Next Generation Sequencing of Colorectal Cancers

Colorectal cancer (CRC) refers to cancer in the bowel and rectum. It is the 4th most common cancer in Wales, with 2,200 people being diagnosed every year.

Genetic testing of colorectal cancers informs diagnosis, prognosis and treatment options. In particular, analysis focuses on DNA changes linked to the effectiveness of targeted *monoclonal antibodies (Mabs).* These targeted drug therapies work by recognising and working with specific proteins on cancer cells, with each Mab working differently depending on the protein they target. Mabs may: slow or stop the cancer growth; deliver drugs or radiation therapy directly to the cancer cells; or support the immune system to attack the cancer.

NICE recommends Mab targeted drug therapies for previously untreated patients with KRAS and NRAS wild-type metastatic colorectal cancer.

Microsatellite analysis is also available in the laboratory for colorectal cancer patients; please see separate information sheet for sample requirements and testing information.

Links for further information

Orphanet www.orpha.net
EDDNAL www.eddnal.com
OMIM www.omim.org
Genetic Test Registry www.ncbi.nlm.nih.gov/gtr
Cancer Research UK www.cancerresearchuk.org
Bowel Cancer UK www.bowelcanceruk.org.uk

All Wales Genomics Laboratory (AWGL)

Phone: 02920 742 641 Fax: 029 2074 4043
Email: lab.genetics.cav@wales.nhs.uk

Website: medicalgenomicswales.co.uk

Colorectal Cancer Multi-Gene Panel Molecular Analysis

The AWGL multi-gene solid tumour panel will be used to test all colorectal cancer (CRC) solid tumour samples (with >50ng DNA available) from July 2019.

Analysis of colorectal cancer samples (with >50ng DNA available) will be performed using a bioinformatics analysis pipeline to target regions within 8 genes relevant to the tumour site; only these regions will be analysed and only genetic variants (>4.5% allele frequency) within these regions reported:

KRAS	Hotspots	Exons 2, 3 and 4 (covers: p.12, p.13, p.59, p.61, p.117, p.146 mutations which account for ~98% of known KRAS mutations in colorectal cancer)
NRAS	Hotspots	Exons 2, 3 and 4 (covers: p.12, p.13, p.59, p.61, p.117, p.146 mutations which account for ~91% of known NRAS mutations in colorectal cancer)
BRAF	Hotspots	Exons 11 and 15 (covers: p.599, p.600 and p.601 mutations, accounting for ~98% of known BRAF mutations)
EGFR	Hotspots	Exons 18, 19, 20 and 21
PIK3CA	Hotspots	Exons 10 and 21
PTEN	Screen	Whole gene sequence
TP53	Screen	Whole gene sequence

For colorectal samples, the AWGL report will provide interpretative analysis on:

- KRAS (OMIM 190070) gene changes occur in about 36-40% of CRC and indicate EGFR Mab therapies are unlikely to be effective.
- NRAS (OMIM 164790) gene changes occur in about 1-6% of CRC and indicate EGFR Mab therapies are unlikely to be effective.
- BRAF (OMIM 164757) gene changes occur in about 10% of CRCs and are linked to poorer prognosis, with mounting evidence suggesting BRAF mutations impair the therapeutic effect of EGFR Mab therapies in RAS wild-type colorectal cancer patients.

Please note: Genetic testing for DPYD variants on colorectal tumour samples is no longer provided as part of the AWGL multi-gene solid tumour panel. This service has been replaced by a new service which detects the five germline polymorphisms within the DPYD gene which are clinically relevant predictors of fluoropyrimidine toxicity. This test is carried out on genomic DNA extracted from a blood sample from the patient.

For information on this service and how to request a test please follow the link:

http://www.wales.nhs.uk/sites3/news.cfm?orgid=525&contentid=52576

Multi-Gene Panel Molecular Analysis

Multi-gene panel molecular analysis refers to the use of Next Generation Sequencing (NGS) on a range of genetic markers in one testing process. The All Wales Genetic Laboratory's (AWGL) multi-gene solid tumour panel enables simultaneous analysis of over 20 genes implicated in diagnosis, prognosis, prediction and treatment of cancer patients. The panel has been validated within AWGL for FFPE-derived DNAs (>50ng) for the detection of variants down to 5% in a background of wild type DNA within the following 23 gene regions (+/-5bp):

Androgen receptor (whole gene)	CDKN2A (whole gene)	IDH1 (exon 6)	PIK3CA (exons 10 and 21)
ARID1A (whole gene)	EGFR (exons 18, 19, 20 and 21)	IDH2 (exon 5)	PTEN (whole gene)
ATRX (whole gene)	ESR1 (exons 5, 6 and 8)	KIT (exons 9, 11, 13, 14 and 17)	RET (whole gene)
BRAF (exons 11 and 15)	ERBB2 (exons 8, 17, 19, 20, 21 and 22)	KRAS (exons 2, 3 and 4)	TERT (promoter mutations)
BRCA1 (whole gene)	H3F3A (exon2)	NRAS (exons 2, 3 and 4)	TP53 (whole gene)
BRCA2 (whole gene)	HRAS (exons 2, 3)	PDGFRA (exons 12, 14 and 18)	

Validation is ongoing at AWGL to assess the multi-gene solid tumour panel's potential to analyse copy number variations within EGFR, ERBB2, MET, and PIK3CA, as well as determining the panel's ability to detect structural variants involving the following genes/gene regions: 1p/19q, ALK, BRAF-KIAA1549, EGFRvIII, MET exon skipping (exons/introns 13 and 14), NTRK1, NTRK2, NTRK3, RET, and ROS1. Progress updates are on our website.

Note: if <50ng of DNA is obtained from the FFPE sample provided, an alternative NGS panel will be utilised for testing, which provides hotspot analysis of all clinically actionable genes relevant to the tumour type but overall a less comprehensive gene analysis; this testing comes with a longer turnaround time of 20 working days.

Information for requesters

All requests for testing should be made on an appropriate request form available on the AWGL website medicalgenomicswales.co.uk. This request form should be sent with the FFPE sample (details below) and a copy of the pathology report to:

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

Please the laboratory for pricing and further information details for:

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

Sample Requirements for Multi-Gene Panel testing

- 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 unstained sections mounted on slides

NOTE: *Additional samples will be required for any FISH analysis required (see website for details).*

Sample Information

- Paraffin-embedded tumour tissue (FFPE) slides should be selected with the maximum quantity of viable tumour.
- Please label samples with three identifiers and date of collection.
- Where possible, the FFPE slides should be accompanied by the relevant histology report.
- All samples must be accompanied by a tumour request form: www.medicalgenomicswales.co.uk

NOTE: *Consent for genetic testing and DNA storage is assumed when a test request and samples are received.*

All results will be reported to the named healthcare professional/s on the request form within a target turnaround time of 14 working days.