Next Generation Sequencing of Gastrointestinal Stromal Tumours (GISTs)

GISTs are a rare type of sarcoma (cancer of the connective tissue) and can be found anywhere in the digestive system, although around 50% are found in the stomach.

Some GISTs are benign but can become cancerous if not treated. Surgery is the most common treatment, but larger tumours cannot always be completely removed. Tyrosine kinase inhibitor (TKI) drugs slow or stop GISTs growth by blocking the chemical signals required for cancer cell growth. They have shown to dramatically increase survival and are most effective for patients with tumours harbouring a mutation in the KIT or PDGFRA genes. Mutations in these genes are mutually exclusive; around 85% of GISTs have a mutation in the KIT gene, and then around one third of tumours with a normal KIT gene (no mutation) have a mutation in PDGFRA.

NICE recommends the use of targeted TKI drug therapies for patients with mutations in KIT and PDGFRA.

GIST Multi-Gene Panel Molecular Analysis

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

Analysis of GIST samples (with >50ng DNA available) will be performed using a bioinformatics analysis pipeline to target regions within 2 genes relevant to the tumour site; only these regions will be analysed and only genetic variants (>4.5% allele frequency) within these regions reported:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hotspots</th>
<th>Exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
<td></td>
<td>9, 11, 13, 14 and 17</td>
</tr>
<tr>
<td>PDGFRA</td>
<td></td>
<td>12, 14 and 18</td>
</tr>
</tbody>
</table>

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

- KIT (OMIM) gene changes occur in about 85% of all GISTs and enables access to TKI treatment imatinib.
- PDGFRA (OMIM 173490) gene changes occur in about 5% of all GISTs and enables access to TKI treatment imatinib.

Links for further information

- Orphanet: [www.orpha.net](http://www.orpha.net)
- OMIM: [www.omim.org](http://www.omim.org)
- EDDNAL: [www.eddnal.com](http://www.eddnal.com)
- Cancer Research UK: [www.cancerresearchuk.org](http://www.cancerresearchuk.org)
- GIST Support UK: [www.gistsupportuk.com](http://www.gistsupportuk.com)
- Cancer Help UK: [www.cancerhelp.org.uk](http://www.cancerhelp.org.uk)
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 24 gene regions (+/-5bp):

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genes/variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androgen receptor</td>
<td>CDKN2A (whole gene) HRAS (exons 2, 3) PDGFRA (exons 12, 14 and 18)</td>
</tr>
<tr>
<td>ARID1A</td>
<td>DPYD (whole gene sequence and intronic variants: c.1905+1G&gt;A, c.483+18G&gt;A, c.680+139G&gt;A, c.959-51T&gt;C) IDH1 (exon 6) PIK3CA (exons 10 and 21)</td>
</tr>
<tr>
<td>ATRX</td>
<td>EGFR (exons 18, 19, 20 and 21) IDH2 (exon 5) PTEN (whole gene)</td>
</tr>
<tr>
<td>BRAF (exons 11 and 15)</td>
<td>ESR1 (exons 5, 6 and 8) KIT (exons 9, 11, 13, 14 and 17) RET (whole gene)</td>
</tr>
<tr>
<td>BRCA1 (whole gene)</td>
<td>ERBB2 (exons 8, 17, 19, 20, 21 and 22) KRAS (exons 2, 3 and 4) TERT (promoter mutations)</td>
</tr>
<tr>
<td>BRCA2 (whole gene)</td>
<td>H3F3A (exon2) NRAS (exons 2, 3 and 4) TP53 (whole gene)</td>
</tr>
</tbody>
</table>

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 http://www.wales.nhs.uk/AWMGS. This request form should be sent with the FFPE sample (details below) and a copy of the pathology report to:

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

Please contact the laboratory for pricing and further information 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: http://www.wales.nhs.uk/AWMGS

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 10 working days.