CYSGODI
CYmru **S**ervice for **G**enomic **O**ncology **DI**agnoses

The All Wales Genomics Laboratory (AWGL) launched the CYSGODI service in August 2021. CYSGODI delivers a high- quality oncology precision medicine service utilizing next generation sequencing to screen specific genes in a tumour or haematological malignancy; this provides information on a patients diagnosis, prognosis and which treatment is most likely to be effective.

This is beneficial as understanding the genomic profile of a tumour or haematological malignancy allows clinicians to tailor treatment options ensuring the best outcome for the patient.

This service information leaflet provides information on the tests that are used, which cancer types are suitable for testing and how to request a test. If any additional information is required, please contact the laboratory directly.

Service Details

The **CYSGODI** service uses the TruSight Oncology 500 High-Throughput assay which facilitates the simultaneous detection of single nucleotide variants, copy number variants, structural variants (gene fusions), MSI and TMB from DNA in 523 genes and RNA in 55 genes. The assay also allows flexible batching of samples from 16 to 192 samples per sequencing flow cell on the NovaSeq 6000. Further flexibility is provided by the ability of the assay to concurrently sequence DNA and RNA extracted from formalin fixed paraffin embedded tissue (FFPE), bone marrow and leukaemic blood samples.

The test consists of two parts, known as ‘panels’, one for DNA and one for RNA. These are processed and sequenced in parallel, for both assays a minimum of 40ng DNA and RNA is required.

The DNA panel consists of 523 genes, this is used to detect single nucleotide variants and small insertions or deletions (indels).

The RNA panel consists of 55 genes, this is use to detect structural variants and gene fusions.

It is important to note that variants from all 523 genes cannot be reported for individual tumour types.

Implementation of the **CYSGODI** service has future proofed the somatic next generation sequencing service as emerging biomarkers are included in the assay. It will also enable Welsh patients and clinicians access to appropriate clinical trials.

The full gene list and further information on the assay can be found here https://emea.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-data-sheet-1170-2018-010/trusight-oncology-500-and-ht-data-sheet-1170-2018-010.pdf

Solid Tumour and Haematological Malignancy Specific Gene Panel

The NHS England Genomics Test Directory (TD) was published in 2018, AWMGS is committed to providing equitable genetic testing to cancer patients in Wales. The TD specifies the gene panel content to be reported for different tumour types for patients in England which in turn is based on those targets which will influence management. This includes those which help to establish the correct diagnosis, predict prognosis, or to identify NICE treatments. Genes which do not influence management are currently not included in the TD. Therefore, following a consultation with service users, the gene panel content for some tumour types has changed to bring the gene panels in line with national guidelines. Tumour specific panel content is shown below:

Tumour Site	DNA Targets	RNA Targets
Lung	*EGFR* (exons 18-21); *BRAF* (exons 11 & 15); *KRAS* (exons 2-4)	ALK RET ROS1 NTRK1 NTRK2 NTRK3 EGFR MET
Melanoma	*BRAF* (exons 11 & 15); *KIT* (exons 9, 11, 13, 14, & 17); *NRAS* (exons 2-4)	NTRK1 NTRK2 NTRK3
Glioma	*IDH1* (codon 132 exon 4); *IDH2* (codon 172 exon 4); *BRAF* (exon 15); *EGFR* (exon 18-21); *ATRX* (whole gene), *H3F3A* (exon 2); *TERT* (promoter region c.-124; c.-126); *PTEN* (whole gene); *TP53* (whole gene); *CDKN2A* (whole gene)	BRAF EGFR NTRK1 NTRK2 NTRK3

