Clinical and in vivo Evidence that *EGFR* S768I Mutant Lung Adenocarcinomas Are Sensitive to Erlotinib

*Matthew D. Hellmann, MD, * Boris Reva, PhD,† Helena Yu, MD, * Valerie W Rusch, MD,‡ Naiyer A Rizvi, MD,* Mark G Kris, MD, * and Maria E Arcila, MD§‖*

Not all *EGFR* mutations are alike and the sensitivity of the less common ("minor") mutations to epidermal growth factor receptor (EGFR)-TKIs is often not well defined. Documenting sensitivity or resistance of these minor mutations is critical for guiding treatment decisions. Here, we use clinical response, in vivo evidence of selective pressure, and in silico modeling to demonstrate that *EGFR* S768I sensitizes tumors to erlotinib.

A five pack-year former smoker was diagnosed with localized, metachronous lung adenocarcinomas that were resected in 2001 and 2003. In 2005, new metastatic lung nodules appeared. Erlotinib 150 mg daily was begun, achieving a partial radiographic response (−40% by Response Evaluation Criteria in Solid Tumors v1.1, Fig. 1), and then continued for 8 years with durable response. In 2013, growth in a left upper lobe nodule consistent with acquired resistance was detected and resected. Mass-spectrometry genotyping of the resistance sample revealed *EGFR* S768I and T790M mutations. The original tumors from 2001 and 2003 were also evaluated and only the *EGFR* S768I mutation was detected. Further characterization of the resistance sample with a locked nucleic acid probe in the polymerase chain reaction step to completely suppress the wild-type allele confirmed that T790M occurred in the same clone and in-*cis* with the S768I mutation (Fig. 2).

We visualized the experimental structure of the EGFR protein (Fig. 3). The three-dimensional modeling suggests the substitution of serine at position 768 for a bulky isoleucine leads to loss of hydrogen bonding between a side chain hydroxyl of the original serine and a main chain oxygen of tyrosine at position 764. We hypothesize that the disturbance of tightly packed residues surrounding this region destabilizes the C-end of the alpha-helix (753–768) and pushes the kinase conformational equilibrium to favor a constitutively active state.[1,2] Notably, the

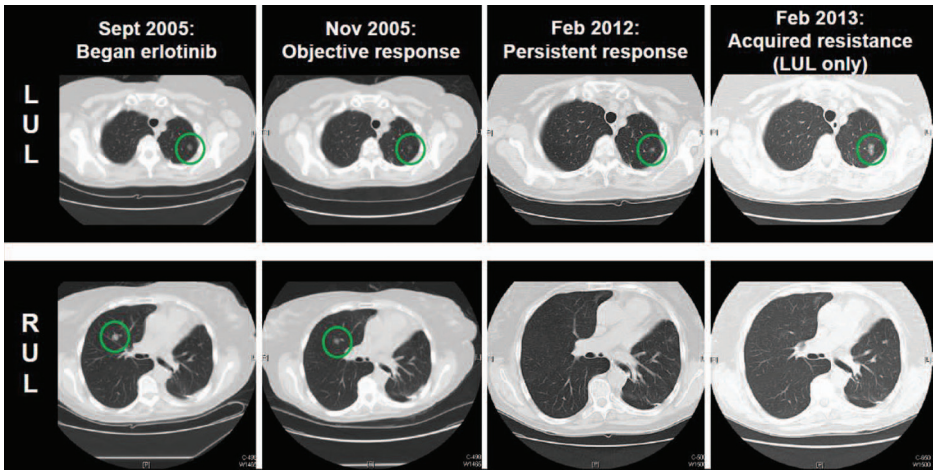

FIGURE 1. Radiographic response to erlotinib in patient with *EGFR* S768I mutation (–40% by Response Evaluation Criteria in Solid Tumors v1.1).

*Thoracic Oncology Service, Department of Medicine; †Computational Biology Center; ‡Thoracic Service, Department of Surgery; §Department of Pathology, Memorial Sloan-Kettering Cancer Center; and ‖Weill Cornell Medical College, New York, NY.

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Address for correspondence: Maria E Arcila, MD, Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY. E-mail: arcilam@mskcc.org

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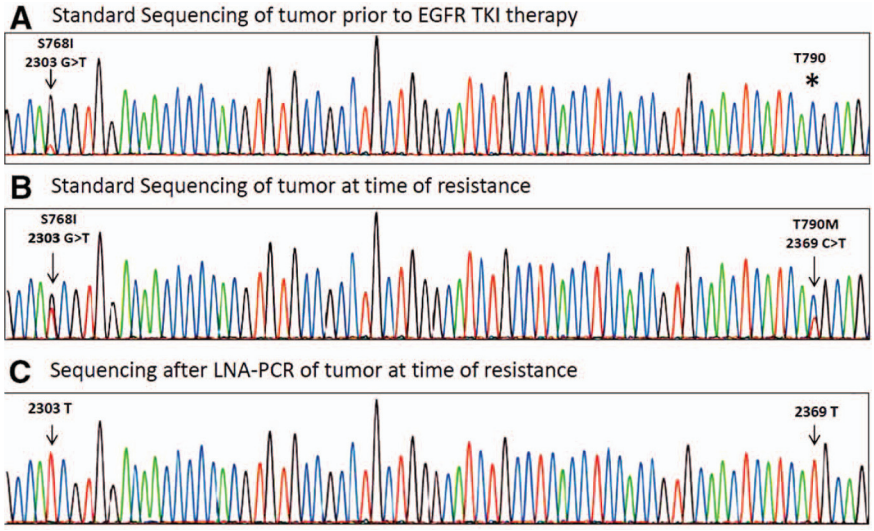

