

NTRK Gene Fusion Testing Clinical Guidance

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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

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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.

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NTRK gene fusion testing request algorithm



NTRK Gene Fusion Testing Request Algorithm



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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).

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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).

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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 (<u>https://medicalgenomicswales.co.uk/index.php/health-professional-information/cysgodi</u>). 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').

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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:

- 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.

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NTRK gene fusion testing request process

NTRK-inhibitors are positioned as last-line treatment options for NTRK fusionpositive 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.

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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 <u>ALL</u> of the following recipients: <u>Domenica.Lear@wales.nhs.uk</u> <u>John.Bibby@wales.nhs.uk</u> <u>Akinwale.Akinola@wales.nhs.uk</u> <u>Gerrard.Fletcher@wales.nhs.uk</u> <u>Susan.Davies2@wales.nhs.uk</u> Louise Nash2@wales.nhs.uk
Cardiff and Vale	Mg.Cellpath@wales.nhs.uk
Hywel Dda	<u>WWGH.Histology@wales.nhs.uk</u> (laboratory) <u>HDD.Secretaries@wales.nhs.uk</u> (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

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Cardiff CF14 4XW Telephone: 02921845347 Email address: <u>Admin.Genetics.cav@wales.nhs.uk</u> Website: <u>http://www.medicalgenomicswales.co.uk</u> 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 <u>NTRK request</u> 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.

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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.

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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').

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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).

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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

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References

- Amatu A, Sartore-Bianchi A, Bencardino K, Pizzutilo EG, Tosi F, Siena S. Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer. Ann Oncol. 2019; 30(Suppl_8): viii5-viii15. doi: 10.1093/annonc/mdz383.
- Chen Y, Chi P. Basket trial of TRK inhibitors demonstrates efficacy in TRK fusion-positive cancers. J Hematol Oncol. 2018; 11:78.
- Hsiao SJ, Zehir A, Sireci AN, Aisner DL. Detection of tumour NTRK gene fusions to identify patients who may benefit from tyrosine kinase (TRK) inhibitor therapy. The Journal of Molecular Diagnostics. 2019; 21 (4):553-571.
- HGVS, 2020. Sequence Variant Nomenclature. <u>https://varnomen.hgvs.org/</u>
- NHS, 2019. The NHS Long Term Plan. https://www.longtermplan.nhs.uk/publication/nhs-long-term-plan/
- NICE, 2020^a. Single technology appraisal: Larotrectinib for treating NTRK fusion-positive advanced solid tumours [ID1299]. Committee papers. <u>https://www.nice.org.uk/guidance/ta630/evidence/appraisal-consultation-committee-papers-pdf-8767837837</u>
- NICE, 2020^b. Final appraisal document: Larotrectinib for treating NTRK fusion-positive solid tumours. <u>https://www.nice.org.uk/guidance/ta630/documents/final-appraisal-determination-document</u>
- NICE, 2020^C. Final appraisal document: Entrectinib for treating NTRK fusion-positive solid tumours. <u>https://www.nice.org.uk/guidance/ta644/documents/final-appraisal-determination-document</u>
- 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.

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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.2002;24:937-946; 8. Ricarte-Filib OJC, 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)

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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.

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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 fusionpositive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncology*. 2020; 21 (2): 271-282.

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Appendix 3: Prescribing information for Larotrectinib

	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).		
Dosage:	Co-administration with a administration with a stron larotrectinib dose should be been discontinued for 3 to should be resumed at the d inhibitor.	strong CYP3A4 inhibitors: If co- ng CYP3A4 inhibitor is necessary, the reduced by 50%. After the inhibitor has 5 elimination half-lives, larotrectinib ose taken prior to initiating the CYP3A4	
	DISCUSS WITH PHARMA	<u>CY</u>	
	Available as 25mg & 100mg 20mg/ml oral solution in 10 C-8°C). Expiry: 30 days afte	hard gelatin capsules. Also available as 00ml bottles. Store in a refrigerator (2° er first opening.	
	Can be taken with or witho juice.	out food. Avoid grapefruit or grapefruit	
Administration	If a dose is missed, the pa same time to make up for a next dose at the next sche taking a dose, the patient make up for vomiting.	tient should not take two doses at the a missed dose. Patients should take the duled time. If the patient vomits after should not take an additional dose to	
Administration:	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.		
	By Consultant / Registra professional.	r / appropriately trained healthcare	
Review clinic:	Clinical review 2 weeks after until disease progression, chooses to stop treatment, place.	starting, then every 4 weeks. Continue or unacceptable toxicity, or patient , or potentially curative surgery takes	

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Anti-emetics:	Nausea & vomiting are very common – consider co-prescribing an anti-emetic.			
Regular investigations:	FBC / U&Es / LFTsMonitor at baseline and every 2 w during the first month of treatm then monthly thereafter (based FDA SPC).		line and every 2 weeks month of treatment, thereafter (based on	
	Serum lipase / amylas	e Monitor at bas indicated.	eline and as clinically	
	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 reactions. Larotrectinib should be withheld until the adverse reactions or improves to baseline or Grade 1. Resume at the dose modification if resolution occurs within 4 weeks. Larotrectinib should be permanently discontinued if an ad reaction does not resolve within 4 weeks. Recommended dose modifications for adverse reactions. 			
Dose modifications:	Dose Modification	Adult and paediatric patients with body surface area of at least 1.0 m ²	Paediatric patients with body surface area less than 1.0 m ²	
	First	75 mg twice daily	75 mg/m ² twice daily	
	Second	50 mg twice daily	50 mg/m ² twice daily	
	Third	100 mg once daily	25 mg/m ² twice daily	
	Larotrectinib should be permanently discontinued in patients who are unable to tolerate treatment after three dose modifications.			
Main toxicities:	The most common adverse drug reactions (\geq 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.			
	vomiting (20%). The majority of advers	se reactions were Grac	le 1 or 2.	

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	Grade 4 adverse reaction and ALT increased (< 1% Grade 3 adverse reaction increased AST, dizzines leukocyte count decrease occurred in less than 5% (7%). Permanent discontinuation adverse reactions, rega patients (one case each perforation, jaundice, sm adverse reactions leading three months of treatme	s were neutrophil count decreased (1.6%) b). s were anaemia, weight increased, fatigue, ss, paraesthesia, nausea, myalgia, and ed. All reported Grade 3 adverse reactions of patients, with the exception of anaemia on of larotrectinib for treatment emergent rdless of attribution, occurred in 3% of of ALT increase, AST increase, intestinal nall intestinal obstruction). The majority of g to dose reduction occurred in the first nt.
	Neurologic reactions in	cluding dizziness gait disturbance and
Neurological toxicity:	For the majority of neuro first three months of trea Withholding, reducing, o be considered, depending symptoms.	r discontinuing larotrectinib dosing should g on the severity and persistence of these
Haematological toxicity:	Grade 3/4 anaemia, neut	ropenia & leukopenia have been reported.
Contraception:	Verify the pregnancy sta prior to initiating. Women effective contraception w month after stopping tr with a non-pregnant w should be advised to u treatment with larotrect final dose.	atus of females of reproductive potential n of childbearing potential must use highly hile taking larotrectinib and for at least one eatment. Males of reproductive potential yoman partner of childbearing potential use highly effective contraception during inib and for at least one month after the
Renal impairment:	No dose adjustment is re	quired for patients with renal impairment.
Hepatic impairment:	The starting dose of lar patients with moderate hepatic impairment. No patients with mild hepati Monitor liver tests includ first month of treatment indicated (based on FD)	otrectinib should be reduced by 50% in (Child-Pugh B) to severe (Child-Pugh C) dose adjustment is recommended for c impairment (Child-Pugh A). ing ALT and AST every 2 weeks during the then monthly thereafter and as clinically A SPC).

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	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.
Interactions:	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.

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Appendix 4:	Prescribing	information	for	Entrectinib
Appendix II				

	Entrectinib (ADULTS)	Initiate at 600mg po once daily continuous therapy.				
	Entrectinib (PAEDIATRICS > 12	Initiate at:				
	years old)	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 BSA>1.50m ² .					
Drugs/Dosage:						
	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.					
	Swallow capsules whole. Do not open, crush, chew, or disso contents of the capsule.					
Administration:	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.					

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	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.				
Anti-emetics:	Nausea & vomiting are very common – consider co-prescribing an anti-emetics.				
	FBC / U&Es / LFTs / serum urate		Monitor at baseline and then every 2 weeks during the first month of treatment, then monthly thereafter (based on FDA SPC) .		
Regular investigations:	Serum lipase / amylase		Monitor at baseline and as clinically indicated.		
	Left ventricular ejection fraction		Consider assessment of LVEF before initiating treatment.		
	ECG		Consider assessment of QT interval in those at risk of prolongation.		
	Recommended d	lose re	ductions f	or adverse react	ions.
Dose modifications:	Action	Adults and Paediatric Patients 12 Years and Older with BSA Greater than 1.50 m2 (Orally once daily)		Paediatric Patients 12 Years and Older with BSA of 1.11 to 1.50 m2 (Orally once daily)	Paediatric Patients 12 Years and Older with BSA of 0.91 to 1.10 m2 (Orally once daily)
	First dose reduction Second dose reduction*	400m 200m	g g	400mg 200mg	300mg 200mg
Main toxicities:	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.				

