms to ALL of the following recipients: Domenica.Lear@wales.nhs.uk John.Bibby@wales.nhs.uk Akinwale.Akinola@wales.nhs.uk Gerrard.Fletcher@wales.nhs.uk Susan.Davies2@wales.nhs.uk Louise.Nash2@wales.nhs.uk
Cardiff and Vale	Mg.Cellpath@wales.nhs.uk
Hywel Dda	WWGH.Histology@wales.nhs.uk (laboratory) HDD.Secretaries@wales.nhs.uk (secretaries)
Swansea Bay	Generic email address not yet available. Please contact the appropriate laboratory directly to request an email address to which the request can be sent.

Table 1: Generic email address details for health boards

Please note: It is not necessary to ask the patient to sign the test request form to indicate their consent for the test to be undertaken. This is a standard pre-printed AWGL form.

The pathology laboratory should prepare the sample in line with the AWGL recommendations (see 'Histopathological sample preparation requirements' section). The pathology laboratory should complete their relevant section of the request form and send a paper copy of the form with the prepared slides directly to the AWGL within a 5 working day turnaround time. It should be noted that historical specimens may be stored off-site and, in such circumstances, the turnaround time for this stage may be longer.

Upon receipt of the sample at AWGL, the result will be available within an estimated 10 working days (or 14 calendar days) turnaround time. Reports will be uploaded to Welsh Clinical Portal and emailed to the requestor/referring clinicians.

The contact details for the AWGL are as follows:

All Wales Genetics Laboratory
Institute of Medical Genetics
University Hospital of Wales
Heath Park

Cardiff
CF14 4XW
Telephone: 02921845347
Email address: Admin.Genetics.cav@wales.nhs.uk
Website: http://www.medicalgenomicswales.co.uk
Opening hours: Monday – Friday 8.30am – 5:00pm

NTRK gene fusion testing for privately funding patients

NTRK gene fusion testing is a WHSSC-funded service. However, it is also available for privately funding patients. Please contact the AWGL directly for further details.

Histopathological sample preparation requirements

The local pathology laboratory housing the diagnostic specimen should prepare the sample as follows before sending the slides to AWGL with the NTRK request form.

Test		*Technology*	*Sample requirements*
Multi-target RNA NGS panel: structural variant - NTRK1, NTRK1, NTRK3	☐	**TSO500 NGS Panel**	**RNA**: 50µM (preferably **5x 10µM**) air dried unstained sections mounted on slides. *Note: slides for RNA - ideally prepared in an RNase-free environment.* **For salvage FISH** testing for NTRK1, NTRK2 and NTRK3 (in the event that RNA-based NGS cannot be performed or is unsuccessful): **2x 3-4µM** sections (singly mounted) on charged/adhesion slides **PER GENE**

For all tumour types please supply **ALL** of the following:

- 1 x H&E stained slide with area of highest neoplastic cell content CLEARLY circled
- 5x10µM air dried unstained sections mounted on slides. Note: slides for RNA ideally prepared in an RNase-free environment
- 6x 3-4 µm sections (singly mounted) on charged/adhesion slides for FISH testing. Note: this material is required for salvage FISH testing in the event that RNA-based NGS cannot be performed or is unsuccessful.

Please note that AWGL will be returning all unused slides to the referring pathology laboratory to file as part of the archive.

Interpreting an NTRK gene fusion test result

NTRK fusions are typically mutually exclusive of KRAS, NRAS, BRAF, MAP2K1, EGFR, ALK, RET, ROS1, KIT, PDGFRA and other MAPK driver mutations/fusions. The most common NTRK partner genes are TPM3, LMNA, TPR, EML4, and SQSTM1. The fusions will be reported in line with Human Genome Variation Society (HGVS) nomenclature guidelines, (HGVS, 2020).

Appendix 5 contains examples of NTRK gene fusion reports.

When an NTRK gene fusion test is reported as a stand-alone test, the following outcomes are possible (the exact wording may differ on a case-by-case basis if clinically appropriate):

1. Actionable NTRK gene fusion identified

The diagnostic comment will describe the type of fusion identified: *e.g.TPM-NTRK1 gene fusion detected (#HGVS nomenclature#). No gene fusions involving NTRK2 or NTRK3 detected.*

Therapeutic comment: *This patient may respond to TRK inhibitors. In patients with tumours harbouring an NTRK gene fusion, treatment with a TRK inhibitor has been shown to be associated with high objective response rates (Drilon, A. et al. (2018) The New Eng J of Med 378,8: 731-739; Doebele, R.C. et al. (2020) Lancet Oncology 21 (2): 271-282).*

If an NTRK gene fusion is identified, the patient should be considered for treatment with TRK-inhibitors (see 'Eligibility criteria for treatment with TRK inhibitors' section) as long as they have a performance status of 0-2, with either locally advanced OR metastatic disease OR surgical resection likely to result in severe health problems, and they have no satisfactory treatment options AND have already been treated with all available NHS- funded systemic therapy options for which a clinical benefit has been established.

2. No actionable NTRK gene fusion detected

Diagnostic comment: *No gene fusions detected in NTRK 1, NTRK2 or NTRK3 detected.*

Therapeutic comment: *This patient has a reduced likelihood of response to treatment with TRK inhibitors.*

If an NTRK gene fusion is not identified, the patient is not eligible for treatment with TRK-inhibitors. The treating clinician should consider whether the patient is a suitable candidate for any clinical trials or offer best supportive care.

3. Failed report: RNA of insufficient quality following FFPE extraction for NGS analysis

Diagnostic comment: *NGS analysis failed; insufficient quality RNA for NGS analysis Conclusive comment:*

FISH analysis for NTRK 1, NTRK2 and NTRK3 has been initiated FISH analysis will be initiated if sufficient material has been provided (as per request form) and results will be reported within 14 calendar days of the date of this failed report. If insufficient material has been provided for the FISH salvage pathway to be initiated, the report will note that additional material will be required in order to proceed with any further analysis. This may require a dialogue between the requesting clinician, local pathology laboratory and AWGL to ascertain whether a further biopsy is clinically indicated.

4. Failed report: Insufficient quantity of RNA following FFPE extraction for NGS analysis

Diagnostic comment: *Insufficient RNA for NGS analysis*

Conclusive comment: *FISH analysis for NTRK 1, NTRK2 and NTRK3 has been initiated*

FISH analysis will be initiated if sufficient material has been provided (as per request form) and results will be reported within 14 calendar days of the date of this failed report. If insufficient material has been provided for the FISH salvage pathway to be initiated, the report will note that additional material will be required in order to proceed with any further analysis. This may require a dialogue between the requesting clinician, local pathology laboratory and AWGL to ascertain whether a further biopsy is clinically indicated.

5. Patients having RNA-based NGS as part of diagnostic work-up via CYSGODI service (e.g. thyroid, glioma, NSCLC)

If NTRK gene fusions are tested for as part of an RNA-based NGS panel, the diagnostic and therapeutic comments will mirror those given above for each of the gene fusions tested, e.g. *ETV6-NTRK3 gene fusion detected (#HGVS nomenclature#). No gene fusions involving ALK, RET, ROS1, NTRK1 or 2 detected. The EGFRvIII structural variant and MET exon 14 skipping variant were not detected.*

Whilst the NTRK gene fusion status may be available at an earlier stage in these patients' treatment pathway, suitable patients will still only be eligible to receive a TRK-inhibitor when there are no satisfactory treatment options available to them (see 'Eligibility criteria for NTRK gene fusion testing section').

