

## **Quantitative Gene Expression in Non-Small Cell Lung Cancer from Paraffin-Embedded Tissue Specimens: Predicting Response to Gefitinib, an EGFR Kinase Inhibitor**

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**Purpose:** To explore the correlation between quantitative gene expression in tumor, using a high-throughput RT-PCR assay, and response to Gefitinib monotherapy in patients (pts) with non-small cell lung cancer.

**Methods:** A total of 46 fixed paraffin-embedded (FPE) tumor specimens, from 37 pts (16 M, 21 F; mean age 63 yrs) collected prior to starting Gefitinib monotherapy, were analyzed. Specimens were obtained most often from lung, involved nodes, or soft tissue. Histologic types included adenocarcinoma (71.8%), bronchoalveolar carcinoma (8.7%), squamous cell carcinoma (6.5%), non-small cell carcinoma, unspecified (6.5%), large cell carcinoma (4.3%), and adeno-squamous carcinoma (2.2%). We extracted RNA from three 5  $\mu$ m sections of FPE tissue, using recently developed methods, and profiled the expression of 192 genes. Genes were selected from published literature and DNA microarray experiments, including genes implicated in HER-kinase signaling.

**Results:** Gene expression data are currently available on 17 pts (9 M, 8F; mean age 61 yrs). Total RNA yield was  $10.7 \pm 5.6$   $\mu$ g (mean  $\pm$ SD). Expression profiles were analyzed by hierarchical clustering. Several sets of genes known to be co-expressed were identified, e.g., STAT5A and STAT5B ( $r=0.89$ ); Amphiregulin and Epiregulin ( $r=0.84$ ); MMP2 and TIMP2 ( $r=0.81$ ); PDGFR $\alpha$  and PDGFR $\beta$  ( $r=0.83$ ); and a 'growth cluster' of Ki-67, Survivin, Chk1, and Topoisomerase II $\alpha$  (avg  $r=0.86$ ). Of the initial 17 pts, 2 had partial response (RECIST criteria) to Gefitinib and 3 pts had stable disease. Expression of several genes correlated with clinical response (e.g., STAT5A, STAT5B, and  $\gamma$ -catenin). The significance of these and other candidate markers of response will be examined in the larger cohort of patients.

**Conclusion:** Quantitative analysis of gene expression in paraffin-embedded tissue is feasible and may be useful to identify correlates of response to EGFR kinase inhibitors.

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