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	Congestive heart f	ailure (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 n fraction, MRI or o diagnosis. For new reassess LVEF and Reduce dose or pe or worsening LVEF	nyocarditis, v cardiac biop v onset or w l institute ap rmanently d	with or without a decreased ejection sy may be required to make the orsening CHF, withhold entrectinib, propriate medical management. iscontinue based on severity of CHF
	0	D	
	Severity	Dosage Mo	
	Grade 2 or 3	 Withhold than or e Resume 	entrectinib until recovered to less equal to Grade 1.
	Grade 1	 Resume Dermane 	ntly discontinue
	Graue 4	• Fernane	
	QTc prolongation:		
Cardiac toxicity:	Monitor patients of prolongation. Asse periodically during or reduced dose, of severity.	who have of ess QT interv treatment. or permanen	r who are at risk for QTc interval val and electrolytes at baseline and Withhold and then resume at same tly discontinue entrectinib based on
	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 pointe polymorphic vent tachycardia;	es; ricular	Permanently discontinue entrectinib.
	signs/symptoms arrhythmia	of serious	
	Other drugs that p	orolong QT ir	iterval:
	QTc interval prolo administration of potential to prolon	ngation can entrectinib g QT/QTc in	occur with entrectinib. Avoid co- with other products with a known terval.

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	CNS adverse reaction disorders, dizziness, a entrectinib. Withhold and then re improvement or perma	s including cognitive impairment, mood and sleep disturbances can occur with esume at same or reduced dose upon nently discontinue based on severity.
	· · · · · · · · · · · · · · · · · · ·	
	Severity	Dosage Modification
Neurological toxicity:	Intolerable Grade 2	 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
	Grade 3	 Withhold entrectinib until recovery to less than or equal to Grade 1 or to baseline. Desume at reduced doce
	Cuede 4	Resume at reduced dose.
	Grade 4	Permanently discontinue.
	the first month of trea clinically indicated. entrectinib based on se same or reduced dose b	atment, then monthly thereafter, and as Withhold or permanently discontinue everity. If withheld, resume entrectinib at based on severity.
	Soverity	Dosage Modification
	Grade 3	 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.
Hepatic impairment:		 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.
	Grade 4	 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.

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NTRK

		Permane Grade 4	ently discontinue for recurrent events.
	ALT or AST >3 t ULN with concur total bilirubin > times ULN (in th absence of cholestasis or haemolysis).	imes • Permane rent 1.5 e	ently discontinue entrectinib.
Hyperuricaemia:	Assess serum un during treatment symptoms of hype medications as of signs and symp reduced dose upo Severity Symptomatic on Grade 4	ic acid levels pric with entrectinib. eruricemia. Initiate clinically indicated toms of hyperur on improvement b Dosage Modifi • Initiate urate • Withhold ent signs or sym • Resume entre dose.	r to initiation and periodically Monitor patients for signs and e treatment with urate lowering and withhold entrectinib for icemia. Resume at same or ased on severity. cation -lowering medication. rectinib until improvement of ptoms. rectinib at same or reduced
Visual disturbances:	Withhold for new activities of daily an ophthalmologi or reduced dose u Severity Grade 2 or above	 visual changes of living until improvised evaluation as upon improvemen Dosage Modifi Withhold ent stabilization. Resume at sa clinically app 	or changes that interfere with ement or stabilization. Conduct appropriate. Resume at same t or stabilization. cation rectinib until improvement or ame dose or reduced dose, as ropriate.
	Se	everity	Dosage Modification

		Severity	Dosage Modification
Haematological toxicity:	Anaemia or Neutropenia	Grade 3 or 4	 Withhold entrectinib until recovery to less than or equal to Grade 2. Resume at the same dose or reduced dose, as clinically appropriate.

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	Severity	Dosage Modification		
	Grade 3 or 4	• Withhold entrectinib until adverse reaction resolves or improves to recovery or improvement to Grade 1 or baseline.		
Other clinically relevant adverse		 Resume at the same or reduced dose, if resolution occurs within 4 weeks. 		
reactions:		 Permanently discontinue if adverse reaction does not resolve within 4 weeks. 		
		 Permanently discontinue for recurrent Grade 4 events. 		
	Entroctinih incroa	sos the rick of fractures. Promptly avaluate		
Skeletal fractures:	patients with signs or symptoms of fractures.			
Serum lipase / mylase;	Raised serum lipase and amylase have been noted in clinical trials and consideration to pancreatitis is needed.			
	Verify the pregnancy status of females of reproductive potential prior to initiating.			
Contraception:	Advise female patients of reproductive potential to use contraception during treatment with entrectinib and for a 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.			
Renal impairment:	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).			
	Co-administration of entrectinib with a strong or moderate CYP3 inhibitor increases entrectinib plasma concentrations, which coul increase the frequency or severity of adverse reactions.			
	Moderate & strong CYP3A inhibitors:			
Interactions:	• Adults and paed Avoid co-administ with entrectinib. I entrectinib.	diatric patients >12 years with BSA>1.50 m2: ration of strong or moderate CYP3A inhibitors of co-administration is unavoidable, reduce the		
	• Paediatric patients 12 years and older with BSA \leq 1.50 m2: Avoid co-administration of entrectinib with moderate or strong CYP3A			