Eligibility criteria for treatment with TRK-inhibitors

If an NTRK gene fusion is identified, the patient must meet all of the following 4 criteria in order to receive treatment with a TRK-inhibitor:

1. Recent baseline imaging performed of disease within last 4 weeks (including CT or MRI brain)
2. Symptomatically stable from any brain metastases (defined as stable neurology and if taking regular steroids, patient has not required an increase in steroid dose in the 7 days preceding treatment)*
3. No previous treatment with a TRK-inhibitor
4. Remains performance status 0-2.

[*Based on inclusion criteria in the NTRK trials]

The patient should provide written consent prior to cycle 1 of treatment.

TRK-inhibitor prescribing information

The choice of TRK-inhibitor (i.e. larotrectinib or entrectinib) should be made by the treating clinician on a case-by-case basis, taking into account patient specific factors (e.g. comorbidities, acceptability of potential toxicities) and clinical experience.

Detailed prescribing information for larotrectinib and entrectinib is provided in appendix 3 and 4, respectively.

Treatment with TRK-inhibitors should continue until disease progression, or unacceptable toxicity, or patient chooses to stop treatment, or potentially curative surgery takes place.

No treatment breaks of more than 6 weeks beyond the expected cycle length are allowed (to allow any toxicity of current therapy to settle or intercurrent comorbidities to improve).

Baseline investigations and on-treatment monitoring for TRK-inhibitors

Table 2 summarises the required baseline investigations and on-treatment monitoring for patients receiving TRK-inhibitors.

Investigation		**Baseline**	**On-treatment**
Bloods	FBC/U+E/LFTs	☐	Every 2 weeks during first month of treatment, then monthly thereafter
	Serum lipase/amylase	☐	As clinically indicated
	Serum urate ***(entrectinib only)***	☐	Every 2 weeks during the first month of treatment, then monthly thereafter
Cardiac assessment	ECG (to assess QTc interval) ***(entrectinib only)***	☐	As clinically indicated
	Assessment of left ventricular ejection fraction ***(entrectinib only)***	☐	As clinically indicated
Imaging	Radiological imaging of disease (including CT or MRI brain)	☐ (Within preceding 4 weeks)	Repeat restaging imaging (including brain) at 10 weeks to assess response; then every three months or as clinically indicated

Table 2: Baseline and on-treatment monitoring

References

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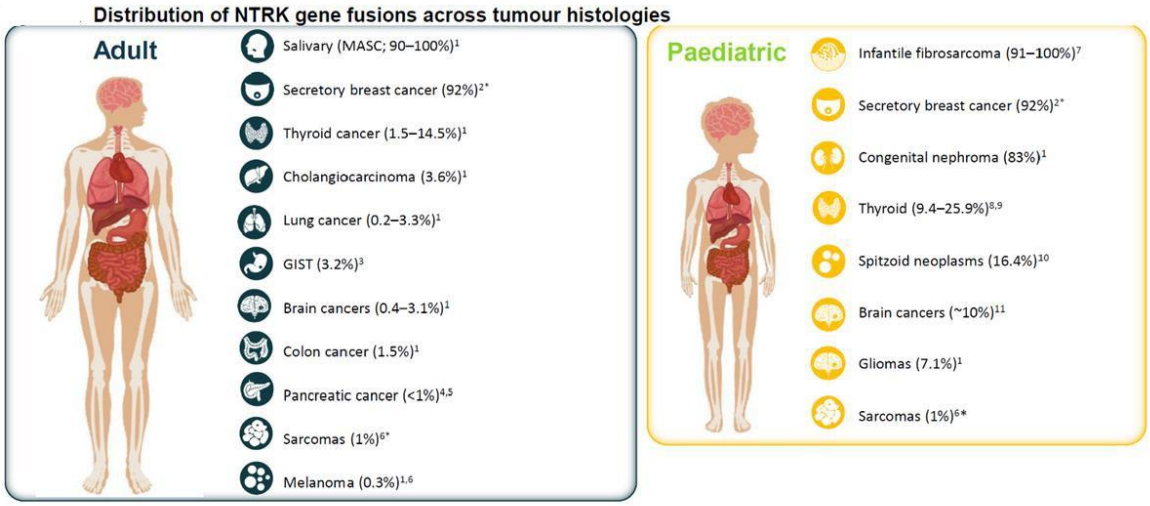
- NICE, 2020[d]. Single technology appraisal: Entrectinib for treating NTRK fusion-positive solid tumours [ID11612]. Committee papers. https://www.nice.org.uk/guidance/ta643/evidence/committee-papers-pdf-8830076941
- Vaishnavi A, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. Cancer Discovery 2015; 5:25-34.

Appendix 1: Prevalence of NTRK gene fusions by tumour type



*Frequency in adult vs. paediatric patients not specified. GIST=gastrointestinal stromal tumour; MASC=mammary analogue secretory carcinoma; NTRK=neurotrophic tyrosine receptor kinase.1. Vaishnavi A, *et al. Cancer Discov.* 2015;5:25-34; 2. TognonC, *et al. Cancer Cell.*2002;2:367-376; 3. Brenca M, *et al. J Pathol.*2016;238:543-549; 4. Pishvaian MJ, *et al.Clin Cancer Res.* 2018; DOI: 10.1158/1078-0432.CCR-18-0531; 5. Cocco E, *et al. Nat Rev Clin Oncol.* 2018 15(12):731-747; 6. Stransky N, *et al. Nat Commun.* 2014 10;5:4846; 7. Bourgeois JM, *et al. Am J Surg Pathol.* 2000;24:937-946; 8. Ricarte-Filho JC, *et al. J Clin Invest.* 2013;123:4935-4944; 9. Prasad ML, *et al. Cancer.* 2016;122(7)1097-1107; 10. Wiesner T, *et al. Nat Commun.* 2014;5:3116; 11. Wu G, *et al. Nat Genet.* 2014;46(5):444-450.

Figure 1: *NTRK* gene fusion prevalence rates by tumour type (NICE, 2020[a])

Appendix 2: Summary of clinical trials using TRK-inhibitors

1.1 Larotrectinib

NICE approved larotrectinib based on the pooled analysis of 102 patients from three trials (NICE, 2020 a). The data was evaluated in two groups; the first included 93 patients with 14 tumour sites, whilst the second included 9 patients with primary CNS tumours.

- NAVIGATE contributed 62 patients to the pooled analysis and is an ongoing basket trial for aged 12 years or older with NTRK gene fusion who had received prior therapy or, in the opinion of the investigator, would be unlikely to derive clinically meaningful benefit from standard of care therapy.
- SCOUT is an ongoing trial which recruits paediatric patients with locally advanced or metastatic solid tumour or primary CNS tumours (32 patients included in pooled analysis).
- LOXO-TRK-14001 a dose-finding study in patients with solid tumours harbouring NTRK fusion from which the data relating to 8 patients was included.

Overall response rate was the primary outcome measure for the 2 larger trials which in the pooled analysis was reported to be 72% across multiple tumour types, ranging from 0% to more than 95%. NICE noted that due to the immaturity of the data, the long-term benefit of larotrectinib on survival cannot be reliably estimated. The reported median overall survival was variable; for common cancer types (including non-small cell lung cancer and colorectal cancer) ranged from 2.3 to 17 months whilst for thyroid carcinoma, GIST and certain soft tissue sarcomas, median overall survival was not reached. Median progression free survival was generally less than 12 months across included tumour types (NICE, 2020 a). Pronounced variability in the percentage of patients experiencing serious adverse events (SAEs) was evident, ranging from less than 10%, to 100% in the included trials. Treatment-related SAEs were reported in patients with all evaluated tumour types.

The following article provides further information: Hong DS et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1-2 clinical trials. *Lancet Oncology*. 2019; 21 (4): 531-540.

