EGFR Gene Polymorphism Predicts Improved Outcome in Patients With *EGFR* Mutation-positive Non–small cell Lung Cancer Treated With Erlotinib

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Abstract

A polymorphism in the *epidermal growth factor receptor* (*EGFR*) gene (181946C>T) was evaluated as a predictor of outcome in 75 patients with *EGFR* mutation-positive, non–small cell lung cancer treated with an EGFR-targeting tyrosine kinase inhibitor. Significantly longer progression-free survival and overall survival was found in patients harboring the 181946CT genotype. The polymorphism could be an important predictor of treatment response.

Background: Patients with advanced-stage non–small cell lung cancer with *epidermal growth factor receptor* (*EGFR*) mutations are successfully treated with tyrosine kinase inhibitors (TKIs). However, treatment outcome varies significantly. Previously, we found the polymorphism 181946C>T (rs2293347) located in exon 25 of the *EGFR* gene to be a predictor of improved outcome. However, these data were based on a subgroup analysis. Furthermore, other minor studies have found conflicting data. Thus, the aim of this study was to demonstrate the association of 181946C>T with clinical outcome in an independent cohort of *EGFR*-mutated patients treated with erlotinib. **Patients and Methods:** Seventy-five patients were prospectively enrolled. Blood samples were collected, and genotype for 181946C>T was determined by allele-specific polymerase chain reaction. Genotype was correlated with outcome. **Results:** In 73 patients, 181946C>T was successfully measured. Patients harboring the 181946CT genotype had a significantly longer median progression-free survival compared with patients harboring the 181946CC genotype (49.9 months [95% confidence interval (CI), 5.9-93.9 months] versus 11.1 months (95% CI, 7.4-14.9 months); $P = .020$). Moreover, a significantly longer median overall survival of 65.6 months (95% CI, 11.0-120.3 months) versus 31.2 months (95% CI, 10.9-51.6 months) was found ($P = .019$). Both results remained significant in a multivariate analysis adjusting for potential confounders. **Conclusion:** We demonstrate that the 181946C>T polymorphism is a significant predictor of prolonged progression-free survival and overall survival in an independent cohort of *EGFR* mutation-positive patients treated with erlotinib. The polymorphism could be an important predictor of treatment response in these patients. A large multicenter cohort study involving other concurrent genetic alterations is warranted.

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Keywords: Epidermal growth factor receptor mutation, Genetic polymorphisms, Non-small cell lung cancer, Tyrosine kinase inhibitor, Treatment response

Introduction

Somatic mutations in the *epidermal growth factor receptor* (*EGFR*) have been identified as important driver mutations in a subset of patients with non–small cell lung cancer (NSCLC).[1,2] This subgroup of *EGFR* mutation-positive patients can be treated with a targeted, small tyrosine kinase inhibitor (TKI) directed against the

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EGFR, which has markedly improved their survival.[3,4] However, substantial individual variability in the response to EGFR-TKIs is observed. Although a considerable proportion of patients initially achieve remarkable disease control, progression inevitably occurs. This can be caused by either intrinsic resistance[5] or resistance acquired during treatment.[6] Several different resistance mechanisms have been identified, with the most important being the resistance mutation T790M,[7,8] MET gene amplification,[9] and histologic transformation to SCLC.[6] Yet, the significant individual variability in response is still not fully understood.