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	Moderate and strong	CYP3A induce	ers:			
	inhibitors. Avoid grape contain inhibitors of CYI	fruit products (P3A.	during treatn	nent, as	the	y
NHS Wales Health	Collaborative			N	ΓRK	
				Paper H	ker:	
	NHS Wales Health	NHS Wales Health Collaborative inhibitors. Avoid grape contain inhibitors of CYI Moderate and strong	NHS Wales Health Collaborative inhibitors. Avoid grapefruit products contain inhibitors of CYP3A. Moderate and strong CYP3A induce	NHS Wales Health Collaborative inhibitors. Avoid grapefruit products during treatn contain inhibitors of CYP3A. Moderate and strong CYP3A inducers:	Paper F NHS Wales Health Collaborative NT inhibitors. Avoid grapefruit products during treatment, as contain inhibitors of CYP3A. Moderate and strong CYP3A inducers:	Paper Ref: NHS Wales Health Collaborative NTRK inhibitors. Avoid grapefruit products during treatment, as the 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

Reason for	Referral :				
Date reported	a :: 01/09/2020			Alt Hosp No	+
Date Rec'd	1 Transe-sides 1 01/09/2020			Your set	1 block ID as
Sex : F Sample type : Tissue s				Hospital No	t A2Z
	5 K	SA37 DEX	5A17 0FX	NHS No	Ŧ
DoB	1 01/01/2018	Address	7 1 dummy address record	Lab No	1-1
Report on	A Dumi JONES				

NTRK1/2/3 gone rearrangement analysis requested on this intrahepatic cholanglocarcinoma 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 ful clinical details.

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

db

UKAS

Analysed by:	in this differ yord
100040000000	Checked by:
Halan Poham	Division Million
Clinical Scientist	Display Claim Calentist
Test details: Following assessment of the tumour samp (dolimated) at xrSi) was identified; this area	As from this patient by a pathologist (block no: xo), the area of highest apoplastic cell content was macrodissected and RNA extracted for analysis. RNA extraction performed caring Maximil
analysis using in-house bioinformatic targe dataction of structural variants in the follow This pamils is targeted for the detection of i site detect nevel fusion performance. A s variantic detected. This custom design Roche pan cencer pan eventuels detected of the ISU or MICO.	Here NTMK samet endpointing fusion prediction software AmBia and STARF samet, and IGV for the ing parties and regions. NTMKH is a social, NTMKA and annum NTMKA all advants, users partners for the following general. NTMKH fusion partner TMMAI, MM, 1522(83,3). The generi will earch of the COBING database (https://wancer.samger.ac.uk/cosmic) is constanted for all shortcome of this a variabled sensitivity of 9% and specificity of 10% for traces having variated throwing the same of the set of the set of the set of the sensitivity.

Its 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 University Hospital Of Wales Heath Park Cardiff CF14 4XW

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Head of Laboratory: Sian Morgan, FRiCPath

Intellate of Medical Genetics, University Hospital of Wales, Health Plan, CARDINF CF14-40W Tel: 109:20742541, Fac: 2019 20744558. Setychist Generalg Feddygal, Ysbyty Athrefaol Cymru, Parcy-Mynydd Bychan, CAERD YOD CF14 4XW, Flan Coll 2014/2014 Flans, rolin bringaran website: http://www.wales.nhs.uk/awmgs

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A Oncologist

University Hospital Of Wales Heath Park Cardiff CF14 4XW

da USAS website: http://www.wales.nhs.uk/awmgs feri tra i latori

Head of Laboratory: San Morgan, FRGPath

Institute of Meeting/ Genetics, University Holg Hearty Park, CARDIPP CP14 400V, Tel: 029 20742541, Fax: 029 20744059 Setudiad Genergy Fedoryd, Ysbyb Atholiau Cyn Parc y Merydd Bydran, CAERDADD China awy Plan 029 20142541, Planz 029 20144059

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2. NTRK analysis report – NTRK gene fusion detected