1.2 Entrectinib

A pooled analysis of data from 66 patients (adults and children) recruited to four clinical trials was initially presented to NICE (NICE, 2020[d]):

- STARTRK-2 is an ongoing phase 2 basket trial in adults with advanced or metastatic solid tumours with NTRK, ROS1 or ALK gene fusions; 51 patients were included in the pooled analysis
- ALKA is an ongoing phase I trial that contributed 1 adult patient
- STARTRK-1 is an ongoing phase I trial which contributed 2 adult patients
- Data relating to children was collected from the STARTRK-NG trial, a dose escalation and expansion study in patients aged 2 to 22 years.

Exact results were not reported by NICE and although a clinically relevant overall response rate across 13 tumour types was demonstrated, median follow-up was short and survival data was immature.

The following article provides further information:

Doebele RC et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncology*. 2020; 21 (2): 271-282.

Appendix 3: Prescribing information for Larotrectinib

Dosage:	Larotrectinib **(ADULTS)**	Initiate at 100mg po twice daily continuous therapy
	Larotrectinib **(PAEDIATRICS)**	Initiate at 100mg/m2 po twice daily continuous therapy. Maximum of 100 mg per dose.
	The starting dose of larotrectinib should be reduced by 50% in patients with moderate (Child-Pugh B) to severe (Child-Pugh C) hepatic impairment. No dose adjustment is recommended for patients with mild hepatic impairment (Child-Pugh A). Co-administration with strong CYP3A4 inhibitors: If co-administration with a strong CYP3A4 inhibitor is necessary, the larotrectinib dose should be reduced by 50%. After the inhibitor has been discontinued for 3 to 5 elimination half-lives, larotrectinib should be resumed at the dose taken prior to initiating the CYP3A4 inhibitor. **DISCUSS WITH PHARMACY**	
	Available as 25mg & 100mg hard gelatin capsules. Also available as 20mg/ml oral solution in 100ml bottles. Store in a refrigerator (2° C-8°C). Expiry: 30 days after first opening.	
Administration:	Can be taken with or without food. Avoid grapefruit or grapefruit juice. If a dose is missed, the patient should not take two doses at the same time to make up for a missed dose. Patients should take the next dose at the next scheduled time. If the patient vomits after taking a dose, the patient should not take an additional dose to make up for vomiting. Larotrectinib has a moderate influence on the ability to drive and use machines. Dizziness and fatigue have been reported in patients receiving larotrectinib, mostly Grade 1 and 2 during the first 3 months of treatment. This may influence the ability to drive and use machines during this time period and patients should be advised not to do so until they are reasonably certain larotrectinib does not affect them adversely.	
Review clinic:	By Consultant / Registrar / appropriately trained healthcare professional. Clinical review 2 weeks after starting, then every 4 weeks. Continue until disease progression, or unacceptable toxicity, or patient chooses to stop treatment, or potentially curative surgery takes place.	

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<tr><td></td><td colspan="2"></td></tr>
<tr><td>Anti-emetics:</td><td colspan="2">Nausea & vomiting are very common – consider co-prescribing an anti-emetic.</td></tr>
<tr><td>Regular investigations:</td><td>FBC / U&Es / LFTs</td><td>Monitor at baseline and every 2 weeks during the first month of treatment, then monthly thereafter (based on FDA SPC).</td></tr>
<tr><td></td><td>Serum lipase / amylase</td><td>Monitor at baseline and as clinically indicated.</td></tr>
<tr><td>Dose modifications:</td><td colspan="2">For all Grade 2 adverse reactions, continued dosing may be appropriate however close monitoring to ensure no worsening of the toxicity is advised.

For Grade 3 or 4 adverse reactions:

- Larotrectinib should be withheld until the adverse reaction resolves or improves to baseline or Grade 1. Resume at the next dose modification if resolution occurs within 4 weeks.

- Larotrectinib should be permanently discontinued if an adverse reaction does not resolve within 4 weeks.

Recommended dose modifications for adverse reactions.
<table>
<tr><th>Dose Modification</th><th>Adult and paediatric patients with body surface area of at least 1.0 m^2</th><th>Paediatric patients with body surface area less than 1.0 m^2</th></tr>
<tr><td>First</td><td>75 mg twice daily</td><td>75 mg/m^2 twice daily</td></tr>
<tr><td>Second</td><td>50 mg twice daily</td><td>50 mg/m^2 twice daily</td></tr>
<tr><td>Third</td><td>100 mg once daily</td><td>25 mg/m^2 twice daily</td></tr>
<tr><td colspan="3">Larotrectinib should be permanently discontinued in patients who are unable to tolerate treatment after three dose modifications.</td></tr>
</table></td></tr>
<tr><td>Main toxicities:</td><td colspan="2">The most common adverse drug reactions (≥ 20%) in clinical trials of larotrectinib in order of decreasing frequency were fatigue (32%), increased ALT (31%), dizziness (30%), increased AST (29%), constipation (29%), nausea (26%), anaemia (24%), and vomiting (20%).

The majority of adverse reactions were Grade 1 or 2.</td></tr>
</table>

	Grade 4 adverse reactions were neutrophil count decreased (1.6%) and ALT increased (< 1%). Grade 3 adverse reactions were anaemia, weight increased, fatigue, increased AST, dizziness, paraesthesia, nausea, myalgia, and leukocyte count decreased. All reported Grade 3 adverse reactions occurred in less than 5% of patients, with the exception of anaemia (7%). Permanent discontinuation of larotrectinib for treatment emergent adverse reactions, regardless of attribution, occurred in 3% of patients (one case each of ALT increase, AST increase, intestinal perforation, jaundice, small intestinal obstruction). The majority of adverse reactions leading to dose reduction occurred in the first three months of treatment.
Neurological toxicity:	Neurologic reactions including dizziness, gait disturbance and paraesthesia were reported in patients receiving larotrectinib. For the majority of neurologic reactions, onset occurred within the first three months of treatment. Withholding, reducing, or discontinuing larotrectinib dosing should be considered, depending on the severity and persistence of these symptoms.
Haematological toxicity:	Grade 3/4 anaemia, neutropenia & leukopenia have been reported.
Contraception:	Verify the pregnancy status of females of reproductive potential prior to initiating. Women of childbearing potential must use highly effective contraception while taking larotrectinib and for at least one month after stopping treatment. Males of reproductive potential with a non-pregnant woman partner of childbearing potential should be advised to use highly effective contraception during treatment with larotrectinib and for at least one month after the final dose.
Renal impairment:	No dose adjustment is required for patients with renal impairment.
Hepatic impairment:	The starting dose of larotrectinib should be reduced by 50% in patients with moderate (Child-Pugh B) to severe (Child-Pugh C) hepatic impairment. No dose adjustment is recommended for patients with mild hepatic impairment (Child-Pugh A). Monitor liver tests including ALT and AST every 2 weeks during the first month of treatment, then monthly thereafter and as clinically indicated **(based on FDA SPC)**.

	ALT and AST increases were reported in patients receiving larotrectinib. The majority occurred in the first 3 months of treatment. Patients with Grade 2 ALT and/or AST increases, should be followed with serial laboratory evaluations every one to two weeks after the observation of Grade 2 toxicity until resolved to establish whether a dose interruption or reduction is required. In patients who develop transaminase elevations, either withhold or permanently discontinue larotrectinib, based on severity. If withheld, the larotrectinib dose should be modified when resumed.
Interactions:	Larotrectinib is a substrate of cytochrome P450 (CYP) 3A, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). Co-administration of larotrectinib with strong CYP3A inhibitors, P-gp and BCRP inhibitors (e.g. atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, troleandomycin, voriconazole or grapefruit) may increase larotrectinib plasma concentrations. If co-administration with a strong CYP3A4 inhibitor is necessary, consult the Summary of Product Characteristics (SPC) for dose reduction advice. Co-administration of larotrectinib with strong or moderate CYP3A and P-gp inducers (e.g. carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, or St. John's Wort) may decrease larotrectinib plasma concentrations and should be avoided. If concomitant use of larotrectinib with CYP3A substrates with narrow therapeutic range is required (e.g. alfentanil, ciclosporin, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, or tacrolimus), a dose reduction of the CYP3A substrate may be required due to adverse reactions.

