CYSGODI
CYmru Service for Genomic Oncology DIagnoses

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.

Tumour Sites

At present, the CYSGODI service can be used to test the following tumour sites –

- ✓ Non-Small Cell Lung Cancer
- ✓ Melanoma
- ✓ Glioma
- ✓ Gastrointestinal Stromal Tumour (GIST)
- ✓ Colorectal Cancer
- ✓ Thyroid Cancer
- ✓ Endometrial Cancer
- ✓ Breast Cancer
- ✓ Myeloid Malignancies
- ✓ Chronic Lymphocytic Leukaemia
- ✓ Cancer of Unknown Primary (non-interpretative report)

For more information please click here.

Test Information

The CYSGODI service utilizes the illumina TruSight Oncology 500 (TSO500) High Throughput DNA/RNA assay, with sequencing performed on the illumina NovaSeq 6000™ as a frontline test.

This 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. A full list of genes on both panels is in Table 4.

Analysis is targeted for a subset of clinically relevant genes, specific to the tumour site.

Table 1. Tumour Specific Panel Content - reports are issued for the following genes with a 14CD turnaround time.

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)	*N/A – NTRK FISH available*
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)	*N/A – NTRK FISH available*
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)	*N/A – NTRK FISH available*
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, RET, EGFR*

Endometrial	*POLE* (exons 9-14); *TP53* (whole gene)	
Breast	*PIK3CA* (exons 8, 10, 21)	
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 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*
Unless otherwise stated, all genes are +/-5bp; RNA sequencing of EGFR detects the vIII structural variant, MET sequencing detects exon 14 skipping variants; further validation is ongoing to expand NTRK testing by RNA-based NGS to all tumour types, for information on NTRK eligibility refer to the clinical guidance.		

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.

2 x 3-4 micron air dried (non-baked, uncharged) unstained sections mounted on charged slides per gene required: ALK, ROS1, NTRK1, NTRK2, NTRK3. Note: these slides will only be used if the FISH salvage pathway is activated. Any un-used slides will be returned to the relevant pathology laboratory.

- ✓ Paraffin-embedded tumour tissue (FFPE) slides should be selected with the maximum quantity of viable tumour, the tumour content (%) must be stated and a marked H&E slide included.
- ✓ 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.
- ✓ All samples must be accompanied by a tumour request form, available on our website www.medicalgenomicswales.co.uk
- ✓ Specimens that have undergone decalcification methods should be avoided.

We strongly advise that FFPE slides for RNA analysis 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.

Requesting a Test

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.

Requesting a Test

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**

Please contact the AWGL business team (businessteam.genetics@wales.nhs.uk) for pricing and further information for requesting tests for-

- non-NHS patients
- NHS patients outside of current government funding (e.g. WHSSC commissioned genetic testing in Wales or NHS England commissioning)
- research purposes

Contact Details
Tel: 029 2074 2641 Fax: 029 2074 4043
Email: lab.genetics@wales.nhs.uk

TSO500 Technical Information

Table 2. Technical Information

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)	Salvage pathway (samples <40ng)	Targeted FISH, available for ALK, ROS1, NTRK1/2/3
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.

Other Available Tests

Where next generation sequencing using the TSO500 assay is not suitable, or additional tests are required, the following tests are also available for oncology diagnostics. Please refer to test information sheets for sample requirements, these are available on our website medicalgenomicswales.co.uk

