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NHS Genomic Laboratory Hub

# A Guide to North West GLH ctDNA Reports

## Report - Page 1

**North West Genomic Laboratory Hub (Manchester Site)**
Manchester Centre for Genomic Medicine
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NHS North West NHS Genomic Laboratory Hub

| | | | | | |
|---|---|---|---|---|---|
| Forename: | **ctDNA** | Report No: | **R25-00FM-1** | | |
| Surname: | **Booking** | Hospital No: | **123456** | | |
| Sex: | **Female** | Other No: | **Not provided** | | |
| DoB: | **01/08/2025** | Sample: | **Blood** | Sample2: | **Blood** |
| NHS No: | **111 111 1111** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Collected: | **Not provided** | Received: | **04/11/2025 11:16** | Activated: | **05/11/2025** |
| | | Received2: | **04/11/2025 11:16** | Reported: | |

Referred by: Dr Matt Krebs, Clinical Oncology, The Christie Hospital, Manchester, M20 4BX

### Genomics Laboratory Report

**Reason for Testing**
This patient has been referred for non-small cell lung cancer circulating tumour DNA (ctDNA) testing (M4.14).

**RESULT SUMMARY:**
**Significant Variant(s) Detected:**
KRAS c.34G>T p.(Gly12Cys)
BRCA2 c.1837_1840del p.(Ile614ThrfsTer29)
TP53 c.488A>G p.(Tyr163Cys)
**ctDNA Tumour Fraction:** 15%
**Tumour Mutational Burden:** 3 Muts/Mb
**MSI Status:** Microsatellite Stable

**Result and Interpretation**
**Essential target gene variant(s) (including SNVs, CNVs and structural variants):**
KRAS c.34G>T p.(Gly12Cys) 15% VAF

This patient's ctDNA sample has a detectable KRAS variant which increases the likelihood of a response to KRAS G12C inhibitor therapy. Please refer to the current NICE guidance regarding KRAS inhibitor therapy[1].

No other clinically significant essential target gene variants were detected in this patient's ctDNA sample.

**Clinical trial target gene variant(s)(including SNVs, CNVs and structural variants):**
BRCA2 c.1837_1840del p.(Ile614ThrfsTer29) 49% VAF
TP53 c.488A>G p.(Tyr163Cys) 0.5% VAF

**Please note that the BRCA2 oncogenic variant is at increased likelihood of being of constitutional origin[2,3].**

We recommend forwarding a blood sample (minimum 3mls EDTA blood) to determine whether the variant is of germline origin. Please see our website for details on referring for this test[6].

**In conclusion, a clinically actionable variant has been detected and further tumour tissue genomic testing is not indicated.**

Note: Please see Page 2 for technical details of the analysis and references (App I) and details of any variants reported (App II). Please find attached the Foundation One Liquid CDx report for comprehensive results and further test methodology details.

Reported By: Authorised By:

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**Patient demographics:** Includes patient identifiers and sample details.

**Reason for Testing:** This will list the testing which has been referred: breast cancer (M3.13) or lung cancer (M4.14). Any prior or ongoing testing will also be listed.

**Results Summary:** Summarises the key findings, including ctDNA tumour fraction, tumour mutation burden, and microsatellite instability status. May include the note "See Result and Interpretation" as a prompt to review the full report thoroughly to ensure appropriate interpretation.

**Result and Interpretation:** Includes details of the variants identified, grouped under Essential Target Genes and Clinical Trial Targets. Each variant is accompanied by an interpretation of its clinical significance. Where relevant, a comment on the likelihood of germline origin or the possibility that a variant may have arisen from clonal haematopoiesis of indeterminate potential (CHIP) is given. Further testing recommendations will be included where appropriate.

**Notes:** Directs the reader to the appendices (page 2), where information on the variants detected, references, and guidelines used is found.

**Conclusion:** This section then concludes with a statement around the informativity of the testing, which is influenced by variant(s) detected, and ctDNA tumour fraction.

# A Guide to North West GLH ctDNA Reports

## Report - Page 2

Forename: **ctDNA**
Surname: **Booking**

Report No: **R25-00FM-1**
DoB: **01/08/2025**

***APPENDIX I - Test Methodology:***

*Extraction Type: KingFisher cfDNA Extraction*
*FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage. This assay also determines: Microsatellite (MS) status, Blood Tumour Mutational Burden (bTMB) and ctDNA Tumour Fraction. For further details please see Foundation Medicine report attached. Clinical trials and treatments mentioned on the Foundation Medicine report are not always up to date or applicable in the UK.*

*Only variants analysed in-house and classified as oncogenic/likely oncogenic in the following target genes have been listed on the GLH report. Variant classification has been performed as per the relevant guidance[5]. Additional variants may be listed on the attached Foundation Medicine report.*