Appendix 4: Prescribing information for Entrectinib

Drugs/Dosage:	Entrectinib **(ADULTS)**	Initiate at 600mg po once daily continuous therapy.
	Entrectinib **(PAEDIATRICS > 12 years old)**	Initiate at: BSA > 1.50m^2: 600mg po once daily; BSA 1.11 to 1.50m^2: 500mg po once daily; BSA 0.91 to 1.10m^2: 400mg po once daily.
	Moderate and strong CYP3A Inhibitors: Adults and paediatric patients 12 years and older with BSA>1.50m^2. Avoid co-administration of entrectinib with moderate or strong CYP3A inhibitors. If co-administration cannot be avoided, reduce the dose as follows: • Moderate CYP3A Inhibitors: 200 mg orally once daily • Strong CYP3A Inhibitors: 100 mg orally once daily After discontinuation of a strong or moderate CYP3A inhibitor for 3 to 5 elimination half-lives, resume the entrectinib dose that was taken prior to initiating the CYP3A inhibitor. **DISCUSS WITH PHARMACY**	
	Available as: Capsules: 100 mg and 200 mg.	
Administration:	Swallow capsules whole. Do not open, crush, chew, or dissolve the contents of the capsule. If a patient misses a dose, instruct patients to make up that dose unless the next dose is due within 12 hours. If a patient vomits immediately after taking a dose, instruct patients to repeat that dose.	
Review clinic:	By Consultant / Registrar / appropriately trained healthcare professional.	

<table>
<tr><td></td><td colspan="2">Clinical review 2 weeks after starting, then every 4 weeks. Continue TRK-inhibitor until disease progression, or unacceptable toxicity, or patient chooses to stop treatment, or potentially curative surgery takes place.</td></tr>
<tr><td>Anti-emetics:</td><td colspan="2">Nausea & vomiting are very common – consider co-prescribing an anti-emetics.</td></tr>
<tr><td rowspan="4">Regular investigations:</td><td>FBC / U&Es / LFTs / serum urate</td><td>Monitor at baseline and then every 2 weeks during the first month of treatment, then monthly thereafter (based on FDA SPC).</td></tr>
<tr><td>Serum lipase / amylase</td><td>Monitor at baseline and as clinically indicated.</td></tr>
<tr><td>Left ventricular ejection fraction</td><td>Consider assessment of LVEF before initiating treatment.</td></tr>
<tr><td>ECG</td><td>Consider assessment of QT interval in those at risk of prolongation.</td></tr>
<tr><td>Dose modifications:</td><td colspan="2">Recommended dose reductions for adverse reactions.
<table>
<tr><th>Action</th><th>Adults and Paediatric Patients 12 Years and Older with BSA Greater than 1.50 m2 (Orally once daily)</th><th>Paediatric Patients 12 Years and Older with BSA of 1.11 to 1.50 m2 (Orally once daily)</th><th>Paediatric Patients 12 Years and Older with BSA of 0.91 to 1.10 m2 (Orally once daily)</th></tr>
<tr><td>First dose reduction</td><td>400mg</td><td>400mg</td><td>300mg</td></tr>
<tr><td>Second dose reduction*</td><td>200mg</td><td>200mg</td><td>200mg</td></tr>
</table></td></tr>
<tr><td>Main toxicities:</td><td colspan="2">Tiredness, constipation, change in taste, swelling, dizziness, diarrhoea, nausea, abnormal touch sensation, shortness of breath, muscle pain, confusion, mental status changes, memory problems, and hallucinations, weight gain, cough, vomiting, fever, joint pain, vision changes.</td></tr>
</table>

Cardiac toxicity:

Congestive heart failure (CHF):

Assess left ventricular ejection fraction prior to initiation of entrectinib in patients with symptoms or known risk factors for CHF. Monitor patients for clinical signs and symptoms of CHF.

For patients with myocarditis, with or without a decreased ejection fraction, MRI or cardiac biopsy may be required to make the diagnosis. For new onset or worsening CHF, withhold entrectinib, reassess LVEF and institute appropriate medical management.

Reduce dose or permanently discontinue based on severity of CHF or worsening LVEF.

Severity	**Dosage Modification**
Grade 2 or 3	• Withhold entrectinib until recovered to less than or equal to Grade 1. • Resume at reduced dose.
Grade 4	• Permanently discontinue.

QTc prolongation:

Monitor patients who have or who are at risk for QTc interval prolongation. Assess QT interval and electrolytes at baseline and periodically during treatment. Withhold and then resume at same or reduced dose, or permanently discontinue entrectinib based on severity.

Severity	**Dosage Modification**
QTc greater than 500ms	• Withhold entrectinib until QTc interval recovers to baseline. • Resume at same dose if factors that cause QT prolongation are identified and corrected. • Resume at reduced dose if other factors that cause QT prolongation are not identified.
Torsade de pointes; polymorphic ventricular tachycardia; signs/symptoms of serious arrhythmia	• Permanently discontinue entrectinib.

Other drugs that prolong QT interval:

QTc interval prolongation can occur with entrectinib. Avoid co-administration of entrectinib with other products with a known potential to prolong QT/QTc interval.

<table>
<tr>
<td>Neurological toxicity:</td>
<td>CNS adverse reactions including cognitive impairment, mood disorders, dizziness, and sleep disturbances can occur with entrectinib.

Withhold and then resume at same or reduced dose upon improvement or permanently discontinue based on severity.

<table>
<tr><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Intolerable Grade 2</td><td>• Withhold entrectinib until recovery to less than or equal to Grade 1 or to baseline.
• Resume at same dose or reduced dose, as clinically appropriate.</td></tr>
<tr><td>Grade 3</td><td>• Withhold entrectinib until recovery to less than or equal to Grade 1 or to baseline.
• Resume at reduced dose.</td></tr>
<tr><td>Grade 4</td><td>• Permanently discontinue.</td></tr>
</table>
</td>
</tr>
<tr>
<td>Hepatic impairment:</td>
<td>Monitor liver tests, including ALT and AST, every 2 weeks during the first month of treatment, then monthly thereafter, and as clinically indicated. Withhold or permanently discontinue entrectinib based on severity. If withheld, resume entrectinib at same or reduced dose based on severity.

<table>
<tr><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Grade 3</td><td>• Withhold entrectinib until recovery to less than or equal to Grade 1 or to baseline.

• Resume at same dose if resolution occurs within 4 weeks.

• Permanently discontinue if adverse reaction does not resolve within 4 weeks.

• Resume at a reduced dose for recurrent Grade 3 events that resolve within 4 weeks.</td></tr>
<tr><td>Grade 4</td><td>• Withhold entrectinib until recovery to less than or equal to Grade 1 or to baseline.

• Resume at reduced dose if resolution occurs within 4 weeks.

