ST21. Improved Detection of the EGFR T790M Mutation in Lung Cancer Patients with Acquired Resistance to EGFR TKIs Using a Locked Nucleic Acid-based Approach

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Background: Somatic mutations of the epidermal growth factor receptor (EGFR) predict sensitivity to EGFR tyrosine kinase inhibitors (TKIs) in patients with lung adenocarcinoma. Despite initial responses, patients develop resistance to EGFR TKIs and approximately 50% of these patients have been reported to show a secondary EGFR mutation, T790M, in their tumor tissue. Previous studies suggest that the incidence of T790M mutations may be higher but is undetected by standard sequencing techniques. To address this, we used a locked nucleic acid (LNA) to develop a higher sensitivity PCR-sequencing assay to allow the detection of EGFR T790M in patients with low mutant allele burden. **Methods:** Clinical cases previously tested for T790M by standard sequencing were retrospectively reviewed and selected for the study. In all cases, initial testing was performed on DNA extracted from biopsies of metastatic or recurrent lung adenocarcinomas from patients with documented activating EGFR mutations, established response to EGFR TKIs and who had disease progression while on treatment. Stored DNA samples from previous mutation negative cases were then retested with modified PCR and sequencing, using standard primers in conjunction with a 10-mer LNA oligonucleotide designed to suppress amplification of non-mutant DNA during the PCR step. **Results:** A total of 39 tumor samples were tested for T790M between 8/08 and 5/09. Based on initial testing using standard PCR and sequencing, 38% (15/39) of cases were positive for the T790M mutation. Stored DNAs from 18 of the 24 negative cases were available for retesting with the LNA based assay and this detected 10 additional cases (10/18, 56%) with the T790M mutation. The detection sensitivity of similar LNA assays for specific point mutations based on serial dilutions of mutated heterozygous cell lines mixed with a normal control sample is at least 0.1%. **Conclusions:** Since 56% of cases negative by conventional sequencing were positive with a more sensitive assay, we estimate that the prevalence of the T790M resistance mutation in patients with established EGFR TKI resistance may be as high as 72%. This percentage is significantly higher than previously reported in the literature (usually approximately 50%). Improved detection of the T790M resistance mutation by higher sensitivity methods, such as the present one, may have significant clinical implications.