***Please note that the variant nomenclature may differ between the NWGLH report and the Foundation Medicine report. Our variant nomenclature follows international HGVS standards. For any queries, please contact the laboratory using the email provided below.***

*Essential target genes:*
*Test Directory Subpanel analysed: M4.14 - EGFR, ALK, BRAF, KRAS, MET, ROS1, RET, NTRK1, NTRK2, NTRK3*
*Clinical Trial Targets: BRAF, BRCA1, BRCA2, CDK12, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, IDH1, IDH2, KRAS, MET, MLH1, MSH2, MSH6, NF1, NF2, NRAS, PALB2, PIK3CA, RAD51C, RAD51D, TP53*

*The FoundationOne Liquid CDs assay detects copy number loss only in the following genes: BRCA1, BRCA2, PTEN*

*Please note germline variants may be identified, however, this is a somatic assay and these results are not intended to substitute germline testing. If a patient meets rare disease eligibility criteria according to the national test directory, they should be referred through routine rare disease pathways.*

*If high clinical suspicion exists of an actionable alteration to due the clinical scenario, tissue testing can still be undertaken. Please contact your local GLH should this testing be required.*

*Local Email: mft.northwest.ctdna@nhs.net*

***References:***
*1) https://www.england.nhs.uk/cancer/cdf/cancer-drugs-fund-list/*
*2) Kuzbari, Z., C. Bandlamudi, C. Loveday, et al., Germline-focused analysis of tumour-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations. Ann Oncol, 2023. 34(3): p. 215-227*
*3) McVeigh, T. P., Hanson, H., Burghel, G. J., Turnbull, C., & Snape, K. (2025). Proposed framework for triage of putative germline variants detected via tumour genomic testing in UK oncology practice. Journal of medical genetics, 62(11), 709–719.*
*4) Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2022;33(8):750-768.*
*5) Burghel, G. J., Mason, J., et al. (2025). Association for Clinical Genomic Science (ACGS) guidelines for the classification of oncogenicity of somatic variants in cancer: recommendations by the UK somatic variant interpretation group (SVIG-UK). Journal of medical genetics, jmg-2025-111046. Advance online publication. https://doi.org/10.1136/jmg-2025-111046*
*6) mft.nhs.uk/nwglh*

***APPENDIX II - Variant Details and Classification Evidence:***

| | |
|---|---|
| ***HGVS description:*** *KRAS c.34G>T p.(Gly12Cys)* | ***Classification:*** *Oncogenic* |
| ***Genomic coordinates:*** *null* | ***Variant allele frequency (%):*** *15* |

| | |
|---|---|
| ***HGVS description:*** *BRCA2 c.1837_1840del p.(Ile614ThrfsTer29)* | ***Classification:*** *Oncogenic* |
| ***Genomic coordinates:*** *null* | ***Variant allele frequency (%):*** *49* |

| | |
|---|---|
| ***HGVS description:*** *TP53 c.488A>G p.(Tyr163Cys)* | ***Classification:*** *Oncogenic* |
| ***Genomic coordinates:*** *null* | ***Variant allele frequency (%):*** *0.5* |

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|---|
| ***Tumour mutational burden (TMB):*** *3* |

| |
|---|
| ***Microsatellite instability (MSI) status:*** *No evidence of microsatellite instability (MSS)* |

| |
|---|
| ***Tumour fraction (%):*** *15* |

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**Test methodology:**
Provides further details of the assay and how variants are classified and reported.

**Genes targeted:**
Lists the Test Directory subpanel analysed and the Clinical Trial targets.

**References:**
A list of references are provided, including the Cancer Drug Fund, any guidelines or recommend-ations utilised, and any references relating to the specific findings identified, where appropriate.

