Non-Small Cell Lung Cancer (NSCLC) – EGFR, ALK & ROS1 Mutation Testing

Background
Lung cancer is the leading cause of cancer death worldwide. There are two main groups of lung cancers – small cell and non-small cell (NSCLC) – and the majority of patients (85%) have NSCLC. Treatment can involve surgery, radiotherapy and combined chemotherapy, depending upon the disease type and progression.

There are also other developing therapies; some involve the inhibition of the Epidermal Growth Factor Receptor (EGFR – OMIM 131550). This regulates cell proliferation and is over-expressed in numerous cancers, but particularly NSCLC. Deregulation of the EGFR pathway is central to tumour pathogenesis in NSCLC and EGFR is the target for tyrosine kinase inhibitors (TKI) therapy. Approximately 10% of NSCLC adenocarcinoma tumours have activating mutations within the EGFR TK domain (exons 18-21) and ~85% of patients with these mutations respond to treatment with TKIs.

Another therapy is the ALK- (Anaplastic Lymphoma Kinase - OMIM 105590) and ROS1- (ROS proto-oncogene 1, receptor tyrosine kinase – OMIM 165020) targeted inhibitor, crizotinib. NSCLC adenocarcinoma patients may be tested for the existence of an ALK- or ROS1-gene rearrangement; around 4% and 1-2% of adenocarcinomas are expected to have ALK or ROS1 rearrangements respectively, and this indicates that the patient should be considered for treatment with crizotinib.

Most responders to these therapies eventually relapse and this is mainly due to the acquisition of resistance mutations; more details on the laboratory’s EGFR testing on ctDNA service is available on this information sheet - [http://www.wales.nhs.uk/sites3/Documents/525/MI-GEN-EGFRctDNA.pdf](http://www.wales.nhs.uk/sites3/Documents/525/MI-GEN-EGFRctDNA.pdf)
Further information about EGFR mutations is available at [http://www.egfr-mutation.com](http://www.egfr-mutation.com)

Recommended Clinical Referral Criteria
- Patients with NSCLC (not squamous cell carcinoma histology) suitable for treatment with biological agent

Molecular Analysis

**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;
- **KIT** - exons 11,13 & 17;
- **EGFR** - Exons 2,18,19,20,21,22,23 & 24;
- **KRAS** - exons 2,3 & 4;
- **NRAS** - exons 2,3 & 4;
- **PDGFRA** - 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 EGFR requests, only the most common EGFR mutations in exons 18, 19, 20 & 21 will be reported (covers 95% of known TKI mutations); there is an additional charge for reporting the additional genes (please see below**)

**Please note – analysis of all 8 genes in NGS panel is funded for Welsh patients**

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

**ALK analysis:** Fluorescence in situ (FISH) analysis of ALK gene
**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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</thead>
<tbody>
<tr>
<td>Mutation screening (exons 18-21 of <em>EGFR</em>)</td>
<td>10</td>
<td>£175</td>
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<tr>
<td>FISH analysis of <em>ALK</em></td>
<td>10</td>
<td>£120</td>
</tr>
<tr>
<td>FISH analysis of <em>ROS1</em></td>
<td>10</td>
<td>£120</td>
</tr>
<tr>
<td>FISH analysis of <em>ALK AND ROS1</em></td>
<td>10</td>
<td>£200</td>
</tr>
<tr>
<td><em>EGFR</em> mutation screening and <em>ALK</em> or <em>ROS1</em> FISH analysis</td>
<td>10</td>
<td>£250</td>
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<tr>
<td><em>EGFR</em> mutation screening and <em>ALK</em> and <em>ROS1</em> FISH analysis</td>
<td>10</td>
<td>£330</td>
</tr>
<tr>
<td>Technical report of all 8 genes analysed by NGS</td>
<td>10</td>
<td>£50**</td>
</tr>
</tbody>
</table>

### Contact Details

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http://www.wales.nhs.uk/AWMGS/  
Accredited to ISO 15189:2012

### Sample Requirements

Paraffin-embedded tumour tissue’ or bronchial brushings. (Should contain the maximum quantity of viable tumour and be accompanied by a histology report.)  
Please label samples with three identifiers and date of collection  
All samples must be accompanied by a NSCLC request form:  
http://www.wales.nhs.uk/sites3/page.cfm?orgid=525&pid=19423  
Consent for testing & DNA storage is

### Links

Orphanet  
http://www.orpha.net/  
EDDNAL  
http://www.eddnal.com/  
OMIM  
http://www.omim.org/  
Genetic Test Registry  
Support  
British Lung Foundation  
http://www.blf.org.uk/Home  
Roy Castle Lung Cancer

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Cardiff and Vale University Health Board  
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