GIST	*KIT* (exons 9, 11, 13, 14 & 17), *PDGFRA* (exons 12, 14 & 18)	NTRK1 NTRK2 NTRK3
Colorectal	*KRAS* (exons 2-4); *NRAS* (exons 2-4); *BRAF* (exons 11& 15); *PTEN* (whole gene); *TP53* (whole gene); *EGFR* (exons 18-21); *PIK3CA* (whole gene)	NTRK1 NTRK2 NTRK3
Thyroid	*BRAF* (exon 15); *KRAS* (exons 2-4), *NRAS* (exons 2-4); *TP53* (whole gene); *HRAS* (exons 2 & 3); *RET* (whole gene)	NTRK1, NTRK2, NTRK3
Endometrial	*POLE* (exons 9-14); *TP53* (whole gene)	
Breast	*PIK3CA* (exons 8, 10, 21)	
Cholangiocarcinoma	IDH1	FGFR2
Chronic Lymphocytic Leukaemia	*TP53* (whole gene)	
Myeloid	*ASXL1, BCOR, CALR, CEBPA, CBL, DNMT3A, EZH2, ETV6, FLT3, GATA2, IDH1, IDH2, JAK2, KRAS, KIT, MPL, NRAS, NPM1, NOTCH1, PDGFRA, RUNX1, SF3B1, SRSF2, SETBP1, TET2, TP53* and *U2AF1.* The whole coding region is sequenced for *DNMT3A, EZH2, ETV6, TP53* and *RUNX1*. For the remaining genes, only oncogenic hotspots within the gene are sequenced.	

Unknown Primary	*AR* (whole gene), *ARID1A* (whole gene), *ATRX* (whole gene), *BRAF* (exons 11 and 15), *BRCA1* (whole gene), *BRCA2* (whole gene), *CDKN2A* (whole gene), *EGFR* (exons 18, 19, 20, 21), *ERBB2* (exon 8: p.S310, exon 17: p.I655, p.R678, exon 19 p.L755, p.I767, p.D769, exon: 20 p.V777, exon: 21 p.V842, exon: 22 p.R896), *ESR1* (exon 5 (p.K303), exon 6 (p.380, p.392), exon 8 (p.463, p.533_538)), *H3F3A* (exon 2), *HRAS* (exons 2, 3 (p.12, p.13, p.61)), *IDH1* (exon 4), *IDH2* (exon 4), *KIT* (exons 9,11,13,14 and 17), *KRAS* (exons 2, 3, 4), *NRAS* (exons 2, 3, 4), *PDGFRA* (exons 12,14 and 18), *PIK3CA* (exons 8, 10, 21), *PTEN* (whole gene), *RET* (whole gene), *TERT* (promoter variants at c.-124 and c.-146), *TP53* (whole gene).	*BRAF, ALK, RET, ROS1, EGFR, MET, NTRK1, NTRK2 NTRK3, FGFR2*

The NHSE TD is reviewed periodically, and any changes in reporting requirements will be actioned accordingly.

Sensitivity and Specificity

The DNA sequencing gene panel has a minimum validated sensitivity of 98.8% and specificity of 99.2% for the detection of gene rearrangements within FFPE solid tumour samples when >40ng FFPE-derived DNA is used.

The RNA-sequencing gene panel has a minimum validated sensitivity of 97.9% and specificity of 98.7% for the detection of gene rearrangements within FFPE solid tumour samples when >40ng FFPE-derived RNA is used.

Further technical information can be found in Table 1.

Limitations

The **CYSGODI** service is not intended for use to detect cancer related germline variants, however, due to the cross-over between hereditary cancer genetic mutations and somatic variants found within cancers, we can analyse the same gene, but we are using tools to only consider somatic variants which are not suitable for detection of germline variants specifically. It is therefore possible that some variants found within the cancerous tissue indicate that the patient may have a germline (inherited) variant, this is known as an incidental finding. However, the presence of a germline variant cannot be confirmed without further testing.

If a report indicates that there is a possible germline variant, Clinical Genetics have outlined the following approach for oncologists:

- Speak with the patient further to explain that there is a possible germline finding in their molecular cancer results and agree the course of action they wish to take next.