• Permanently discontinue if adverse reaction does not resolve within 4 weeks.</td></tr>
</table>
</td>
</tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><td></td><td>• Permanently discontinue for recurrent Grade 4 events.</td></tr>
<tr><td>ALT or AST >3 times ULN with concurrent total bilirubin > 1.5 times ULN (in the absence of cholestasis or haemolysis).</td><td>• Permanently discontinue entrectinib.</td></tr>
</table>
</td></tr>
<tr><td>Hyperuricaemia:</td><td>Assess serum uric acid levels prior to initiation and periodically during treatment with entrectinib. Monitor patients for signs and symptoms of hyperuricemia. Initiate treatment with urate lowering medications as clinically indicated and withhold entrectinib for signs and symptoms of hyperuricemia. Resume at same or reduced dose upon improvement based on severity.
<table>
<tr><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Symptomatic or Grade 4</td><td>• Initiate urate-lowering medication.
• Withhold entrectinib until improvement of signs or symptoms.
• Resume entrectinib at same or reduced dose.</td></tr>
</table>
</td></tr>
<tr><td>Visual disturbances:</td><td>Withhold for new visual changes or changes that interfere with activities of daily living until improvement or stabilization. Conduct an ophthalmological evaluation as appropriate. Resume at same or reduced dose upon improvement or stabilization.
<table>
<tr><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Grade 2 or above</td><td>• Withhold entrectinib until improvement or stabilization.
• Resume at same dose or reduced dose, as clinically appropriate.</td></tr>
</table>
</td></tr>
<tr><td>Haematological toxicity:</td><td>
<table>
<tr><th></th><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Anaemia or Neutropenia</td><td>Grade 3 or 4</td><td>• Withhold entrectinib until recovery to less than or equal to Grade 2.
• Resume at the same dose or reduced dose, as clinically appropriate.</td></tr>
</table>
</td></tr>
</table>

<table>
<tr><td>Other clinically relevant adverse reactions:</td><td>
<table>
<tr><th>Severity</th><th>Dosage Modification</th></tr>
<tr><td>Grade 3 or 4</td><td>• Withhold entrectinib until adverse reaction resolves or improves to recovery or improvement to Grade 1 or baseline.

• Resume at the same or reduced dose, if resolution occurs within 4 weeks.

• Permanently discontinue if adverse reaction does not resolve within 4 weeks.

• Permanently discontinue for recurrent Grade 4 events.</td></tr>
</table>
</td></tr>
<tr><td>Skeletal fractures:</td><td>Entrectinib increases the risk of fractures. Promptly evaluate patients with signs or symptoms of fractures.</td></tr>
<tr><td>Serum lipase / mylase;</td><td>Raised serum lipase and amylase have been noted in clinical trials and consideration to pancreatitis is needed.</td></tr>
<tr><td>Contraception:</td><td>Verify the pregnancy status of females of reproductive potential prior to initiating.

Advise female patients of reproductive potential to use effective contraception during treatment with entrectinib and for at least 5 weeks following the final dose.

Advise male patients with female partners of reproductive potential to use effective contraception during treatment with entrectinib and for 3 months following the final dose.</td></tr>
<tr><td>Renal impairment:</td><td>No dose adjustment is recommended for patients with mild or moderate renal impairment (CrCl 30 to < 90 mL/min calculated by Cockcroft-Gault equation). Entrectinib has not been studied in patients with severe renal impairment (CrCl < 30 mL/min).</td></tr>
<tr><td>Interactions:</td><td>Co-administration of entrectinib with a strong or moderate CYP3A inhibitor increases entrectinib plasma concentrations, which could increase the frequency or severity of adverse reactions.

Moderate & strong CYP3A inhibitors:

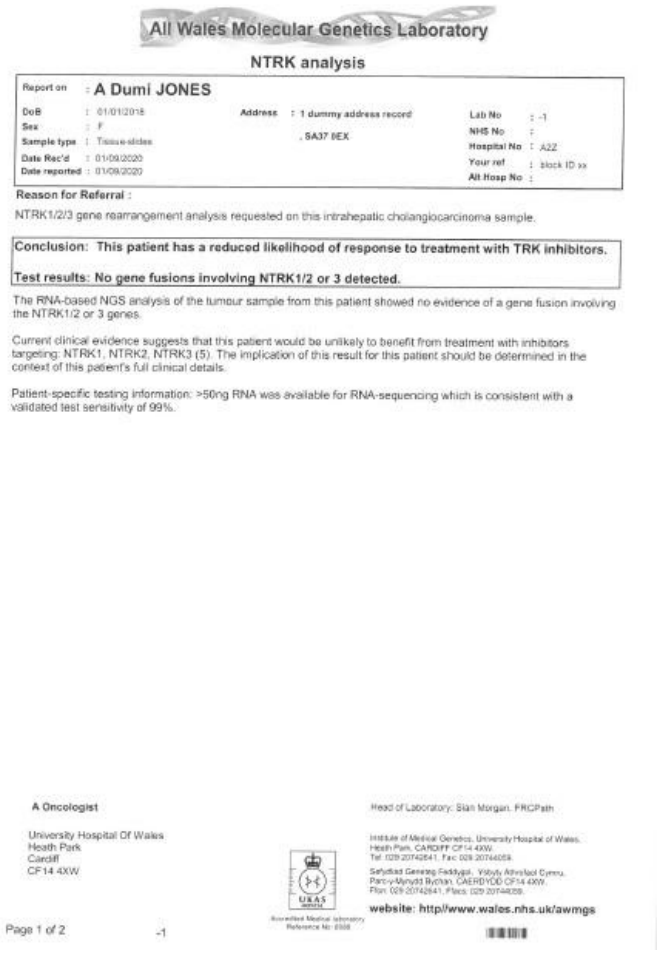
• Adults and paediatric patients >12 years with BSA>1.50 m2: Avoid co-administration of strong or moderate CYP3A inhibitors with entrectinib. If co-administration is unavoidable, reduce the entrectinib.

• Paediatric patients 12 years and older with BSA ≤1.50 m2: Avoid co-administration of entrectinib with moderate or strong CYP3A</td></tr>
</table>

	inhibitors. Avoid grapefruit products during treatment, as they contain inhibitors of CYP3A. **Moderate and strong CYP3A inducers:** Co-administration of entrectinib with a strong or moderate CYP3A inducer decreases entrectinib plasma concentrations which may reduce entrectinib efficacy. Avoid co-administration of strong and moderate CYP3A inducers with entrectinib.

Appendix 5: Example Genetic Reports

1. NTRK analysis report – no NTRK gene fusion detected



All Wales Molecular Genetics Laboratory

NTRK analysis

Report on : A Dumi JONES

DoB : 01/01/2018 Address : 1 dummy address record
Sex : F , SA37 0EX
Sample type : Tissue-slides
Date Rec'd : 01/09/2020
Date reported : 01/09/2020

Lab No : -1
NHS No :
Hospital No : A22
Your ref : block ID xx
Alt Hosp No :

Reason for Referral :

NTRK1/2/3 gene rearrangement analysis requested on this intrahepatic cholangiocarcinoma sample.

Conclusion: This patient has a reduced likelihood of response to treatment with TRK inhibitors.

Test results: No gene fusions involving NTRK1/2 or 3 detected.

The RNA-based NGS analysis of the tumour sample from this patient showed no evidence of a gene fusion involving the NTRK1/2 or 3 genes.

Current clinical evidence suggests that this patient would be unlikely to benefit from treatment with inhibitors targeting: NTRK1, NTRK2, NTRK3 (5). The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Patient-specific testing information: >50ng RNA was available for RNA-sequencing which is consistent with a validated test sensitivity of 99%.