It has been proposed that the variability in part could be explained by genetic variation.[10,11] Germline variations located in genes involved in drug transport, drug metabolism, and in genes directly affected by the drugs could affect the pharmacokinetics and pharmacodynamics of a drug by changing protein expression, structure, and/or function. Several pharmacogenetics studies have investigated the association between different genetic polymorphisms and treatment response in patients with NSCLC treated with an EGFR-TKI.[12,13] The synonymous single nucleotide polymorphism (SNP) 181946C>T (rs2293347) located in exon 25 of the *EGFR* gene has shown promising results in several studies. However, data have been conflicting. In two studies with Chinese patients, a better outcome on gefitinib was found in patients with NSCLC harboring the 181946CC genotype.[14,15] In contrast, we demonstrated a positive correlation between the 181946CT/TT genotype and clinical outcome in a cohort of erlotinib-treated patients with NSCLC.[16] In the latter study, a subgroup analysis based on EGFR mutation status revealed that the strongest correlation was found in the subset of EGFR mutation-positive patients. In contrast, no investigation based on EGFR mutation status was performed in the two other studies[14,15] as complete data on EGFR mutation status was lacking. Thus, in these studies, the importance of the SNP in relation to the EGFR-TKI treatment remains unclear in both the *EGFR* mutated and *EGFR* wild-type patients.

In order to address the contradictory data, the aim of this study was to reevaluate the 181946C>T in an independent validation cohort of *EGFR* mutation-positive NSCLC patients treated with erlotinib.

Patients and Methods

Patients

Patients with advanced stage, *EGFR* mutation-positive NSCLC starting treatment with erlotinib were enrolled between September 2014 and March 2017 at the Department of Oncology, Aarhus University Hospital Denmark. Patients were eligible for enrollment if they fulfilled the following criteria: age $\geq$ 18 years, histologically or cytologically proven NSCLC, *EGFR* mutation-positive in a tumor sample, and no prior treatment with a TKI. All patients gave informed written consent before inclusion in the study, and the study was approved by the Central Denmark Region Committees on Biomedical Research Ethics (no. 1-10-72-83-14).

All patients had a pretreatment computed tomography (CT) scan of the chest and abdomen performed prior to start of erlotinib. Follow-up was done by CT scans every 12 weeks during the treatment period combined with clinical and biochemical evaluation every fourth week in the first 12 weeks and subsequently every sixth week as standard of care in our institution. Treatment response evaluated on CT scans was defined according to Response Evaluation Criteria in Solid Tumors, version 1.1 criteria. Neuroimaging was performed on clinical indication. If side effects appeared during the treatment, erlotinib was continued together with symptomatic management of toxicity before any dose reduction. However, if the patient had continuous side effects of grade 2 or more according to the Common Terminology Criteria for Adverse Events, version 4.0, dose reduction was performed. The patients were treated until radiologic or clinical progression, unacceptable toxicity, or death. At the end of treatment, the patient was evaluated regarding subsequent treatment with a platinum-based chemotherapy or best supportive care according to the standard of care at our institution. From May 2017, osimertinib became available at our institution for treatment of patients with the resistance mutation T790M.

Testing for somatic EGFR mutations in exon 19-21 was performed as part of the diagnostic workup in all patients and was performed by use of the Therascreen EGFR RGQ polymerase chain reaction (PCR) kit (QIAGEN, Manchester, UK) according to the manufacturer's protocol.

DNA Extraction and Genotyping

Genomic DNA was extracted from a blood sample. A 10 mL blood sample was centrifuged (1400 g for 15 minutes), and peripheral white blood cells were isolated in the buffy coat. Genomic DNA was isolated using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was eluted in 20 µl elution buffer.

Genotyping of 181946C>T was determined using allele-specific PCR with a blocking reagent as described by Morlan et al.[17] Primers and blocker for detection of the C-allele were: 5′CCTGCT GGCAATAGACCCCT-3′ (forward), 5′-GCACGGTAGAAGTT GGAG-3′ (reverse) and 5′GGTAGAAGTTGGAATCTGTA GGACTTG-3′ (blocker), and for the T-allele: 5′CCTGCTGGCA ATAGACCCCT-3′ (forward), 5′GCACGGTAGAAGTTGGAA-3′ (reverse) and 5′GTAGAAGTTGGAGTCTGTAGGACTTG-3′ (blocker). PCR amplification was performed in a 10 µl reaction volume containing 5 µl Lightcycler 480 Probe Master (Roche Diagnostics, Mannheim, Germany), 1 µl H_2O, 0.6 µl forward primer (15 pmol/µl), 0.6 µl reverse primer (15 pmol/µl), 1.8 µl blocker (20 pmol/µl), and 1 µl genomic DNA. Samples were initially denatured at 95°C for 10 seconds followed by 45 cycles (each cycle at 95°C for 10 seconds, 62°C for 30 seconds, and 72°C for 1 second). As control for the C allele, the lung cancer cell line H1568 was used, and for the T allele, a plasmid was constructed with the variant sequence.

Statistical Analysis

The χ^2 goodness-of-fit test was used to compare observed frequencies of alleles and genotypes against the Hardy-Weinberg equilibrium prediction. The association between genotype and clinical characteristics was calculated with the Fisher exact test. The last follow-up date was December 6, 2017. Progression-free survival (PFS) was the primary endpoint, and overall survival (OS) was the second endpoint. PFS was defined as the time from first administration of erlotinib to first documentation of radiologic or clinical progression or death. OS was determined as the time from start of erlotinib until death of any course or last follow-up date. Estimates of median PFS and OS were calculated using the Kaplan-Meier

method and analyzed by the log-rank test. Univariate and multivariate hazard ratios (HRs) were determined using Cox proportional hazards model. Multivariate analysis of PFS was adjusted for 4 variables: age (continuous), performance status (0-1 vs. 2-3), smoking status (never or former vs. current), and line of erlotinib therapy (first vs. second), and multivariate analysis of OS was adjusted for 2 variables: performance status (0-1 vs. 2-3) and line of erlotinib therapy (first vs. second). All tests were 2-sided, and *P* values less than .05 were considered to be statistically significant. Statistical analyses were performed using SPSS statistics version 24.0 for windows (IBM SPSS Statistics, Chicago, IL).

Results

Patients

Seventy-five patients were included. A flow diagram of inclusion is shown in Figure 1. Patient characteristics are summarized in Table 1. As can be seen, the majority of patients were females, never-smokers, and received erlotinib as the first line of treatment. All patients had adenocarcinoma. An exon 19 deletion was the most frequent *EGFR* mutation (55%) followed by the point mutation L858R in exon 21 (32%). No patients were lost to follow-up. On the last follow-up day, 37 patients (49%) were still alive, and 14 patients (19%) were still on treatment with erlotinib. The median PFS of all patients was 13.5 months (95% confidence interval [CI], 10.5-16.6 months), and the median OS was 41.3 months (95% CI, 22.0-60.6 months).

Genotype Distribution

Genotyping succeeded in 73 (97%) patients. Sixty (82%) patients were identified with the 181946CC genotype and 13 (18%) patients with the 181946CT genotype. No patients were homozygous for the SNP. The distribution of the SNPs was in concordance with the Hardy-Weinberg expectation ($P = .732$). The minor allele frequency was 0.089, which was as expected in Caucasians according to the National Center for Biotechnology Information SNP database.[18] Distribution of genotypes in correlation with patient characteristics is shown in Supplemental Table 1 (in the online version).

Figure 1 Flow Diagram of Patient Inclusion

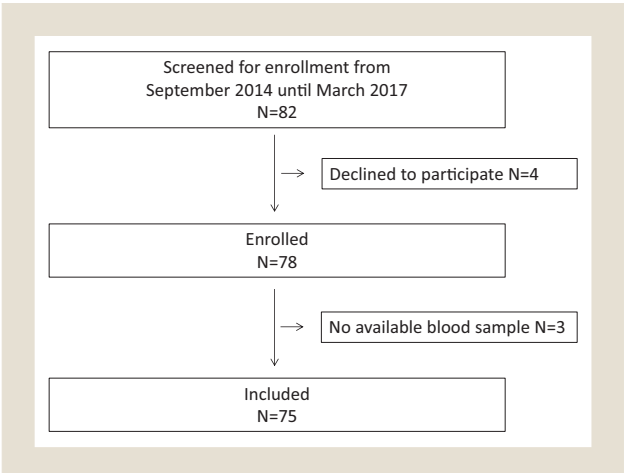


Table 1 Basic Characteristics for All Patients (N = 75)