FIGURE 2. Mutation analysis of exon 20 of *EGFR* in the tumor sample using standard sequencing. *A*, Electropherogram from tumor before EGFR-tyrosine kinase inhibitor shows the mutation S768I and absence of T790M. (*B*) Electropherogram from tumor at acquired resistance shows both S768I and T790M. Both the wild-type and the mutant alleles are present. (*C*) Electropherogram corresponds to sequencing after LNA-polymerase chain reaction with a probe targeting the wild-type sequence in the region encompassing codons 789–792. With suppression of the wild-type allele, only the mutant peaks for S768I and T790M are present, indicating mutations are in-*cis* (same allele).

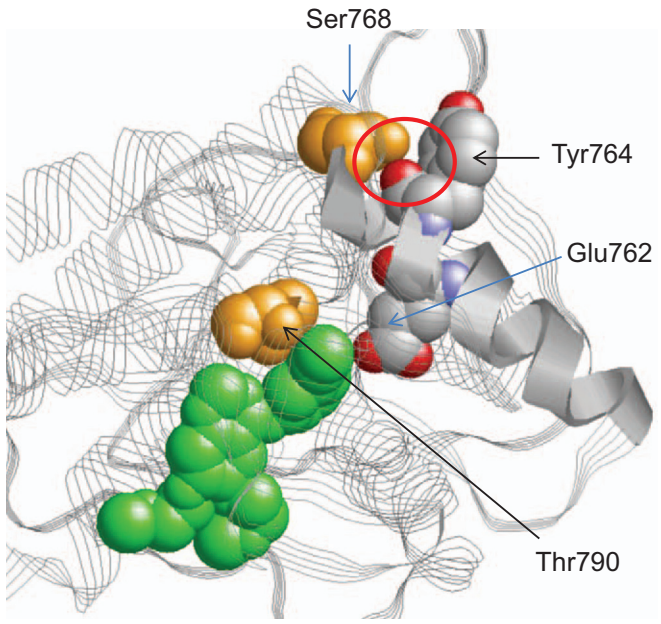

FIGURE 3. Three-dimensional structure of the EGFR kinase domain illustrating the location of the EGFR S768I and the T790M mutations (Protein Data Bank code of EGFR structure used is 1m17). The substitution of serine for a bulky isoleucine at position 768 will result in a loss of hydrogen bonding between a side chain hydroxyl of Ser768 and a main chain oxygen of Tyr764 (circled in red). The loss of the hydrogen bond and the disturbance of the tight residue packing in the mutation position is predicted to destabilize the C-end of the alpha-helix (753–768). We hypothesize that the conformational changes at the C-end of the alpha-helix will lead to an equilibrium shift favoring an active conformation of the kinase. Importantly, the location of this change is outside the binding pocket of erlotinib (green), thereby permitting binding of erlotinib in this pocket. By contrast, the substitution of a small threonine residue for a bulky methionine in the T790M mutation, directly affects the drug binding pocket rendering the protein resistant to the drug.

location of S768 is outside the erlotinib binding pocket, which would assert no immediate steric hinderance to erlotinib binding and is consistent with the sensitivity to erlotinib seen in vivo.

The *EGFR* S768I point mutation occurs in 1–2% of *EGFR* mutant lung cancers.[3] In vitro studies demonstrate the transforming capacity of this mutation.[4] However, sensitivity to EGFR-TKIs has been controversial; in vitro studies reported relative resistance,[4] whereas one prior clinical case of an *EGFR* S768I mutant lung adenocarcinoma reported apparent sensitivity to gefitinib.[5] However, it was unknown if the responding tumors were distinct subclones with a different or additional *EGFR* mutation. In other previously reported cases, *EGFR* S768I is often coincident with other *EGFR* mutations, which has further limited conclusions.

This case provides conclusive evidence that *EGFR* S768I mutant lung adenocarcinomas are sensitive to erlotinib. The in vivo emergence of *EGFR* T790M in the same allele confirms the selective pressure of erlotinib on the *EGFR* S768I mutant kinase. We believe molecular analyses of lung adenocarcinomas should evaluate for *EGFR* S768I mutations and patients harboring this mutation should receive an EGFR-tyrosine kinase inhibitor as initial therapy.

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