A Oncologist

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Ffon: 029 20742641, Ffacs: 029 20744059.

website: http//www.wales.nhs.uk/awmgs

NTRK analysis

Analysed by: Checked by:

Helen Roberts
Clinical Scientist

Rhian White
Principal Clinical Scientist

Test details:
Following assessment of the tumour sample from this patient by a pathologist (block no: xx), the area of highest neoplastic cell content (estimated at xx%) was identified; this area was macrodissected and RNA extracted for analysis. RNA extraction performed using Maxwell RSC FFPE RNA kit (Promega AS1440); concentration of RNA using Zymo RNA clean and concentrator kit performed where required.
Next generation sequencing was performed using a Roche pan cancer panel (custom-design) and sequenced on an Illumina platform. Data analysis using in-house bioinformatic targeted NTRK panel, employing fusion prediction software Arriba and STARFusion, and IGV for the detection of structural variants in the following genes and regions: NTRK1 all exons; NTRK2 all exons; NTRK3 all exons.
This panel is targeted for the detection of fusion partners for the following genes: NTRK1 fusion partner TPM3 (NM_152263.3). The panel will also detect novel fusion partner genes. A search of the COSMIC database (https://cancer.sanger.ac.uk/cosmic) is conducted for all structural variants detected.
This custom design Roche pan cancer panel has a validated sensitivity of 99% and specificity of 100% for known fusion variants (those previously identified by FISH or NGS), as determined by in-house validation using an input RNA amount of >50ng. A minimum of 38K total mapped reads (duplicates removed) is required for detection of non-intragenic fusion variants. For samples with <50ng input RNA the validated sensitivity of this assay is 88% and specificity is 100%. Sensitivity for rearrangement detection may be reduced in: samples with <30% neoplastic cells (in-house data) or samples with low expressed fusions [1].
Rearrangements are reported according to HGVS nomenclature (https://varnomen.hgvs.org/bg-material/consultation/vd-wg007/). Owing to differential expression of genes, it is not possible to determine the proportion of tumour cells carrying any structural variants detected using this assay.
Only actionable NTRK fusions are reported. To be considered actionable, the gene fusion must retain the continuous open reading frame at the fusion junction and the tyrosine kinase domain of the protein [2]. Actionable NTRK fusions are also noted by the absence of the extracellular ligand binding domain [3].
NTRK1/2/3 fusions have been reported in 3.6% of patients with cholangiocarcinoma [4].
The reported results are dependent upon the analysed tissue representing the molecular makeup of the tumour in this patient.
Reference sequences: NTRK1 NM_001007792.1; NTRK2 NM_006180.4; NTRK3 NM_001012338.2
Please note that this test is not currently accredited by UKAS.
References: [1] Haas, B.J., et al (2019) Genome Biology 20:213. [2] Kumar-Sinha, C., et al 2015 Genome Medicine 7:129; Cocco, E., et al 2018 Nat Rev Clin Oncol 15(12):731-747. [3] Hsiao, S.J. et al (2018) J of Mol Diag 21(4):553-571. [4] #NICE, 2020 https://www.nice.org.uk/guidance/ta630/evidence/appraisal-consultation-committee-papers-pdf-8767837879. [5] References re treatment response:
NTRK1/2/3 – Drilon, A. et al. (2018) The New Eng J of Med 378.8: 731-739; Doebele, R.C. et al. (2020) Lancet Oncology 21 (2): 271-282.
[6] Reference re TRK drug use: NICE, 2020 https://www.nice.org.uk/guidance/gid-ta10229/documents/final-appraisal-determination-document and https://www.nice.org.uk/guidance/ta644/chapter/1-Recommendations

Copies to:

Results are dependent on samples being correctly labelled and family relationships as indicated.
Please note, any remaining DNA will be stored in the laboratory.

A Oncologist

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website: http//www.wales.nhs.uk/awmgs

2. NTRK analysis report – NTRK gene fusion detected

All Wales Molecular Genetics Laboratory

NTRK analysis

Report on : A Dumi JONES

DoB : 01/01/2018 Address : 1 dummy address record Lab No : -1
Sex : F , SA37 0EX NHS No :
Sample type : Tissue-slides Hospital No : A2Z
Date Rec'd : 01/09/2020 Your ref : block ID xx
Date reported : 01/09/2020 Alt Hosp No :

Reason for Referral :

NTRK1/2/3 gene rearrangement analysis requested on this intrahepatic cholangiocarcinoma sample.

Conclusion: This patient may respond to TRK inhibitors.

Test results: TMP3-NTRK1 gene fusion detected; #HGVS nomenclature#.
No gene fusions involving NTRK2 or NTRK3 detected.

The RNA-based NGS analysis of the tumour sample from this patient detected a TMP3-NTRK1 gene fusion. Analysis showed no evidence of a gene fusion involving the NTRK2, or NTRK3 genes.

In patients with tumours harbouring an NTRK gene fusion, treatment with TRK inhibitors has been shown to be associated with high objective response rates [5].

TRK inhibitors are recommended for use 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 the patient has no other satisfactory treatment options [6]. The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Patient-specific testing information: <50ng RNA was used for RNA-sequencing, providing an 88% sensitivity for rearrangement detection.

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website: http//www.wales.nhs.uk/awmgs

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NTRK analysis

Analysed by: Checked by:

Helen Roberts, Clinical Scientist
Rhian White, Principal Clinical Scientist

Test details:
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This panel is targeted for the detection of fusion partners for the following genes: NTRK1 fusion partner TPM3 (NM_152263.3). The panel will also detect novel fusion partner genes. A search of the COSMIC database (https://cancer.sanger.ac.uk/cosmic) is conducted for all structural variants detected.
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The reported results are dependent upon the analysed tissue representing the molecular makeup of the tumour in this patient.
Reference sequences: NTRK1 NM_001007792.1; NTRK2 NM_006180.4; NTRK3 NM_001012338.2
Please note that this test is not currently accredited by UKAS.
References: [1] Haas, B.J., et al (2019) Genome Biology 20:213. [2] Kumar-Sinha, C., et al 2018 Genome Medicine 7:129; Cocco, E., et al 2018 Nat Rev Clin Oncol 15(12):731-747. [3] Hsiao, S.J. et al (2019) J of Mol Diag 21(4):553-571. [4] NICE, 2020 https://www.nice.org.uk/guidance/ta630/evidence/appraisal-consultation-committee-papers-pdf-8767837839.
[5] References re treatment response: NTRK1/2/3 – Drilon, A. et al. (2018) The New Eng J of Med 378.8: 731-739. Doebele, R.C. et al. (2020) Lancet Oncology 21 (2): 271-282.
[6] Reference re TRK drug use: NICE, 2020 https://www.nice.org.uk/guidance/gid-ta10229/documents/final-appraisal-determination-document and https://www.nice.org.uk/guidance/ta644/chapter/1-Recommendations

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Results are dependent on samples being correctly labelled and family relationships as indicated.
Please note, any remaining DNA will be stored in the laboratory.

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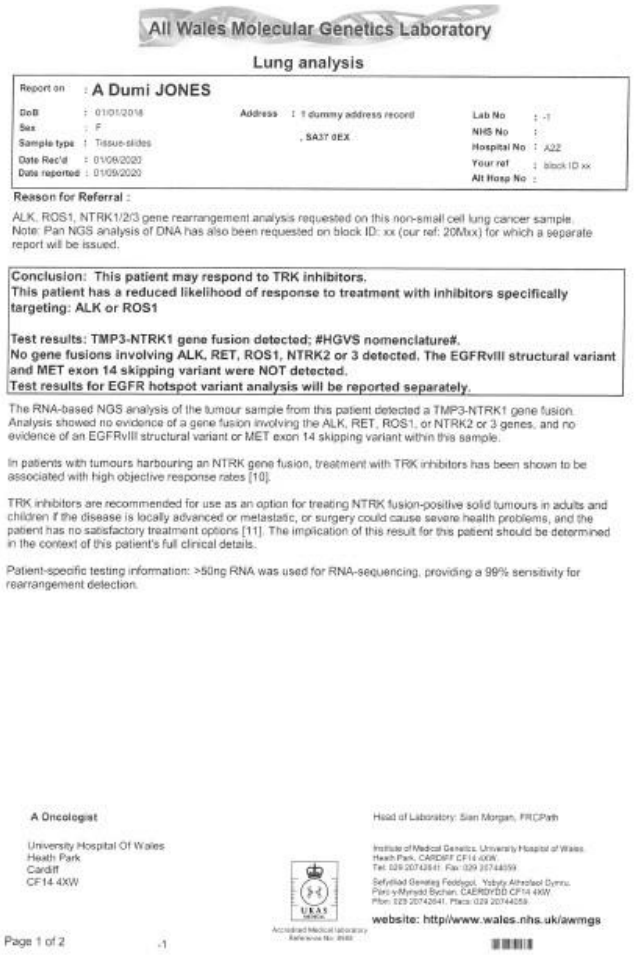
3. RNA-based NGS report (including NTRK gene fusion analysis where NTRK gene fusion is detected)