Patient Characteristics	N (%)
Age, y	
Median (range)	69 (44-89)
Gender	
Female	56 (75)
Male	19 (25)
Smoking	
Never-smoker	29 (38)
Former smoker[a]	38 (51)
Current smoker	7 (10)
Missing data	1 (1)
ECOG performance status	
0	24 (32)
1	35 (47)
2	14 (19)
3	1 (1)
Missing data	1 (1)
Histology	
Adenocarcinoma	75 (100)
Stage	
IV	75 (100)
EGFR mutation	
Del19	41 (55)
L858R	24 (32)
L861Q	3 (4)
S768I and G719X	3 (4)
G719X	2 (3)
Exon 20 insertion	2 (3)
Erlotinib therapy	
First-line	71 (95)
Second-line	4 (5)
Osimertinib treatment	
Second-line	14 (19)
Third-line	2 (3)
Fourth-line	5 (7)
No treatment	54 (71)

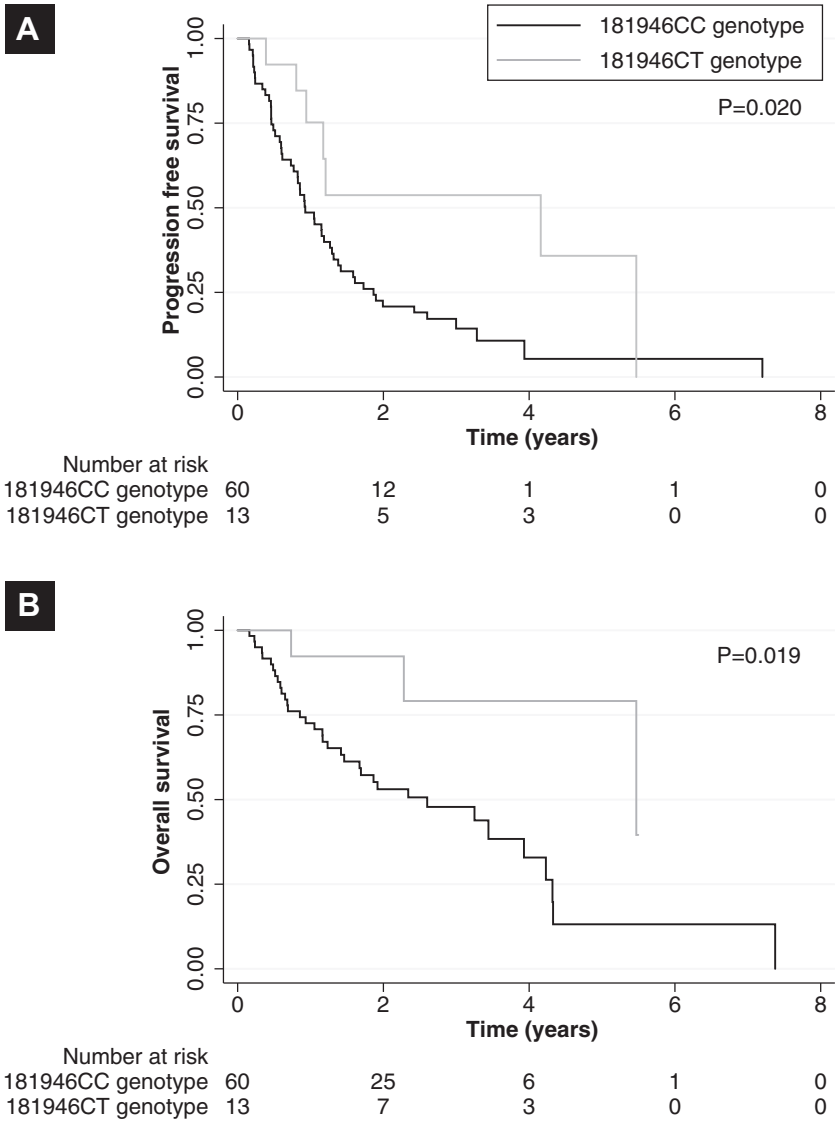
Abbreviations: ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor.
[a]Former smoker was defined as having stopped smoking at time of diagnosis.

181946C>T and Clinical Outcome

Patients harboring a T allele at position 181946 had a significantly longer median PFS of 49.9 months (95% CI, 5.9-93.9 months) compared with patients harboring the 181946CC genotype (11.1 months [95% CI, 7.9-14.9 months] $P = .020$) (Figure 2A). Moreover, patients with the 181946CT genotype had a significantly longer median OS of 65.6 months (95% CI, 11.0-120.3 months) compared with patients with the 181946CC genotype (31.2 months [95% CI, 10.9-51.6 months] $P = .019$) (Figure 2B).

A multivariate Cox regression analysis was conducted. Harboring the T allele at position 181946 remained an independent predictive factor for both longer median PFS with an adjusted HR of 0.348

Figure 2 Kaplan-Meier Survival Curves for Progression-free Survival (A) and Overall Survival (B) according to 181946C>T Genotype (N = 73). Differences Between Groups Were Calculated Using the Log-Rank Test



(95% CI, 0.141-0.857; $P = .022$) and for longer median OS (adjusted HR, 0.210; 95% CI, 0.058-0.764; $P = .018$). All regression data are shown in Table 2.

Discussion

This study aimed at evaluating the association between the 181946C>T SNP and outcome on erlotinib in patients with advanced-stage, *EGFR* mutation-positive NSCLC. The significance of the 181946C>T in EGFR–TKI-treated patients with NSCLC is controversial as data from former studies have been conflicting. Two studies performed in 83[14] and 128[15] Chinese patients treated with gefitinib in the second or third line found a superior outcome in patients carrying the 181946CC genotype. In contrast, we demonstrated in 331 patients of Caucasian ethnicity a significant longer PFS and OS on erlotinib in patients carrying the T allele.[16] A subgroup analysis in our study indicated that the strongest correlation was found in the 82 patients harboring an activating *EGFR* mutation. However, only 44% of the *EGFR* mutation-positive patients in this study received erlotinib in the first-line of treatment, whereas the rest were treated in the second or third-line. Data on *EGFR* mutation status was incomplete or lacking in the 2 other studies and therefore not evaluated. Overall, the conflicting data could therefore be owing to the mixture of EGFR mutation-positive and EGFR wild-type patients in the studies and a suboptimal treatment of the mutation-positive patients. Moreover, differences in the importance of the SNP between ethnicities cannot be excluded. Lastly, owing to the low frequency of the SNP, the number of patients carrying the CT/TT genotype was limited in all studies, making survival calculations prone to statistical uncertainty because of the low number of events in this group of patients.

Table 2 Univariate and Multivariate Cox Regression Analysis for Progression-free Survival and Overall Survival (N = 73)

	Progression-free Survival		Overall Survival	
	HR (95% CI)	Adjusted HR (95% CI)	HR (95% CI)	Adjusted HR (95% CI)
Age[a]	0.98 (0.96-1.01)	0.97 (0.95-1.00)	1.01 (0.98-1.04)	
Gender				
Male versus female	1.13 (0.63-2.03)		1.11 (0.52-2.36)	
Smoking				
Current versus never or former[b]	2.48 (1.10-5.60)	1.61 (0.66-3.91)	1.79 (0.62-5.14)	
Line of treatment				
First versus second	6.28 (1.39-28.36)	6.66 (1.21-36.76)	6.90 (0.90-53.16)	4.93 (0.63-38.77)
ECOG PS				
0-1 versus 2-3	0.88 (0.47-1.64)	0.76 (0.37-1.57)	0.57 (0.27-1.20)	0.36 (0.17-0.78)
181946C>T				
CT versus CC	0.39 (0.17-0.88)	0.35 (0.14-0.86)	0.26 (0.08-0.87)	0.21 (0.06-0.76)

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio.
[a]Age was tested as a continues variable.
[b]Former smoker was defined as having stopped smoking at time of diagnosis.

In order to investigate some of these concerns and validate our previous data, we evaluated the SNP in an independent cohort solely consisting of *EGFR* mutation-positive patients of Caucasian ethnicity where 95% of patients received erlotinib as first-line treatment. We were able to confirm our previous findings, which significantly increases the validity of our results. We genotyped the SNP in 75 patients prospectively enrolled. A significantly longer PFS and OS were found in patients harboring a T allele. Surprisingly, the median PFS was found to be nearly 40 months longer in patients with the T allele compared with patients who were homozygous for the C allele. Further, this resulted in a nearly 30 month longer median survival. After adjustment for potential confounding factors, the results still remained significant, and data indicates an independent value of the SNP on clinical outcome. The 181946C>T could therefore be an important marker of clinical outcome on first-line erlotinib in *EGFR* mutation-positive patients.

The 181946C>T SNP is located in exon 25 of the *EGFR* gene, which is a region considered to control the protein kinase activity. Thus, the SNP is located in the coding part of the gene; nonetheless, the SNP is synonymous as the nucleotide change does not result in any change in the amino acid sequence. Historically, synonymous SNPs were considered to be without any function and consequently only received very little attention. Recently, however, data have revealed that synonymous variants can significantly impact the amount, structure, and/or function of the protein by leading to alternative splicing, changing the structure, and/or stability of the mRNA.[19,20] The synonymous SNP 1236C>T located in the *Multidrug Resistance 1* gene was shown to alter function of the protein product (P-glycoprotein) owing to an effect on the timing of the protein folding.[21] Moreover, a synonymous SNP located in the *Catechol-O-methyltransferase* gene was shown to lead to an increased protein expression aiding to alterations in mRNA secondary structure.[22] Yet, the functionality of the 181946C>T has not been determined.

This study is strengthened by its prospective nature, consecutive sampling of the cohort, and the complete clinical data on all patients. Still, the study also has some limitations to consider. Owing to the design of the study, no control group of untreated patients was included, and we were therefore unable to decide whether the observed association was truly predictive or prognostic. Further studies are needed to discriminate between the predictive and prognostic value of the SNP. Moreover, the number of patients was limited. Yet, the study is still the largest to evaluate the SNP in a cohort of exclusively *EGFR* mutation-positive patients of Caucasian ethnicity.

Conclusion

This study indicates an association between the SNP 181946C>T and clinical outcome in TKI-treated patients with advanced-stage, *EGFR* mutation-positive NSCLC. The study confirms previous findings, and the SNP could be an important predictor of treatment response in this subgroup of patients with NSCLC. A well-designed large multicenter cohort study involving other concurrent genetic alterations is warranted.

Clinical Practice Points

- Treatment response on TKIs varies significantly in patients with advanced stage NSCLC with *EGFR* mutations.
- The 181946C>T polymorphism located in exon 25 of the *EGFR* gene has proven to be a potential predictor of improved clinical outcome. Yet, data have been conflicting.
- Here, we validate the SNP as a predictor of outcome in 75 patients with *EGFR* mutation-positive NSCLC treated with erlotinib.
- A significant longer PFS and OS was found in patients harboring the 181946CT genotype.
- The polymorphism could be an important predictor of treatment response in these patients in the future.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2019.02.011.