All Wales Molecular Genetics Laboratory			NTRK analysis			
N	TRK analysis		Analysed by:	Checked by:		
Report of : A Dumi JONES DeB : 01101/2018 Add Sex : P	reas : 1 dummy address record	Lab No I -1	Helen Roberts	Rhian White		
Sample type 1 Tissue-sides Date Roa'd 7 01.092020 Date reported 1 01.092020	, SAJ7 DEX	Hospital No 1 A22 Your ref : block (Dive Alt Hose No 1	Test datatis Poloaring assessment of the turnour sample hom field/mailed at xx5-1 was identified, the area was n	this patient by a pathologist (Nock no. ss), the area of highest neighbolic cell content		
Reason for Referral : NTRK1/2/3 gene rearrangement analysis reque	ssted on this intrahepatic cholangio	carcinoma sample.	RSC PTPE RNA kit (Promega AS 1440): concern Nost generation sequencing was performed using analysis using in-house bioinformatic largeted NT detection of structural variants in the following get	Individual of the source of th		
Conclusion: This patient may respond Test results: TMP3-NTRK1 gene fusion No gene fusions involving NTRK2 or NT	to TRK inhibitors. detected; #HGVS nomenclatu RK3 detected.	iro#.	This panel is targeted for the detection of Vasion p also detect novel fusion partner genes. A sainch or variants detected. This custom design Roche pan cancer panel has proviously identified by FIBH or NOB), as dearmin mapped roads (duplicates removal) is required for	atman for the following period. INTEXT fistion pattern FPK0 (ML, 162263.2). The pares of the COSMC distance (https://amore.emisperiod.cos/cosmo/bits/conducted for all athouts a validated semilihety of 99% and specificity of 100% for shown fusion validates (these med to in-house validation using on input RNA emount of >60m), A minimum of 38k data of administon dimensionalization variants. For somalizes with <50m (the) RNA RNA with the specific semilation of the state of		
The RNA-based NGS analysis of the tumour sa Analysis showed no evidence of a gene fusion i	mple from this patient detected a T rivolving the NTRK2, or NTRK3 get	IMP3-NTRK1 gene fusion. nes.	Benefit way or the assay is 50% and specificity is 1 nooptastic cells, in-house later or sampling with te Reprivingements are reported according to HOVS differential expression of genes, it is not possible t assay.	30%. Semistrikt for normsgement debc/son may be reduced in: samples with <20% exemptions durings [1], non-endature (https://www.exemption.com/bg/material/consultation/sed-eg007/). Owing to determine the proportion of tumour cells carrying any shudatal variants detected using a determine the proportion of tumour cells carrying any shudatal variants detected using a determine the proportion of tumour cells carrying any shudatal variants detected using a determine the proportion of tumour cells carrying any shudatal variants detected using a determine the proportion of tumour cells carrying any shudatal variants.		
In params with functions harbouring and trick g associated with high objective response raties [5 TRX inhibitors are recommended for use as an children if the disease is locally advanced or me patient has no other satisfactory treatment optio determined in the context of this patient's ful oil Patient-specific testing information: <50ng RNA rearrangement detection.	ene usion, treatment with 3 rec and option for treating NTRK fusion-po- tastatic, or surgery could cause se rs [6]. The implication of this result rical debats, was used for RNA-sequencing, pro	buons has been shown to be silve solid turnours in adults and vere health problems, and the for this patient should be sviding an 88% sensitivity for	Only addressible INTRK fusions are reported. To be the fusion purchasm and the typestre interaction addressibilitie ligant branching domain [2]. NTRK-0220 suscerim news down reported at 3.05% of The typestrat requires an dependent copion the restly the typestrat requires an dependent copion the restly Presses are the fit has test in the C4 annothy Accredite References [1] Hams, bull, et al. 2019 (Second B 2018 Nat Rev Cille Choose (12) (2131-174-17, 12) Halps trips. Jowns nets ong uksgutteen extend/of/weddenceb [2] References in Timot days are RVCE, 2000 https:// 18] References net Timot days are RVCE, 2000 https://	considered aclicrable. The gard facies must reach the continuous apain reaching theme a of the protein (2). Aclicinative IRTRA Nasions are also facial by the abaance of the patients with chicking occardioma (4), nod tissue report only the insteadar makeup of the tamour in this patient, and the control (2), and the other advances and the tamour in this patient, body (VAR). In Original Control (2), and the other advances (2), and the tamour in this patient, body (VAR). S - 3, et al (2)(2) of MV Diag 2), and (2)(3)(3)(3), and (2)(3), and (2), and (2), the opportable consultation committee-papers and F8783378379. In Distances (2)(3)(1) The New Eng. J of Med 205, 731-739. Doebeles: R, C, et al. (2) Verse recency, articulation committee-papers and F8783378379.		
			Coales to. Results are dependent on Plasses not	semples being conrectly labelled and family relationships as indicated. a any nemaining DNA will be stored in the laboratory.		
A Omcologist University Hospital Df Wales	Head of Labora	tory Sian Morgan, FRCPath				
Heath Park Cardiff CF14 4XW	Heath Pain, CARD Tel 102 AUT2341 UKA55 Material Mediat Menuty Material Mediat Menuty	AFF CF14.40W, Factors 2014/008 Factors 2014/008 Factors 2014/008 Factors 2014/008 F. Place CdR 2014/2018 P./Www.wales.nhs.uk/awmgs	A Oncologist University Hospital Of Wales Heath Park Cardiff	Head of Laboratory. Sites Morgan, FRCPain Instate of Network Greeks, University Hispate of Wales, Instant Park, CARONT FC 711, 2019, Tel (09) 20742141, 545, 529 20744039.		
Page 1 of 2 -1	Roberto Mr. 520	1011	CF14.4XW	Sefeliet Gerang Pethogal, Ystyp Athonas Cyrvu, Petroy-Merged Bychur, CAERDrub CFI 8, 000, Plan, CO241644, France Car 20044649, worked bits http://www.selie.com/petro/en/		

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

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

All Wales Melsoules Constine Labor	atom	Lung analysis		
All Wales Molecular Genetics Labor	atory	Analysed by:	Checked by:	
Lung analysis				
Report on : A Dumi JONES DoB : 0101/2016 Address 1 t dummy address record	Lab No. 1. 1	Helen Roberts Clinical Scientist	Rhian White Principal Clinical Scientist	
Sar : F Sample type : Trissue stides Date Rect : 0.1008/2020 Date reports : 0.1008/2020	NHS No : Hospital No : A22 Your ref : block ID xx Alt Hosp No :	Test details: Following assessment of the tamour sample from (estimated at whi) was identified; this area was RSC FFPE RNA at (Promaga AS1440); concent Noti generation sequencing was performed usin	In this partiant by a pathologist (block no: w), the area of highest negatable cell contant microblasected and IRNA estracted for analysis. RNA estraction pathomed using Maxwall Instance of RNA using Zymo RNA clean and concentrator is a performed where regard, a Roche can concerp pare (outpendicated) and solutioned on an ill marve pathom. Data	
Reason for Referral :		analysis using in-house bioinformatic targeted to splicing software and IGV for the detection of stri	ng panel, employing fusion prediction software Amba, STARFusion, and rMATS transcript uctural variants in the following gates and regions: EGFR exons 1-8 (region relevant for	
ALK, ROS1, NTRK1/2/3 gene rearrangement analysis requested on this non-small Note: Pan NGS analysis of DNA has also been requested on block ID: xx (our ref: 2 report will be issued.	cell lung cancer sample. (OMix) for which a separate	detection of EXPRAID through departs, ALX exp eleipting events, RET all exons, ROS1 all exons This panel is targeted for the detection of fusion (NRL 0045212); RET fusion partners; CCDC6 (NI SLC3442 (MML 0046412); EZR (NML 001116077) (NML 152246.31). The panel will also detect novel.	onts 10 and 22, McT evont 13, 14 and 15 (regon nilewarit to divertification of MET evon 16 NTRK1 intervals, NTRK2 all evones. NTRK3 all evolutions. partners for the reflowing genes. Ack fusion partners ENA (NL, 010023 4) and KP50 (NL 00216 4), and KF56 (NL, 0026923 2) all K015 that is partners (TAVI (NL, 01023163 2) (NL 00216 4), and KF56 (NL, 0026923 2) and K154 (NL 010523 4) and K550 (NL 00216 4), and KF56 (NL, 002692 2) and K154 (NL 010523 2) (NTRK1 haven gentine THMS) (NL 00216 4), and (NL 022692 2), and THK2 (NL 052262 2) (NTRK1 haven gentine THMS) (NL 00216 4), and (NL 022692 2) and (NL 022692 2) (NTRK1 haven gentine THMS) (NL 00216 4), and (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692 2) (NTRK1 haven gentine THMS) (NL 022692 2) (NL 022692 2) (NL 022692 2) (NTRK1 (NL 022692	
Conclusion: This patient may respond to TRK inhibitors. This patient has a reduced likelihood of response to treatment with in' 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 and MET exon 14 skipping variant were NOT detected. Test results for EGFR hotspot variant analysis will be reported separat	nibitors specifically #. EGFRvIII structural variant lely.	Is consistent for all structural variants elected. This spaced begins Roche para conserte para la lange previously, identified by FIBH or NGS, as a determ mode is negative for diffeomory on intragenic, fusione Fer samples with SGNg input RNA Rev variabled Restangements are regolarized according to HOV otherential expression of genes, it is not possible 5669. Only actionate ALK, RET, RDS1 and MTRS (has com stading Tama at The busine junction and more statistical according to HOV.	a validated sensitivity of 20% and appecticity or 100% for known factors variants (Risos med by in-backs variants and any an input RNA encourt of *20%), a mission of 5 using a major RNA encourt of *20% and an encourted and encourted of *20% and any	