All Wales Molecular Genetics Laboratory

Lung analysis

Report on : A Dumi JONES

DoB : 01/01/2018 Address : 1 dummy address record Lab No : -1
Sex : F , SA37 0EX NHS No :
Sample type : Tissue-slides Hospital No : A22
Date Rec'd : 01/09/2020 Your ref : block ID xx
Date reported : 01/09/2020 Alt Hosp No :

Reason for Referral :

ALK, ROS1, NTRK1/2/3 gene rearrangement analysis requested on this non-small cell lung cancer sample.
Note: Pan NGS analysis of DNA has also been requested on block ID: xx (our ref: 20Mxx) for which a separate report will be issued.

Conclusion: This patient may respond to TRK inhibitors.
This patient has a reduced likelihood of response to treatment with inhibitors specifically targeting: ALK or ROS1

Test results: TMP3-NTRK1 gene fusion detected; #HGVS nomenclature#.
No gene fusions involving ALK, RET, ROS1, NTRK2 or 3 detected. The EGFRvIII structural variant and MET exon 14 skipping variant were NOT detected.
Test results for EGFR hotspot variant analysis will be reported separately.

The RNA-based NGS analysis of the tumour sample from this patient detected a TMP3-NTRK1 gene fusion. Analysis showed no evidence of a gene fusion involving the ALK, RET, ROS1, or NTRK2 or 3 genes, and no evidence of an EGFRvIII structural variant or MET exon 14 skipping variant within this sample.

In patients with tumours harbouring an NTRK gene fusion, treatment with TRK inhibitors has been shown to be associated with high objective response rates [10].

TRK inhibitors are recommended for use 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 the patient has no satisfactory treatment options [11]. The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Patient-specific testing information: >50ng RNA was used for RNA-sequencing, providing a 99% sensitivity for rearrangement detection.

A Oncologist

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Lung analysis

Analysed by: Checked by:

Helen Roberts, Clinical Scientist
Rhian White, Principal Clinical Scientist

Test details:
Following assessment of the tumour sample from this patient by a pathologist (block no: xx), the area of highest neoplastic cell content (estimated at xx%) was identified; this area was macrodissected and RNA extracted for analysis. RNA extraction performed using Maxwell RSC FFPE RNA kit (Promega AS1440); concentration of RNA using Zymo RNA clean and concentrator kit performed where required. Next generation sequencing was performed using a Roche pan cancer panel (custom-design) and sequenced on an Illumina platform. Data analysis using in-house bioinformatic targeted lung panel, employing fusion prediction software Arriba, STARFusion, and rMATS transcript splicing software and IGV for the detection of structural variants in the following genes and regions: EGFR exons 1-8 (region relevant for detection of EGFRvIII structural variant); ALK exons 19 and 20; MET exons 13, 14 and 15 (region relevant to identification of MET exon 14 skipping event); RET all exons; ROS1 all exons; NTRK1 all exons; NTRK2 all exons; NTRK3 all exons.
This panel is targeted for the detection of fusion partners for the following genes: ALK fusion partners EML4 (NM_019063.4) and KIF5B (NM_004521.2); RET fusion partners CCDC6 (NM_005436.4), and KIF5B (NM_004521.2); ROS1 fusion partners CD74 (NM_001025159.2), SLC34A2 (NM_006424.2), EZR (NM_001111077.1), SDC4 (NM_002999.3), and TPM3 (NM_152263.3); NTRK1 fusion partner TPM3 (NM_152263.3). The panel will also detect novel fusion partner genes. A search of the COSMIC database (https://cancer.sanger.ac.uk/cosmic) is conducted for all structural variants detected.
This custom design Roche pan cancer panel has a validated sensitivity of 99% and specificity of 100% for known fusion variants (those previously identified by FISH or NGS), as determined by in-house validation using an input RNA amount of >50ng. A minimum of 5 unique reads is required for detection of intragenic fusions, and 38K total mapped reads (duplicates removed) for detection of other fusion variants. For samples with >50ng input RNA the validated sensitivity of this assay is 88% and specificity is 100%. Sensitivity for rearrangement detection may be reduced in: samples with <30% neoplastic cells (in-house data) or samples with low expressed fusions [1].
Rearrangements are reported according to HGVS nomenclature (https://varnomen.hgvs.org/bg-material/consultation-old-wg007/). Owing to differential expression of genes, it is not possible to determine the proportion of tumour cells carrying any structural variants detected using this assay.
Only actionable ALK, RET, ROS1 and NTRK fusions are reported. To be considered actionable, the gene fusion must retain the continuous open reading frame at the fusion junction and the tyrosine kinase domain of the protein [2]. Actionable NTRK fusions are also noted by the absence of the extracellular ligand binding domain [3].
Within patients with NSCLC, the frequency of structural variants is: EGFRvIII: 0-1% [4]; ALK fusions: 3-7% [5]; MET exon 14 skipping events: 1.5-6% [6]; RET fusions: 0.9% [7]; ROS1 fusions: 1-2% [8]; NTRK fusions: up to 3% [9].
The reported results are dependant upon the analysed tissue representing the molecular makeup of the tumour in this patient.
Reference sequences: EGFR NM_005228.3 (LRG_304); ALK NM_004304.3 (LRG_488); MET NM_001127500.1 (LRG_862); RET NM_020975.4 (LRG_518); ROS1 NM_002944.2 (LRG_997); NTRK1 NM_001007792.1; NTRK2 NM_006180.4; NTRK3 NM_0010123382.2
Please note that this test is not currently accredited by UKAS.
References: [1] Haas, B.J., et al (2019) Genome Biology 20:213. [2] Kumar-Sinha, C., et al 2019 Genome Medicine 7:129. Cocco, E., et al. 2018 Nat Rev Clin Oncol. 15(12):731-747. [3] Hsiao, S.J. et al (2019) J of Mol Diag 21(4):553-571. [4] Gan, H.K., et al (2013) The FEBS Journal 280(21):5350-5370. [5] Sasaki, T., and Janne, P.A. (2011) Clin Cancer Res 17:7213-7218. [6] Hirsh, R.S. et al. (2018) The Oncologist 21(4):481-488. [7] Cancer Genome Atlas Research Network (2014) Nature 511(7511):543–550. [8] Davies, K., and Doebele, R. (2013) Clinical Cancer Research, 19(15):4040-4045. [9] Vaishnavi, A. et al. (2013) Nat Med 19(11):1469–1472).
[10] References re treatment response:
EGFR – no currently approved treatments target EGFRvIII structural rearrangements in NSCLC
ALK – Hallberg, B., and Palmer, R.H. (2011) F1000 Medicine Reports 3:21.
MET – no currently approved treatments target MET rearrangements in NSCLC
RET – no currently approved treatments target RET rearrangements in NSCLC
ROS1 – Shaw, A.T., et al (2014). N Engl J Med. 20; 371(21): 1963-1971. Drilon, A. et al. (2020) The Lancet Onc 21(2):261-270.
NTRK1/2/3 – Drilon, A. et al. (2018) The New Eng J of Med 378.8: 731-739. Doebele, R.C. et al. (2020) Lancet Oncology 21 (2): 271-282
[11] Reference re TRK drug use: NICE, 2020 https://www.nice.org.uk/guidance/gid-ta10229/documents/final-appraisal-determination-document and https://www.nice.org.uk/guidance/ta844/chapter/1-Recommendations

Copies to:

Results are dependent on samples being correctly labelled and family relationships as indicated.
Please note, any remaining DNA will be stored in the laboratory.

