EGFR, ALK & ROS1 analysis in Non-Small Cell Lung cancer (NSCLC)

**Background**
Over 40,000 cases of lung cancer are diagnosed each year in the UK, of which about 85% will be Non-Small Cell Lung cancer (NSCLC). NSCLC can be divided into histological subtypes; squamous cell carcinoma and adenocarcinoma account for 43% and 33% respectively. Locally advanced (stage IIIIB) or metastatic (stage IV) NSCLC can be treated with systemic therapy: cytotoxic chemotherapy or first and second generation EGFR tyrosine kinase inhibitors (TKIs) erlotinib (Tarceva®), gefitinib (Iressa®) or afatinib (Giotrif®). Patients who are progressing on first/second line TKIs may have developed the EGFR c.2369C>T, p.(Thr790Met) resistance mutation (known as T790M) and so can be treated with osimertinib (Tagrisso®).

Data presented at ASCO 2009 from the IPASS trial (of over 1200 patients) suggest that those tumours with an EGFR mutation are most likely to benefit from Iressa whilst those without a mutation are most likely to benefit from chemotherapy. In addition, it was demonstrated that EGFR mutations are found in about 10% of adenocarcinomas but only rarely in squamous cell carcinomas. AstraZeneca have a license for first or second line therapy in stage IIIB or IV EGFR- positive NSCLC.

The All Wales Laboratory Genetics (AWLG) also provides alternative testing for a limited number of EGFR sensitizing and resistance mutations on blood when the patient cannot have a biopsy, biopsy testing has failed or to monitor for the T790M resistance mutation.

Another therapy is the inhibitor, crizotinib. NSCLC adenocarcinoma patients may be tested for the existence of ALK gene or ROS1 gene rearrangements (using FISH-based analysis); around 4% of adenocarcinomas are expected to have ALK rearrangements and 1-2% are expected to have ROS1 rearrangements (tumours only very rarely have both) and this indicates that the patient should be considered for crizotinib.

Following NICE guidance regarding the use of Iressa in EGFR mutation-positive patients in 2010, EGFR molecular analysis for Welsh patients with NSCLC is funded by WHSSC (Welsh Health Specialised Services Committee). For non-Welsh patients the laboratory will invoice the referring clinician or the relevant health insurance company for patients with private insurance. ALK testing is funded by pharma and ROS1 analyses are also funded by WHSSC.

**Tissue molecular analysis and pathology requirements**
A diagnostic service for EGFR, ALK and ROS1 mutations is established at the AWLG in Cardiff. It is essential for this analysis that the correct tissue sample is supplied.

1. The sample should be subtyped and indicated on the request form: **Adenocarcinomas**, **large cell carcinomas** and **NOS** should be tested for EGFR mutations. (Necrotic samples, carcinoid tumours and squamous carcinomas should not be included). Adenocarcinoma patients will also be tested for ALK and ROS1 rearrangements (if specifically requested, and sufficient and appropriate material is provided).

2. The laboratory will require the tissue section to be marked to highlight the area of highest neoplastic cell content. This allows the laboratory staff to determine that the molecular assays performed are sufficiently sensitive to detect a mutation if it is present within the sample.

3. If the available sample contains regions with <10% tumour, please contact the molecular laboratory to discuss.

4. There is currently no lower limit to the size of biopsy sample accepted for analysis. However it should be noted that, where neoplastic cell content is determined to be <10%, normal test
results will be reported with caution, as such low level tumour samples are at the limits of assay sensitivity, especially in samples with low cellularity.

4. Cytology samples may also be analysed for EGFR mutations; the % neoplastic cell content should be recorded on the referral form.

5. As the molecular analysis will determine the treatment that the patient receives, it is essential that blocks are sent, received and analysed within a short time-frame. The laboratory aims to achieve a 10 working day turnaround from when the sample is received.

6. Where a tissue block or unmarked/unassessed slides are sent, a copy of the pathology report should be included where possible. All blocks will be returned to the requesting pathologist, with a copy of the molecular report.

**Blood molecular analysis and sample requirements**

A diagnostic service for testing of EGFR sensitizing and resistance mutations from circulating tumour DNA (ctDNA) from a blood sample has also been established. Due to the labile nature of ctDNA, it is important that blood samples are sent in the correct preservative tube and within 96 hours of collection.

1. 10ml of blood should be taken in one Streck Cell-Free DNA BCT® tube or Janssen CellSave preservative tube, at room temperature, and labeled with patient details. EDTA tubes do not preserve the quality of the ctDNA as efficiently as Streck or CellSave and so are not recommended for collection of blood for ctDNA analysis.

2. Please include the relevant ctDNA request form with sample and indicate the time and date taken.

3. The blood sample should not be frozen or refrigerated before or during delivery.

4. If Streck or CellSave blood samples are being sent to Laboratory Genetics, they should be dispatched as soon as possible to reach the lab within 96 hours; the Royal Mail Blue Guaranteed Delivery boxes are recommended. Please do not send samples to the lab on a Friday as these are unlikely to reach the laboratory within the specified time frame. Please notify lab of sample dispatch via email at: lab.genetics@wales.nhs.uk

An analysis schematic for sample preparation and dispatch is attached below for easy reference.
**EGFR, ALK, ROS1 analysis in NSCLC**

**Requirement for pathology sample**

Biopsy received by local pathologist

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*EGFR* mutation analysis indicated for all NSCLC subtypes except squamous cell carcinoma.

Adenocarcinoma patients will also be tested for the presence of *ALK* and *ROS1* gene rearrangements if specifically requested

Sample requirements and preparation for molecular analysis:

- 1 x 5µm H&E stained slide with tumour-rich regions highlighted and the approximate % tumour nuclei noted and:
  - For *EGFR* analysis – 4 x 10µm unstained slide mounted sections
  - For *ALK* analysis – 3 x 3 or 4µm sections (for FISH-based testing) mounted on SuperFrost® Plus slides (Thermo Fisher Scientific)
  - For *ROS1* analysis – 3 x 3 or 4µm sections (for FISH-based testing) mounted on SuperFrost® Plus slides (Thermo Fisher Scientific)

  OR

- The tumour block

OR

- 1x10 ml blood sample in a Streck Cell-Free DNA BCT® tube or Janssen CellSave preservative tube (at room temperature) (*EGFR* only)

The sample should be accompanied by an *EGFR* tumour request form that includes the patient, sample and referring lung oncologist details. A copy of the pathology report should also be included where possible

Send sample as soon as possible to:

**All Wales Laboratory Genetics**

**Institute of Medical Genetics, University Hospital of Wales**

**Heath Park**

**Cardiff CF14 4XW**

*EGFR* mutation report (including *ALK* or *ROS1* results where applicable) will be sent to lung oncologist or named clinical lead and will be copied to referring pathologist (Any blocks received will be returned to the named pathologist)

If you have questions about this process, please contact:

Laboratory Genetics Phone: 02920 742641 Email: lab.genetics@wales.nhs.uk

Or:

Dr Allen Gibbs and Dr Richard Attanoos, Consultant Pathologists with pulmonary expertise, Llandough Hospital.