Table 3. Non-NGS Oncology Services

Tumour Site	Test	TAT (CD)
Lung	Circulating tumour DNA analysis - Droplet digital PCR analysis of common exon 21 sensitising mutation c.2573T>G (p.L858R) and exon 19 deletions, and exon 20 resistance mutation c.2369C>T (p.T790M) (*EGFR*)	7
	Fluorescent in situ hybridization (FISH)*	14
Melanoma	BRAF exon 15 p.V600E/D/K/R/M pyrosequencing analysis	7
Glioma	1p36/19q13 LOH (FISH) and/or *MGMT* Methylation Pyrosequencing and/or Pyrosequencing of codon 132 (*IDH1)* & codon 172 (*IDH2*)	14
Colorectal	Microsatellite Instability (MSI)**	14
	p.V600E/D/K/R/M pyrosequencing analysis of exon 15 (*BRAF*)	7
	Circulating tumour DNA analysis – Droplet digital PCR analysis of most common KRAS (codon 12, 13, 61) and NRAS (codon 61) variants .	7
For any of the above, plus unknown primary with <40ng DNA.	Clinically Relevant Mutations (CRM) GeneRead NGS panel - 24 genes (*AKT1, ALK, AR, BRAF, CTNNB1, DDR2, EGFR, ERBB2, FGFR3, GNA11, GNAQ, IDH1, IDH2, KIT, KRAS, MAP2K1, MET, NRAS, PDGFRA, PIK3CA, PTEN, RET, STK11, TP53*)	14

*For RNA samples that do not meet the 40ng input requirements of TSO500.
**Additional samples would be required, refer to test information sheets.

ABL1	BCL2L1	CDK12	DICER1	ETV5	FH	HGF	IKZF1
ABL2	BCL2L11	CDK4	DIS3	ETV6	FLCN	HIST1H1C	IL10
ACVR1	BCL2L2	CDK6	DNAJB1	EWSR1	FLI1	HIST1H2BD	IL7R
ACVR1B	BCL6	CDK8	DNMT1	EZH2	FLT1	HIST1H3A	INHA
AKT1	BCOR	CDKN1A	DNMT3A	FAM123B	FLT3	HIST1H3B	INHBA
AKT2	BCORL1	CDKN1B	DNMT3B	FAM175A	FLT4	HIST1H3C	INPP4A
AKT3	BCR	CDKN2A	DOT1L	FAM46C	FOXA1	HIST1H3D	INPP4B
ALK	BIRC3	CDKN2B	E2F3	FANCA	FOXL2	HIST1H3E	INSR

ALOX12B	BLM	CDKN2C	EED	FANCC	FOXO1	HIST1H3F	IRF2
ANKRD11	BMPR1A	CEBPA	EGFL7	FANCD2	FOXP1	HIST1H3G	IRF4
ANKRD26	BRAF	CENPA	EGFR	FANCE	FRS2	HIST1H3H	IRS1
APC	BRCA1	CHD2	EIF1AX	FANCF	FUBP1	HIST1H3I	IRS2
AR	BRCA2	CHD4	EIF4A2	FANCG	FYN	HIST1H3J	JAK1
ARAF	BRD4	CHEK1	EIF4E	FANCI	GABRA6	HIST2H3A	JAK2
ARFRP1	BRIP1	CHEK2	EML4	FANCL	GATA1	HIST2H3C	JAK3
ARID1A	BRIP1	CIC	EP300	FAS	GATA2	HIST2H3D	JUN
ARID1B	BTG1	CREBBP	EPCAM	FAT1	GATA3	HIST3H3	KAT6A
ARID2	BTK	CRKL	EPHA3	FBXW7	GATA4	HLA-A	KDM5A
ARID5B	C11orf30	CRLF2	EPHA5	FGF1	GATA6	HLA-B	KDM5C
ASXL1	CALR	CSF1R	EPHA7	FGF10	GEN1	HLA-C	KDM6A
ASXL2	CARD11	CSF3R	EPHB1	FGF14	GID4	HNF1A	KDR
ATM	CASP8	CSNK1A1	ERBB2	FGF19	GLI1	HNRNPK	KEAP1
ATR	CBFB	CTCF	ERBB3	FGF2	GNA11	HOXB13	KEL
ATRX	CBL	CTLA4	ERBB4	FGF23	GNA13	HRAS	KIF5B
AURKA	CCND1	CTNNA1	ERCC1	FGF3	GNAQ	HSD3B1	KIT
AURKB	CCND2	CTNNB1	ERCC2	FGF4	GNAS	HSP90AA1	KLF4
AXIN1	CCND3	CUL3	ERCC3	FGF5	GPR124	ICOSLG	KLHL6
AXIN2	CCNE1	CUX1	ERCC4	FGF6	GPS2	ID3	KMT2B
AXL	CD274	CXCR4	ERCC5	FGF7	GREM1	IDH1	KMT2C
B2M	CD276	CYLD	ERG	FGF8	GRIN2A	IDH2	KMT2D
BAP1	CD74	DAXX	ERRFI1	FGF9	GRM3	IFNGR1	KRAS
BARD1	CD79A	DCUN1D1	ESR1	FGFR1	GSK3B	IGF1	LAMP1
BBC3	CD79B	DDR2	ETS1	FGFR2	H3F3A	IGF1R	LATS1
BCL10	CDC73	DDX41	ETV1	FGFR3	H3F3B	IGF2	LATS2
BCL2	CDH1	DHX15	ETV4	FGFR4	H3F3C	IKBKE	LMO1