The RNA-based NGS analysis of the tumour sample from this patient detected a TM Analysis showed no evidence of a gene fusion involving the ALK, RET, ROS1, or NT evidence of an EGFRVIII structural variant or MET exon 14 skipping variant within the	P3-NTRK1 gene fusion. RK2 or 3 genes, and no is sample.	Writing of an extension of NSCLC. The theorem, of certain 1.54% [b]; RET (solens: 0.0% [r]) ROST Inserts: The regorded results are dependent upon the area Reference sequences. ECPR NM, 0052ES, 3(1/6) NM, 005075 4.7(1/6), 51% ROST NM, 0052ES, 3(1/6)	11-0 Local variants in: EIGFRVIII: 0-1% (4): ALK fusions: 3,7% (5): MET even 14 skipping events 1-3% (5): NTRK fusions: up to 35% (5) synd trover prepending the molecular makeup of the tumour in this patient 3, 240 (ALK NM DODDA STURE): 48811; MET NM D01127500: 1 (LRC) 4882; HET URG; 3071; NTRK 1M, D010075; 1: NTRK 2M, MO10127500; 1 (LRC) 4882; HET URG; 3071; NTRK 1M, D010075; 1: NTRK 2M, MO10127500; 1 (LRC) 4882; AME	
In patients with tumours harbouring an NTRK gene fusion, treatment with TRK inhibit associated with high objective response rates [10].	tors has been shown to be	Please note that this test is not currently accredity References [1] Haas, B.J., et al (2019) Genome I 2018 Nat Rev Clin Oncis (5(12):731-747, [3] Heat	ed by UKAS Biology 20:213. [2] Kuman-Sinha. C., et al 20:16 Genome Medicine 7:129: Cocco. E., et al c. S. J. et al (20:19). J of Mol Dag 21(4):553-571. [41 Gan. H.K., et al (20:13). The PEBS	
TRK inhibitors are recommended for use as an option for treating NTRK fusion-positi children / the disease is locally advanced or metastatic, or surgery could cause seve patient has no statisfactory treatment options [11]. The implication of this result for the in the context of this patient's full clinical details.	ive solid tumours in adults and ne health problems, and the s patient should be determined	Journal 200(21):0350-9370, (3) Saussi, T., and Ju 21(4):431-438. (7) Cancer Centrem Artise Researc Cancer Research. 10(15):4040-4045. (8) Visatima (10) References in treatment response. EOFR – in currently approved treatments target 1 AUX – Halberg, B. and Patriner, R.H. (2011) FTO	rame, P.A. (2011) Clin Cencer Res 17 22(3):724 (2):	
Patient-specific testing information: >50ng RNA was used for RNA-sequencing, provi rearrangement detection.	ding a 99% sensitivity for	MET – no currently approved treatments target MI RET – no currently approved treatments target RI ROST – Shaw, AT, et al. (2014). N Engl J Med. 2 NTRK1/2/2 – Onton, A. et al. (2018). The New Eng (11) Reference ne TRK drug user NICE, 2020 https and https://www.nice.org.uk/guidance/ta/644/chogu	ET reamagnements in NSGLC 17 maamagnements in NSGLC 0. 371(2) / 1905-1977. Drivin, A. et al. (2020), The Lancet One 21(2), 281-270. 1 of Med 374.8, 71-38. Doteber R. C. et al. (3020), Lancet Oneology 21 (2), 271-382. knowne new org vikguidanterijed stri0229/boouments/inal-appraisal-defermination-document mil-Recommendations	
		Copies to: Results are dependent or Please not	s samplies being connectly lisbelied and family relationships as indicated. In: any remaining GNA will be stored in the lationatory.	

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Institute of Medical Genetics. University Hospital of Wales, Heath Park, CARD/EF CF14 40W. Tel: 539 20742541, Park 029 20742059 Setvitiad General Fedfigol, Tsbyly Alimotaci Cymru. Paro y Mynydd Sychae, CAEPROVDD CF14 4007. Pfor: 129.20742041, Placa 029.20744010.

