Next Generation Sequencing of Colorectal Cancers

Overview

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 instability testing is also available in the laboratory for colorectal cancer patients; please see separate information sheet for sample requirements and testing information. For further details please refer to Lynch Syndrome service information and HNPCC Service Information. If these services are required, please use the Lynch Syndrome tumour testing form.

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.

The All Wales Genomics Laboratory utilises the Illumina TruSight Oncology 500 High Throughput DNA/RNA assay for next generation sequencing using the Illumina NovaSeq 6000™ to identify nucleotide variants and gene rearrangements (fusions) in patients with solid tumours. More information on this service is available here.

Table 1. Colorectal Cancer DNA Gene Panel

Gene	Hotspots/Screen	Regions covered
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

NTRK1 (OMIM 191315), NTRK2 (OMIM 600456), and NTRK3 (OMIM 191316) fusion testing is available by FISH as required for eligible patients, please refer to the clinical guidance and use the NTRK FISH request form.

Interpretation

A fully interpreted report will be issued for *NRAS, KRAS* and *BRAF*.

Please be aware that variants of uncertain significance (VUS) may be identified with this test, these will be further investigated if they are in clinically relevant gene regions and reported as appropriate. This test is performed to evaluate somatic variants within tumour samples and is not designed to assess for germline variants within the targeted genes.

Genetic testing for DPYD variants in patients with colorectal tumours is currently performed on genomic DNA extracted from a blood sample from the patient. This PCR-based assay detects the five germline polymorphisms within the DPYD gene, which are clinically relevant predictors of fluoropyrimidine toxicity. For information on this service and how to request a test please refer to the following link: http://www.wales.nhs.uk/sites3/news.cfm?orgid=525&contentid=52576

Specimen Requirements

For information on sending FFPE samples refer to the CYSGODI service information sheet.

Please use the FFPE solid tumour request form and complete all fields.

Links for further information

- Orphanet www.orpha.net
- OMIM www.omim.org
- Genetic Test Directory https://www.england.nhs.uk/publication/national-genomic-test-directories/

Colorectal cancer specific links

- Cancer Research UK for Bowel cancer https://www.cancerresearchuk.org/about-cancer/bowel-cancer
- Bowel Cancer UK www.bowelcanceruk.org.uk

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