A Oncologist

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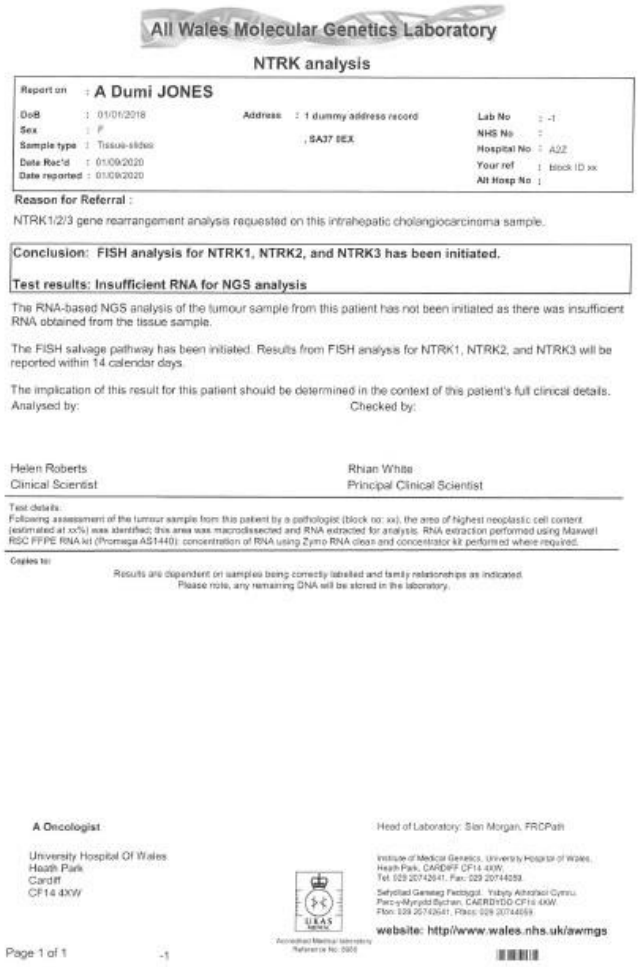
4. RNA-based NGS not possible (insufficient quantity or quality of RNA)



All Wales Molecular Genetics Laboratory

NTRK analysis

Report on : A Dumi JONES

DoB : 01/01/2018 Address : 1 dummy address record Lab No : -1
Sex : F , SA37 0EX NHS No :
Sample type : Tissue-slides Hospital No : A2Z
Date Rec'd : 01/09/2020 Your ref : block ID xx
Date reported : 01/09/2020 Alt Hosp No :

Reason for Referral :

NTRK1/2/3 gene rearrangement analysis requested on this intrahepatic cholangiocarcinoma sample.

Conclusion: FISH analysis for NTRK1, NTRK2, and NTRK3 has been initiated.

Test results: Insufficient RNA for NGS analysis

The RNA-based NGS analysis of the tumour sample from this patient has not been initiated as there was insufficient RNA obtained from the tissue sample.

The FISH salvage pathway has been initiated. Results from FISH analysis for NTRK1, NTRK2, and NTRK3 will be reported within 14 calendar days.

The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Analysed by: Checked by:

Helen Roberts, Clinical Scientist
Rhian White, Principal Clinical Scientist

Test details:
Following assessment of the tumour sample from this patient by a pathologist (block no: xx), the area of highest neoplastic cell content (estimated at xx%) was identified; this area was macrodissected and RNA extracted for analysis. RNA extraction performed using Maxwell RSC FFPE RNA kit (Promega AS1440); concentration of RNA using Zymo RNA clean and concentrator kit performed where required.

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All Wales Molecular Genetics Laboratory

NTRK analysis

Report on : A Dumi JONES

DoB : 01/01/2018 Address : 1 dummy address record Lab No : -1
Sex : F , SA37 0EX NHS No :
Sample type : Tissue-slides Hospital No : A2Z
Date Rec'd : 01/09/2020 Your ref : block ID xx
Date reported : 01/09/2020 Alt Hosp No :

Reason for Referral :

NTRK1/2/3 gene rearrangement analysis requested on this intrahepatic cholangiocarcinoma sample.

Conclusion: FISH analysis for NTRK1, NTRK2, and NTRK3 has been initiated.

Test results: NGS analysis failed; insufficient quality RNA for NGS analysis

The RNA-based NGS analysis of the tumour sample from this patient has unfortunately failed to give a result: The required quality metrics were not achieved for this sample. This failure is most likely due to poor quality RNA which is commonly associated with FFPE tissue.

The FISH salvage pathway has been initiated. Results from FISH analysis for NTRK1, NTRK2, and NTRK3 will be reported within 14 calendar days.

The implication of this result for this patient should be determined in the context of this patient's full clinical details.

Analysed by: Checked by:

Helen Roberts, Clinical Scientist
Rhian White, Principal Clinical Scientist

Test details:
Following assessment of the tumour sample from this patient by a pathologist (block no: xx), the area of highest neoplastic cell content (estimated at xx%) was identified; this area was macrodissected and RNA extracted for analysis. RNA extraction performed using Maxwell RSC FFPE RNA kit (Promega AS1440); concentration of RNA using Zymo RNA clean and concentrator kit performed where required.
Next generation sequencing was performed using a Roche pan cancer panel (custom-design) and sequenced on an Illumina platform. Data analysis using in-house bioinformatic targeted NTRK panel, employing fusion prediction software Arriba and STARFusion, and IGV for the detection of structural variants in the following genes and regions: NTRK1 all exons; NTRK2 all exons; NTRK3 all exons.
This panel is targeted for the detection of fusion partners for the following genes: NTRK1 fusion partner TPM3 (NM_152263.3). The panel will also detect novel fusion partner genes. A search of the COSMIC database (https://cancer.sanger.ac.uk/cosmic) is conducted for all structural variants detected.
This custom design Roche pan cancer panel has a validated sensitivity of 99% and specificity of 100% for known fusion variants (those previously identified by FISH or NGS), as determined by in-house validation using an input RNA amount of >50ng. A minimum of 38K total mapped reads (duplicates removed) is required for detection of non-intragenic fusion variants. For samples with <50ng input RNA the validated sensitivity of this assay is 88% and specificity is 100%. Sensitivity for rearrangement detection may be reduced in: samples with <30% neoplastic cells (in-house data) or samples with low expressed fusions [1].
NTRK1/2/3 fusions have been reported in 3.6% of patients with cholangiocarcinoma [4].
The reported results are dependent upon the analysed tissue representing the molecular makeup of the tumour in this patient.
Reference sequences: NTRK1 NM_001007792.1; NTRK2 NM_006180.4; NTRK3 NM_001012338.2.2
Please note that this test is not currently accredited by UKAS.
References: [1] Haas, B.J., et al (2019) Genome Biology 20:213. [2] Kumar-Sinha, C., et al 2015 Genome Medicine 7:129; Cocco, E., et al 2018 Nat Rev Can Oncol 15(12):731-747. [3] Hirao, S.J. et al (2019) J of Mol Diag 21(4):553-571. [4] #NICE, 2020 https://www.nice.org.uk/guidance/ta630/evidence/appraisal-consultation-committee-papers-pdf-87678378379

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