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

All Wales Molecular Genetics Laboratory NTRK analysis			All Wales Molecular Genetics Laboratory NTRK analysis			
Reason for Referral :		All Hosp no. 1	Reason for Referral :			
TRK1/2/3 gene rearrangement analysis re	quested on this intrahepatic cholangios	arcinoma sample.	NTRK1/2/3 gene rearrangement analys	is requested on this intrahepatic cholanging	ocardinoma sample,	
conclusion: FISH analysis for NTRK	1, NTRK2, and NTRK3 has been i S analysis	nitiated.	Conclusion: FISH analysis for N Test results: NGS analysis failed	TRK1, NTRK2, and NTRK3 has been insufficient quality RNA for NGS a	i initiated. nalysis	
he RNA-based NGS analysis of the turnou NA obtained from the tissue sample.	r sample from this patient has not been	initiated as there was insufficient	The RNA-based NGS analysis of the tu required quality metrics were not achiev commonly associated with FFPE tissue	mour sample from this patient has unfortu red for this sample. This failure is most like	nately failed to give a result. The sly due to poor quality RNA which is	
he FISH salvage pathway has been initiate eported within 14 calendar days.	d. Results from FISH analysis for NTRI	K1, NTRK2, and NTRK3 will be	The FISH salvage pathway has been in reported within 14 calendar days.	tiated. Results from FISH analysis for NTI	RK1, NTRK2, and NTRK3 will be	
the implication of this result for this patient Analysed by:	should be determined in the context of t Checked by:	his patient's full clinical details.	The implication of this result for this pati Analysed by:	ent should be determined in the context of Checked by:	f this patient's full clinical details.	
lelen Roberts Jinical Scientist	en Roberts Rhian White ical Scientist Principal Clinical Scientist		Helen Roberts Clinical Scientist	Rhian White Principal Clinical Scientist		
diaverg a sessement of Pan Lurour avergin from this bitmetic at xx50 kines bitmetic averages near wars more DC FPPE RNA MI (Promegin AS1H40), concentration rakes tei Recuts are digerented on sam Please note, an	alteret tre a certrologie (Block no: ski) the energi descend and RNA extended for analysis. RNA of RNA using Zymo RNA others and concentrato of RNA using Zymo RNA others and concentrato plete training CNA will be altered in the latteretory y remaining CNA will be altered in the latteretory	Pulphet inexplaisit cell context extraction performed using Maxwell kit performed where required.	Following basesment of the tumour sample how (settrated et Jol) was devided, the area was in RBC. FFRE RNA to Promega AS1440; concern RSG settration sourced and the settrate of the statistic using in-house bioinformatic supposed to the setting and the setting and the setting and setting and the setting and the setting of source also detect invert lister participation of source parel has detect in any setting and the setting and the setting and the setting and the setting and the Please node that this set is not conserve and the Reference [1] Has as [1, et al (2019) Genome E 2016 Nat Riv Cin Chaol Tol (15/12) 71-71,71 [2] de- tates and the setting and the setting and the setting the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting the setting and the setting and the setting and the setting and the setting and the setting and the setting and the setting the set of the setting and the setting and the setting and the setting the setting and the setting and	This patient by a pathologist Dirock no: white uses a proceedings of the MA estance for the manyles. RNM above or RAMA using Zyme RNMA clean and concernant RNC panel, employing Laking presection address and use manyles and the second second second second second second second second second second second second address and the RAMA second second second second address address and the RAMA second second second address add	of higher modelacts cell content or higher heritage in prevention and the provide profitting and there required. The entroped in previous particular and STARFusion, and GMV for the an and STARFusion, and GMV for the Accesser is accessed and an analysis to a set of the analysis of the structural between the structural the structural is determined. The structural is determined the structural in samples with <30% we function if this patient. anne Medicine 7:120; Cosco, E., et al MVCE, 2000 756128	
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VERSION			A Oncologist	Head of Labor	atory: Sian Morgan, PRCPath	
A University Hospital Of Wales. Haath Plan Candiff CP14 45W	read of Laboration	ny, aran margan, marganar ol wake, PF OF14 400, Pre D23 4400, Pre D23 44633, Pre D23 2074453, Pre D43 5074453, Pre D43 5074453, Pre D43 5074453, Pre D43 5074453, Pre D43 5074453,	University Hospital Of Wales Health Park Cardiff CF14 4XW	Verbolic Made Verbolic terror australia Verbolic terror australia Verbolic terror australia Verbolic terror australia	al Develop Linkers University Respiral of Water. Diff CPT 4 4500 15 Aug 082 ACT 44353 9 Footback, Yoshy Attributed Cyress and Chern Develop CPT4 2000 417 Place 023 ACT 44523 bp//www.wales.hs.uk/atwimgs	

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