

# Letters

## RESEARCH LETTER

### Acquired Resistance of *EGFR*-Mutant Lung Cancer to a T790M-Specific *EGFR* Inhibitor: Emergence of a Third Mutation (C797S) in the *EGFR* Tyrosine Kinase Domain

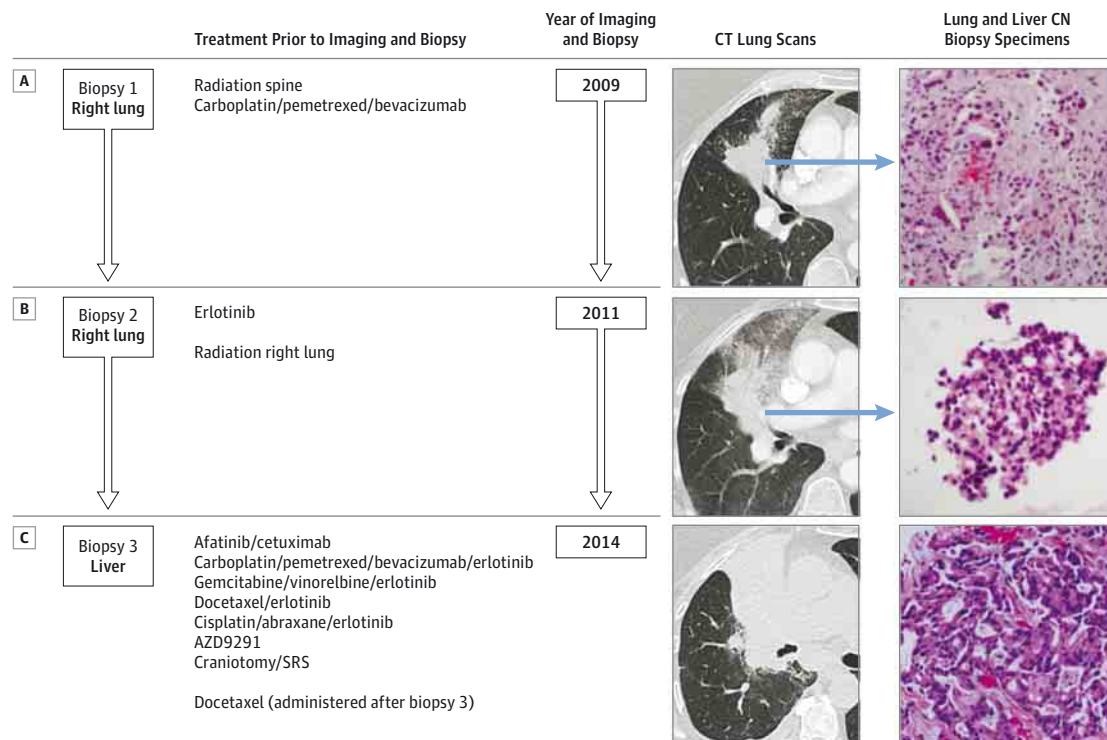
*EGFR*-mutant lung cancers represent a paradigm for the use of tyrosine kinase inhibitors (TKIs) to treat molecular subsets of cancer, with randomized trials demonstrating the efficacy of first-generation, reversible epidermal growth factor receptor (*EGFR*) TKIs. However, acquired resistance invariably develops, and rebiopsy of patients with clinical progression has elucidated *EGFR* T790M mutation as the major resistance mechanism.<sup>1</sup>

To address this clinical challenge, third-generation pyrimidine-based *EGFR* TKIs have been designed to have selectivity for *EGFR* T790M over wild-type *EGFR*.<sup>2</sup> They form a covalent bond with cysteine 797 in the adenosine triphosphate-binding cleft. Early-phase clinical trials have demonstrated

their efficacy in patients with double-mutant tumors (*EGFR* L858R/T790M and ex19del/T790M) and acquired resistance to first-generation *EGFR* inhibitors.<sup>3</sup> Specificity for *EGFR* T790M may be the result of hydrophobic interactions between the bulky methionine moiety of *EGFR* T790M and these pyrimidine-based drugs.