It is possible that the patients family history has already shown known cancer-causing variants present in the family, therefore this finding will come as no surprise to the patient, e.g. in ovarian high grade cancer patients in whom a variant in the BRCA gene is a contributing cause in the cancer, as well as being indicative of treatment options and prognostic information.

- Contact the on-call duty genetic counsellor to explore the next steps and whether a referral to medical genetics is required to confirm the germline finding.
- Organise a confirmation test directly, where you are confident to do so, by completing the relevant referral form and providing the required blood sample
- If a germline variant is confirmed a referral to clinical genetics will need to be arranged for further discussion about the implications for the patient and wider family.

Sample Requirements - Solid Tumour

For DNA-based NGS testing, please send –

1 x ~5 micron H&E stained slide with area of highest neoplastic cell content highlighted and the approximate % tumour nuclei noted

6 x 10 micron air dried (non-baked, uncharged) unstained sections mounted on slides for microdissection and DNA extraction

For RNA-based NGS testing, please send -

1 x ~5 micron H&E stained slide with area of highest neoplastic cell content highlighted and the approximate % tumour nuclei noted

5 x 10 micron air dried unstained sections mounted on slides and cut in RNase free conditions (see below) for microdissection and DNA extraction.

We strongly advise that <u>FFPE slides for RNA analysis</u> are prepared in RNase free conditions wherever possible, which include:

- ✓ The work area and all equipment (including microtome blade) should be wiped down with RNase decontamination solution prior to use.

- ✓ Operator gloves should be wiped with RNase decontamination solution regularly or changed regularly when handling slides destined for RNA extraction.
- ✓ Glass slides are wiped with a tissue soaked in RNase decontamination solution.
- ✓ Containers used for transporting slides destined for RNA extraction should be new (and reused for slides for DNA analysis only) or, alternatively, cleaned with RNase decontamination solution before use.

- Please label samples with 3 identifiers and date of collection, block number must be on all slides and paperwork.
- Where possible, FFPE slides should be accompanied by the relevant histology report.
- Specimens that have undergone decalcification methods should be avoided.

Completed request forms should be sent to the local Cellular Pathology laboratory for slide preparation. The request form, FFPE sample, and a copy of the pathology report can then be sent to:

All Wales Genomics Laboratory, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF14 4XW

Sample Requirements – Haematological Malignancies

2ml of bone marrow or peripheral blood in an EDTA tube**.**

Completed request forms PD-GEN-ReqMyeloidNGS4.pdf (medicalgenomicswales.co.uk) should be sent directly to **All Wales Genomics Laboratory, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF14 4XW**

About the Genetic Report

The final report clinicians receive has been designed to meet their needs with findings laid out simply in the following sections:

- Result summary outlines clinically actionable results relevant for the tumour type which the test was requested for
- Interpretation provides more detail on the test undertaken and specific genetic markers identified and classified within the report
- Technical information ensures we are ISO 15189 compliant and provides the variants nomenclature in line with HGVS, which clinicians may wish to refer to when considering eligibility for clinical trials
- Other information clarifies the somatic nature of the test and if applicable, will identify any suspected germline mutation

Reporting times

The reporting time for this test is 14 calendar days for solid tumour samples and 21 calendar days for haemtological samples.

Table 1

DNA-based NGS		RNA-based NGS	
Sensitivity	98.77%	**Sensitivity**	97.99%
Specificity	99.22%	**Specificity**	98.73%
Input requirement	40ng	**Input requirement**	40ng
Types of variants	Single nucleotide variants, small indels	**Types of variants**	Gene fusions, structural variants (MET exon 14 skipping, EGFR vIII)
Salvage pathway (samples <40ng)	Alternative NGS panel – CRM (see table 2)		
Limitations	Assay not validated for detection of copy number variants. Not designed to detect germline variants. The limit of detection (LOD) of this assay is 5% with a minimum coverage of 270x, and 10% with a minimum coverage of 135x. A negative (wild-type) result does not rule out the presence of a variant below the assay LOD.	**Limitations**	A minimum of 9 million reads per sample is required in order to obtain a result.