**Appendix II:**
Provides further details about any relevant findings identified, including the classification applied.

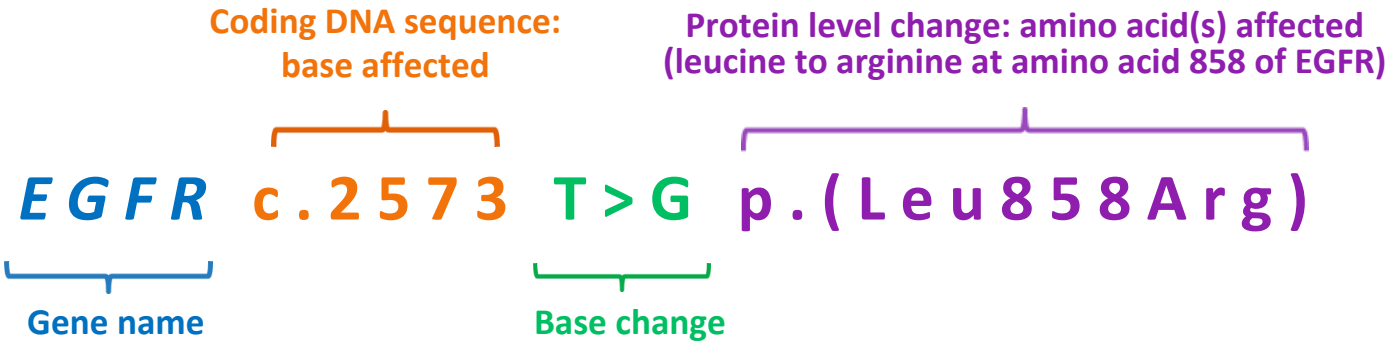
# Key terms and statements

| | |
|---|---|
| **ctDNA Tumour Fraction** | *Represents the proportion of circulating tumour DNA within the total cell-free DNA. Low tumour fraction may limit the ability to detect variants and will be highlighted in the report, with a comment regarding the increased risk of false negative results where appropriate.* |
| **Tumour Mutational Burden** | *Indicates the total number of somatic mutations per megabase of DNA. This is reported as a numerical value (mut/Mb). High TMB may indicate potential eligibility for immunotherapy treatment or clinical trials.* |
| **MSI status** | *Microsatellite instability (MSI) status will be reported as stable (MSS) or MSI-high (MSI-H). MSI-high results may indicate potential eligibility for immunotherapy. Indeterminate results will include a comment to consider repeat sampling or alternative testing if clinically indicated.* |
| **See results and interpretation** | *If any important further details need considering, the note "See Results and Interpretation" will appear in the Results Summary box as a prompt to carefully review the full report, e.g. if there is a risk of a false negative result, or potential germline implications.* |
| **CHIP** | *Clonal haematopoiesis of indeterminate potential (CHIP) refers to somatic variants arising from aging-related clonal expansion of variants in haematopoietic cells rather than from the tumour. Variants suspected to originate from CHIP will be annotated as such in the report, with comments in the 'Results and Interpretation' section to guide clinical interpretation.* |
| **Variant classification** | *This will be detailed in the body of the report and in **Appendix II**. If there are any relevant caveats or difficulties in variant analysis then this will be highlighted in the 'Results and Interpretation' section, such as specific elements which made interpretation difficult, or for any variants which have been regarded as reportable variants of uncertain significance.* |
| **Minority clone variants** | *If the variant allele frequency is significantly lower than expected based on the ctDNA fraction, this will be highlighted to indicate that it may be derived from a minority tumour clone. In such cases, predicting treatment response may be more difficult, therefore clinical judgement should be applied.* |
| **Germline risks and referral recommendation** | *If there are any germline implications to consider, recommendations for further testing (including sample requirements) will be highlighted in 'Result and Interpretation', in bold. The comment 'See Results and Interpretation' will appear in the Results Summary box to flag to the clinical team there is more information to impart other than just the variant identified.* |
| **Bold text** | *Any information of particular importance. such as details relating to a variant's actionability, or any potential germline testing implications, will **appear in bold** on the report.* |
| **Coverage information** | *This indicates the overall quality of the sample. Any key hotspots or regions which fall below the reportable threshold will be indicated to allow the clinical team to make decisions regarding further testing, if appropriate. Failed hotspots will be mentioned in 'Results and Interpretation'. Other failed regions will be recorded in the coverage section of **Appendix I**.* |

## *Variant nomenclature*

The NWGLH follows the internationally recognised Human Genome Variation Society (HGVS) standard nomenclature for variant descriptions.

The example below highlight the different parts of a full variant descriptor as per HGVS standards. This full description provides a unique identifier for variants to avoid misinterpretation / miscalling. For more information, please see the HGVS Recommendations for the Description of Sequence Variant or the HGVS nomenclature website.




Please note that in the **protein level** description, we apply the **3 -letter amino acid abbreviations**. However, some clinical guidance, databases or alternative reports may refer to the variant with a **single-letter amino acid abbreviation**.

The above variant, *EGFR c.2573T>G p.(Leu858Arg)*, is also commonly referred to as *L858R* where L is equivalent to Leu (leucine) and R is equivalent to Arg (arginine). This can cause confusion, particular for amino acids where the single-letter and three-letter abbreviations are less intuitive. Other common examples include:

- *KRAS c.35G>A p.(Gly12Asp) = KRAS G12D*
- *KRAS c.34G>T p.(Gly12Cys) = KRAS G12C*
- *BRAF c.1799T>A p.(Val600Glu) = BRAF V600E*
- *EGFR c.2369C>T p.(Thr790Met) = EGFR T790M*

The table (shown right) below provides a complete list of amino acids, including their three-letter and single-letter codes, to support interpretation of full HGVS descriptions.

| Amino acid | 3-letter | 1-letter |
|---|---|---|
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Glycine | Gly | G |
| Histidine | His | H |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| Valine | Val | V |