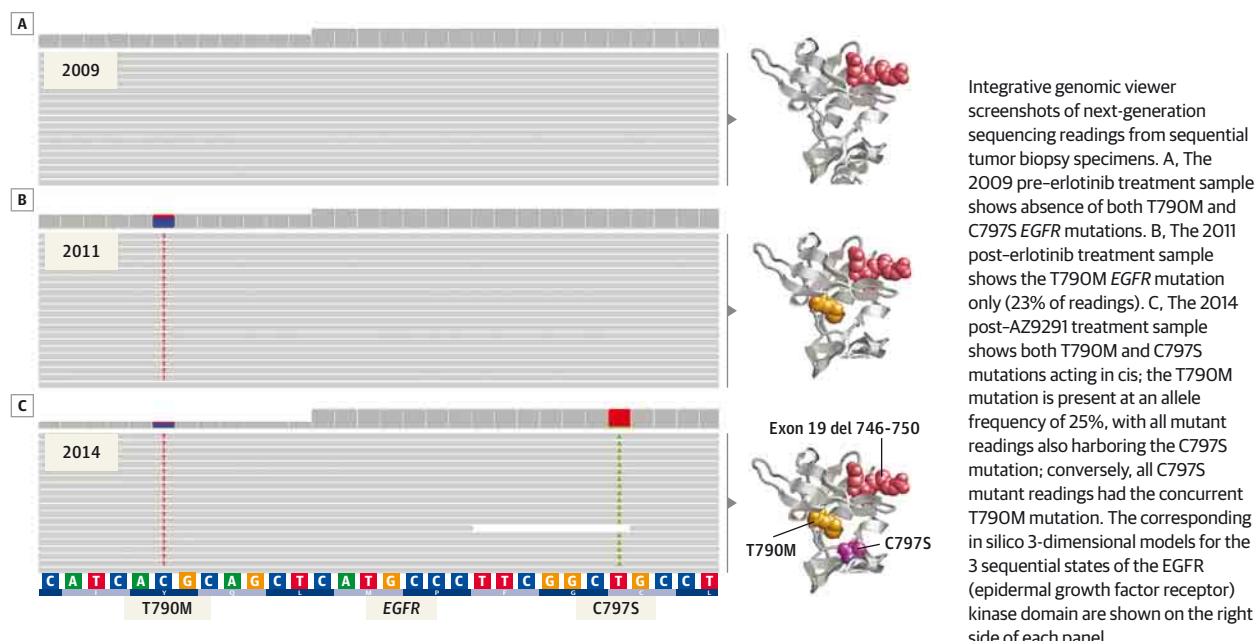
Mechanisms of resistance to these third-generation *EGFR* TKIs have been identified in preclinical in vitro models, but none have so far been identified in patients. Mutations at the *EGFR* C797 codon, located within the kinase-binding site, are a predicted resistance mechanism to irreversible *EGFR* inhibitors also confirmed in vitro.<sup>4</sup> Loss of the potential for covalent bond formation at position 797 by the missense mutation C797S results in a markedly reduced cellular potency of this class of TKIs.<sup>5</sup> Interestingly, an analogous cysteine-to-serine mutation is a mechanism of resistance to the irreversible Bruton tyrosine kinase inhibitor ibrutinib in patients with chronic lymphocytic leukemia.<sup>6</sup>

Figure 1. Patient Clinical Course Including Treatment History and Relevant Imaging Studies and Tumor Biopsy Specimens Demonstrating Adenocarcinoma



A, Right lung tumor before treatment with erlotinib (lung biopsy specimen positive for *EGFR* exon 19 deletion). B, Enlarging right lung tumor after acquired resistance to erlotinib (lung biopsy specimen positive for *EGFR* exon 19 deletion and *EGFR* T790M mutation). C, Further enlarged lung tumor CT after acquired resistance to AZD9291. In 2014, the pictured biopsy specimen is from a

metastatic liver lesion and was positive for *EGFR* exon 19 deletion, *EGFR* T790M mutation, and *EGFR* C797S mutation. All biopsy specimens were stained with hematoxylin-eosin, original magnification  $\times 200$ ; CN indicates core needle; CT, computed tomographic; SRS, stereotactic radiosurgery.

Figure 2. Mutation Analysis of Exon 20 of *EGFR* in the 2009, 2011, and 2014 Tumor Samples

**Report of a Case** | A former smoker in her 60s presented with stage IV lung adenocarcinoma metastatic to the lung, lymph nodes, bone, and brain. After progression during first-line chemotherapy, a biopsy of the right lung tumor (biopsy 1, Figure 1) identified a 15-base pair deletion in *EGFR* exon 19. She experienced a partial response with erlotinib treatment for 15 months. After further progression, the enlarging right lung mass was rebiopsied (biopsy 2, Figure 1), and in addition to the previous *EGFR* exon 19 deletion, Sanger sequencing identified *EGFR* T790M.

The patient was treated with a sequence of *EGFR* TKIs and chemotherapies (Figure 1). She also enrolled in a phase 1 study of AZD9291 (NCT01802632) and received AZD9291 for 9 months until further disease progression. She then underwent a biopsy of a hepatic metastasis (biopsy 3, Figure 1) in which Sanger sequencing identified a third mutation, *EGFR* C797S, in addition to the exon 19 deletion and T790M.

The patient signed an institutional biospecimen protocol, and next-generation sequencing (NGS) was performed on all 3 biopsy specimens: before treatment with erlotinib (biopsy 1, Figure 1), after acquired resistance to erlotinib (biopsy 2, Figure 1), and after acquired resistance to AZD9291 (biopsy 3, Figure 1). The *EGFR* mutations seen on Sanger sequencing were confirmed by NGS (Figure 2), which also showed that the C797S mutation was acting in cis with the T790M; ie, all alleles with C797S also had T790M, and conversely all alleles with T790M had C797S. In addition, *PTEN* Y27C and *CTNNB1* S37F mutations were present in all 3 samples, and a *TSC2* N486I was seen in the third biopsy sample only.

