

Assay Summary

Epidermal Growth Factor Receptor Gene Mutation Analysis **Iressa® (Gefitinib) and Tarceva® (Erlotinib) responsiveness in lung cancer** **EGFR Gene Analysis**

Synopsis

Non small cell lung cancer (NSCLC) is one of the most common cancers worldwide. Pulmonary malignancies are the leading cause of death in both men and women¹. Treatment generally consists of surgical resection, radiation therapy and/or chemotherapy. Gefitinib, or Iressa, was recently approved for the FDA for the treatment of refractory NSCLC, in part, due to the impressive response seen in only about 10-15% of patients². Two recent journal publications have shed new light on what separates the “responders” from the “non-responders” to Gefitinib. Both journals reported that virtually all those who responded to Iressa (Gefitinib) were found to have a somatic mutation in a mutational “hotspot” within the kinase domain of the EGFR gene (Exons 18-21)^{3,4}. This is the precise location where Gefitinib interacts with the EGFR protein. These studies went on to demonstrate that cells with mutations in the protein kinase domain are more sensitive to stimulation by EGFR, and more sensitive to inhibition by Gefitinib. A similar association was subsequently reported by Pao et al. for responsiveness to Tarceva (Erlotinib)⁵, another small molecule drug directed at the kinase domain of the EGFR protein.

Indications for testing

Individuals with a clear diagnosis of non-small cell lung carcinoma. To help predict which patients are likely to benefit from treatment with Iressa.

Methodology

Mutation Analysis of the portion of the EGRF protein kinase domain associated with NSCLC responsiveness to Iressa (Gefitinib), [EGFR-KD]: The coding exons 18-21 and associated intron junctions of the EGFR gene are analyzed by direct DNA sequence analysis of normal and tumor tissue, using an automated fluorescent sequencer. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction from a second, independent PCR product. If no mutation is found, sequence analysis is performed in both directions.

Performance/Limitations

Based on the literature, the assay has an expected detection rate of 10-30%, depending on ethnicity. This method will not detect mutations located in regions of the genes that are not analyzed (non-coding exon sequences, intron sequences other than the splice junctions, and upstream and downstream sequences). The method also will not detect gross genetic alterations including most large deletions, duplications, and inversions. Some sequence alterations that may be detected (such as those causing uncharacterized missense or silent changes) will be of uncertain clinical significance. Interpretation of test results should be in the context of the patient’s ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

The tissue sample should preferably be large enough to provide at least two visual fields (low magnification) with predominantly tumor cells (at least 50%). We have been successful in testing samples that smaller, such as trans-bronchial biopsies, but these generally require more work because of increased failure rates, and may end up not giving any interpretable results. We would prefer to receive formalin-fixed, paraffin-embedded tissue blocks, but slides are also accepted. We require 5 slides of tumor sample, 10 micron serial sections, unstained, with no coverslips. If the sample is a needle biopsy or clearly has very little tumor, please send additional slides (2 or 3). Please include 1 H&E slide, 4 micron section, with a coverslip as a reference slide for the unstained slides. Ensure that the slides are clearly labeled with the patient name or identifier and date of birth and type of sample. Place slides in appropriate container(s) to ensure against breakage. Call for any questions regarding sample requirements.

Test Request Form (TRF)

- a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [EGFR Patient Information Form](#): Include a completed EGFR Patient Information .

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
EGFR-KD (EGFR gene, full sequencing of kinase domain)	83890, 88323, 88381, 83892, 83898(x4), 83894, 83904(x4), 83912	2 wks

References

1. Ries LAG, Kosary CL, Hankey BF, et al, editors. "SEER Cancer Statistics Review, 1973 1996". Bethesda, MD: National Cancer Institute; 1999.
2. Fukuoka, M., Yano, S., Giaccone, G., et al., "Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer., J. Clin. Oncol.;21:2237-46, 2003.
3. Lynch et al., Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib. NEJM, 2004; 350(21): 2129-2139.
4. Paez et al., EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy. Science, 2004; 304: 1497-1500.
5. Pao et al., EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. PNAS, 2004; 101(36): 13306-13311.

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc