Colorectal cancer – RAS & BRAF Mutation Testing; MSI Analysis

Background
Mutations in the KRAS oncogene (OMIM 190070) and NRAS oncogene (OMIM 164790) are frequently found in a number of human cancers. Clinical data has established that 51% of metastatic colorectal tumours may carry a mutated RAS gene. An activating mutation indicates that small molecule drugs (such as Erlotinib and Gefitinib) or drugs which target the Epidermal Growth Factor Receptor (EGFR) (such as Cetuximab and Panitumumab) will be ineffective in terms of control of tumour proliferation. BRAF mutations are a significant negative prognostic marker for patients with metastatic colorectal cancer, and there is mounting evidence to suggest that BRAF mutations impair the therapeutic effect of EGFR monoclonal antibody therapy in RAS wild-type colorectal cancer patients. It is clinically recommended that patients with a surgically resected stage II (i.e. non node positive) colorectal cancer, who are being considered for adjuvant chemotherapy, should have their dMMR or MSI status assessed.

Details on the laboratory’s RAS mutation testing on ctDNA service is available on this information sheet – MI-GEN-RASctDNA

Recommended Clinical Referral Criteria
- Patients with metastatic colorectal cancer, suitable for treatment with Cetuximab
- Patients with stage II colorectal cancer being considered for adjuvant chemotherapy

Molecular Analysis

BRAF only:
Pyrosequence analysis of selected tumour tissue for mutations in codons 599, 600 and 601 of the BRAF gene [covers the common c.1799T>A (p.Val600Glu)]; this assay detects ~5% of mutant in a background of wild-type genomic DNA.

Mutation hotspot screen:
Next Generation Sequencing (NGS): Target genes are enriched using the Qiagen GeneRead Tumour Actionable Mutations panel and sequenced on an Illumina MiSeq. This NGS assay covers the following regions of each gene: BRAF - exon 15; KRAS - exons 2, 3 & 4; NRAS - exons 2, 3 & 4; KIT - exons 11, 13 & 17; EGFR - Exons 2, 18, 19, 20, 21, 22, 23 & 24; PDGFRα - exons 12 & 18; IDH1 – exon 4; IDH2 – exon 4

The analysis performed aims to cover these gene regions to a minimum vertical depth of 500X, allowing the detection of approximately 5% of mutant in a background of wild-type genomic DNA.

For specific RAS requests, only the most common KRAS and NRAS mutations in codons 12, 13, 61, 117 & 146 will be reported (covers 98% and 91% of known mutations in colorectal cancer respectively); there is an additional charge for reporting the additional genes (please see below**). Where BRAF is requested, only BRAF mutations in codons 599,600 & 601 will be reported

If the NGS service is unavailable, alternative testing methods will be used as appropriate e.g. pyrosequencing

MSI analysis: Microsatellite instability analysis of selected tumour tissue to check mismatch repair deficiency

Prices* & Turnaround Times (TAT)
*Valid until March 2019 – prices apply to NHS referrals; non-NHS patients are subject to 20% surcharge

<table>
<thead>
<tr>
<th>Test</th>
<th>TAT(working days)</th>
<th>Price</th>
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<tbody>
<tr>
<td>Mutation screening (codons 12, 13, 61, 117 and 146 of KRAS)</td>
<td>10</td>
<td>£120</td>
</tr>
<tr>
<td>Mutation screening (codons 12, 13, 61, 117 and 146 of NRAS)</td>
<td>10</td>
<td>£120</td>
</tr>
<tr>
<td>Mutation screening (KRAS and NRAS) – as above</td>
<td>10</td>
<td>£200</td>
</tr>
<tr>
<td>Mutation analysis (codons 599, 600 and 601 in exon 15 of BRAF)</td>
<td>10</td>
<td>£85</td>
</tr>
<tr>
<td>Mutation screening (KRAS, NRAS and BRAF exon 15) – as above</td>
<td>10</td>
<td>£250</td>
</tr>
<tr>
<td>Microsatellite instability (MSI)</td>
<td>10</td>
<td>£120</td>
</tr>
<tr>
<td>Technical report of all 8 genes analysed by NGS</td>
<td>10</td>
<td>£50**</td>
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</tbody>
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Contact Details
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http://www.wales.nhs.uk/AWMGS/
Accredited to ISO 15189:2012 (8988)

Sample Requirements
Paraffin-embedded tumour tissue (or frozen tissue). Blocks should contain the maximum quantity of viable tumour and be accompanied by the histology report. Please label samples with three identifiers and date of collection.
All samples must be accompanied by a RAS request form:
http://www.wales.nhs.uk/sites3/page.cfm?orgid=525&pid=19423
Consent for testing & DNA storage is assumed when request for test received

Links
UKGTN
http://ukgtn.nhs.uk/
Orphanet
http://www.orpha.net/
EDNICAL
http://www.ednical.com/
OMIM
http://www.omim.org/
Genetic Test Registry
Support
Bowel Cancer UK
http://www.bowelcanceruk.org.uk/