**Discussion** | We describe herein a patient whose tumor acquired an *EGFR* C797S mutation after treatment with a third-generation *EGFR* TKI. The acquired *EGFR* C797S should confer resistance to all third-generation *EGFR* TKIs, similar to the

emergence of *EGFR* T790M and its cross-resistance to all first-generation *EGFR* TKIs. The original sensitizing *EGFR* mutation was present in all obtained tumor samples, demonstrating the continued dependence of the tumor on *EGFR* signaling for growth and survival. Under the strong selective pressure of *EGFR* TKIs, the tumor developed secondary and tertiary mutations in *EGFR* (T790M and C797S, respectively) to maintain *EGFR* signaling. To our knowledge, this is the first report of a tertiary acquired mutation identified in a clinical lung cancer sample.

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## Efficacy of Prostate-Specific Antigen Screening: Use of Regression Discontinuity in the PLCO Cancer Screening Trial

The Prostate Lung Colorectal and Ovarian (PLCO) cancer screening trial randomized 76 693 men from 1993 to 2001 to usual care or annual prostate-specific antigen (PSA) screening for 6 years and annual digital rectal examination for 4 years. This study found that PSA screening results in increased detection of prostate cancer but does not reduce prostate cancer-specific or overall mortality. The findings of the PLCO cancer screening trial are controversial largely because of a high rate of PSA screening in the control group, which reached 52% by the sixth year of the trial.<sup>1,2</sup> Despite this shortcoming, the PLCO

trial is likely to remain the only major trial of PSA screening in the United States.

We used regression discontinuity (RD), a statistical technique used in the social sciences but rarely applied to clinical data, to address the above criticism.<sup>3</sup> This technique allows us to examine the effect of PSA screening on outcomes using only the screening arm of the PLCO trial.

**Methods** | The statistical basis of RD has been described previously.<sup>4</sup> Regression discontinuity allows us to leverage that a PSA of 4.0 ng/mL was used as the threshold for further workup in the PLCO trial (to convert PSA to micrograms per liter, multiply by 1). In the absence of a treatment effect, the regression of PSA and a given outcome should be continuous around the PSA cutoff. However, if a biopsy based on PSA screening affects an outcome, we would expect to find a discontinuity in the regression around a PSA of 4.0 ng/mL. Since confounders should be evenly distributed right below and above this cutoff, RD allows us to isolate the effect of screening on outcomes.

We obtained the 13-year screening and outcome data from the PLCO trial. The control arm of the study was dropped from all analyses. We used a first-degree local polynomial approach with the Imbens and Kalyanaraman mean squared error minimizing bandwidth.<sup>5</sup> Our results are not sensitive to this bandwidth choice. We used STATA/ICv13.1 (StataCorp) for statistical analysis. An RD analysis code was generated, and we confirmed its accuracy using a Stata module for RD estimation.<sup>6</sup> A waiver was obtained from the Office of Research Integrity at Weill Cornell Medical College; institutional review board review was not required as data was deidentified.

**Results** | The probability of a PLCO trial participant undergoing a biopsy as a function of the maximum PSA value from all tests increased at the 4.0 ng/mL PSA cutoff by 27.3% (95% CI, 23.3%-31.3%;  $P < 1 \times 10^{-10}$ ) (Figure). This translates into a relative 445% increase in the biopsy rate for those with a PSA just above 4.0 ng/mL compared with those just below that cutoff.

At a PSA of 4.0 ng/mL, biopsy based on screening increased the absolute detection rate of low-risk (Gleason score  $\leq 6$  at clinical stage T1-2a) prostate cancer by 7.2% (95% CI, 3.6%-10.8%;  $P = 8.5 \times 10^{-5}$ ) (Figure and Table). There was no effect on the detection of intermediate-risk (Gleason score = 7 or clinical stage T2b) ( $P = .94$ ) (Table) or high-risk (Gleason score  $\geq 8$  or clinical stage T2c-3a) ( $P = .98$ ) (Figure and Table) prostate cancer.

Examining the pathology from those who underwent prostatectomy yields similar results. There was a discontinuity in the detection of cancers with a Gleason score of 6 or lower (5.6% [95% CI, 2.6%-8.7%];  $P < .001$ ) and no discontinuity in the detection of scores of 7 ( $P = .52$ ) or 8 to 10 ( $P = .56$ ) (Table). We found no discontinuity in prostate cancer-specific mortality ( $P = .27$ ) or overall mortality ( $P = .62$ ) (Figure and Table).

**Discussion** | Using RD in the screening arm of the PLCO trial, we were able to effectively instrument for biopsy based on PSA screening. Despite excluding the control arm of the study, we