References

1. Pao W, Miller V, Zakowski M, 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. *Proc Natl Acad Sci U S A* 2004; 101: 13306-11.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004; 350:2129-39.
3. Rosell R, Carcereny E, Gervais R, et al. Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13:239-46.
4. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; 361:947-57.
5. Blakely CM, Watkins TBK, Wu W, et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. *Nat Genet* 2017; 49:1693-704.
6. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; 3:75ra26.
7. Rosell R, Molina MA, Costa C, et al. Pretreatment EGFR T790M mutation and BRCA1 mRNA expression in erlotinib-treated advanced non-small-cell lung cancer patients with EGFR mutations. *Clin Cancer Res* 2011; 17:1160-8.
8. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. *Cancer Res* 2006; 66: 7854-8.
9. Turke AB, Zejnullahu K, Wu Y-L, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* 2010; 17:77-88.
10. Wheeler HE, Maitland ML, Dolan ME, et al. Cancer pharmacogenomics: strategies and challenges. *Nat Rev Genet* 2013; 14:23-34.
11. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. *Trends Mol Med* 2011; 7:201-4.
12. Galvani E, Peters GJ, Giovannetti E. EGF receptor-targeted therapy in non-small-cell lung cancer: role of germline polymorphisms in outcome and toxicity. *Future Oncol* 2012; 8:1015-29.
13. Soh SX, Siddiqui FJ, Allen JC, et al. A systematic review and meta-analysis of individual patient data on the impact of the BIM deletion polymorphism on treatment outcomes in epidermal growth factor receptor mutant lung cancer. *Oncotarget* 2017; 8:41474-86.
14. Ma F, Sun T, Shi Y, et al. Polymorphisms of EGFR predict clinical outcome in advanced non-small-cell lung cancer patients treated with gefitinib. *Lung Cancer* 2009; 66:114-9.
15. Zhang L, Yuan X, Chen Y, Du X-J, Yu S, Yang M. Role of EGFR SNPs in survival of advanced lung adenocarcinoma patients treated with gefitinib. *Gene* 2013; 517:60-4.
16. Winther-Larsen A, Nissen PH, Jakobsen KR, Demuth C, Sorensen BS, Meldgaard P. Genetic polymorphism in the epidermal growth factor receptor gene predicts outcome in advanced non-small cell lung cancer patients treated with erlotinib. *Lung Cancer* 2015; 90:314-20.
17. Morlan J, Baker J, Sinicropi D. Mutation detection by real-time PCR: a simple, robust and highly selective method. *PLoS One* 2009; 4:e4584.
18. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* 2001; 29:308-11.
19. Hunt RC, Simhadri VL, Iandoli M, Sauna ZE, Kimchi-Sarfaty C. Exposing synonymous mutations. *Trends Genet* 2014; 30:308-21.
20. Sauna ZE, Kimchi-Sarfaty C, Ambudkar SV, Gottesman MM. Silent polymorphisms speak: how they affect pharmacogenomics and the treatment of cancer. *Cancer Res* 2007; 67:9609-12.
21. Kimchi-Sarfaty C, Oh JM, Kim IW, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 2007; 315:525-8.
22. Nackley AG, Shabalina SA, Tchivileva IE, et al. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 2006; 314:1930-3.

Supplemental Data

Supplemental Table 1 Genotype Distribution of 181946C>T in Correlation With Patient- and Treatment-related Characteristics

Characteristics	CC N (%)	CT N (%)	*P* Value[a]
Age			
< 69 years	26 (43)	10 (77)	.035
> 69 years	34 (57)	3 (23)	
Gender			
Male	13 (22)	5 (39)	.286
Female	47 (78)	8 (61)	
Smoking			
Current	7 (12)	0 (0)	.339
Never or former[b]	53 (88)	13 (100)	
ECOG PS			
0-1	48 (81)	10 (77)	.708
2-3	11 (19)	3 (23)	
Line of treatment			
First-line	58 (97)	11 (85)	.143
Second-line	2 (3)	2 (15)	
EGFR mutation			
Del19	29 (48)	11 (84)	.042[c]
L858R	22 (37)	1 (8)	
L861Q	3 (5)	0 (0)	
S768I and G719X	3 (5)	0 (0)	
G719X	1 (2)	1 (8)	
Exon 20 insertion	2 (3)	0 (0)	

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor.
[a] *P* value was calculated with the Fisher exact test.
[b] Former smoker was defined as having stopped smoking at time of diagnosis.
[c] Difference between Del19 and L858R.