Table 4. TSO500 full gene list, RNA panel in blue.

LRP1B	MTOR	PALB2	POLE	RECQL4	SMARCD1	TFE3
LYN	MUTYH	PARK2	PPARG	REL	SMC1A	TFRC
LZTR1	MYB	PARP1	PPM1D	RET	SMC3	TGFBR1
MAGI2	MYC	PAX3	PPP2R1A	RFWD2	SMO	TGFBR2
MALT1	MYCL1	PAX5	PPP2R2A	RHEB	SNCAIP	TMEM127
MAP2K1	MYCN	PAX7	PPP6C	RHOA	SOCS1	TMPRSS2
MAP2K2	MYD88	PAX8	PRDM1	RICTOR	SOX10	TNFAIP3
MAP2K4	MYOD1	PBRM1	PREX2	RIT1	SOX17	TNFRSF14
MAP3K1	NAB2	PDCD1	PRKAR1A	RNF43	SOX2	TOP1
MAP3K13	NBN	PDCD1LG2	PRKCI	ROS1	SOX9	TOP2A
MAP3K14	NCOA3	PDGFRA	PRKDC	RPS6KA4	SPEN	TP53
MAP3K4	NCOR1	PDGFRB	PRSS8	RPS6KB1	SPOP	TP63
MAPK1	NEGR1	PDK1	PTCH1	RPS6KB2	SPTA1	TRAF2

MAPK3	NF1	PDPK1	PTEN	RPTOR	SRC	TRAF7
MAX	NF2	PGR	PTPN11	RUNX1	SRSF2	TSC1
MCL1	NFE2L2	PHF6	PTPRD	RUNX1T1	STAG1	TSC2
MDC1	NFKBIA	PHOX2B	PTPRS	RYBP	STAG2	TSHR
MDM2	NKX2-1	PIK3C2B	PTPRT	SDHA	STAT3	U2AF1
MDM4	NKX3-1	PIK3C2G	QKI	SDHAF2	STAT4	VEGFA
MED12	NOTCH1	PIK3C3	RAB35	SDHB	STAT5A	VHL
MEF2B	NOTCH2	PIK3CA	RAC1	SDHC	STAT5B	VTCN1
MEN1	NOTCH3	PIK3CB	RAD21	SDHD	STK11	WISP3
MET	NOTCH4	PIK3CD	RAD50	SETBP1	STK40	WT1
MGA	NPM1	PIK3CG	RAD51	SETD2	SUFU	XIAP
MITF	NRAS	PIK3R1	RAD51B	SF3B1	SUZ12	XPO1
MLH1	NRG1	PIK3R2	RAD51C	SH2B3	SYK	XRCC2
MLL	NSD1	PIK3R3	RAD51D	SH2D1A	TAF1	YAP1
MLLT3	NTRK1	PIM1	RAD52	SHQ1	TBX3	YES1
MPL	NTRK2	PLCG2	RAD54L	SLIT2	TCEB1	ZBTB2
MRE11A	NTRK3	PLK2	RAF1	SLX4	TCF3	ZBTB7A
MSH2	NUP93	PMAIP1	RANBP2	SMAD2	TCF7L2	ZFHX3
MSH3	NUTM1	PMS1	RARA	SMAD3	TERC	ZNF217
MSH6	PAK1	PMS2	RASA1	SMAD4	TERT	ZNF703
MST1	PAK3	PNRC1	RB1	SMARCA4	TET1	ZRSR2
MST1R	PAK7	POLD1	RBM10	SMARCB1	TET2	