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Clinical Cancer Research

Predictive Biomarkers and Personalized Medicine

Pretreatment EGFR T790M Mutation and BRCA1 mRNA Expression in Erlotinib-Treated Advanced Non–Small-Cell Lung Cancer Patients with EGFR Mutations

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Abstract

Purpose: Advanced non–small-cell lung cancer (NSCLC) patients harboring epidermal growth factor receptor (EGFR) mutations (deletion in exon 19 or L858R) show an impressive progression-free survival of 14 months when treated with erlotinib. However, the presence of EGFR mutations can only imperfectly predict outcome. We hypothesized that progression-free survival could be influenced both by the pretreatment EGFR T790M mutation and by components of DNA repair pathways.

Experimental Design: We assessed the T790M mutation in pretreatment diagnostic specimens from 129 erlotinib-treated advanced NSCLC patients with EGFR mutations. The expression of eight genes and two proteins involved in DNA repair and four receptor tyrosine kinases was also examined.

Results: The EGFR T790M mutation was observed in 45 of 129 patients (35%). Progression-free survival was 12 months in patients with and 18 months in patients without the T790M mutation ($P = 0.05$). Progression-free survival was 27 months in patients with low BRCA1 mRNA levels, 18 months in those with intermediate levels, and 10 months in those with high levels ($P = 0.02$). In the multivariate analysis, the presence of the T790M mutation (HR, 4.35; $P = 0.001$), intermediate BRCA1 levels (HR, 8.19; $P < 0.0001$), and high BRCA1 levels (HR, 8.46; $P < 0.0001$) emerged as markers of shorter progression-free survival.

Conclusions: Low BRCA1 levels neutralized the negative effect of the T790M mutation and were associated with longer progression-free survival to erlotinib. We advocate baseline assessment of the T790M mutation and BRCA1 expression to predict outcome and provide alternative individualized treatment to patients based on T790M mutations and BRCA1 expression. *Clin Cancer Res; 17(5); 1160–8. ©2011 AACR.*

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Introduction

Lung cancer is the leading cause of cancer-related deaths, with current chemotherapies lacking adequate specificity and efficacy. In a mouse model of lung cancer, cisplatin treatment promoted the emergence of resistant tumors expressing elevated levels of multiple DNA damage repair genes. (1) In contrast, high sensitivity to cisplatin was found in breast cancer-related gene 1 (BRCA1)-deficient mouse mammary tumors. (2) It is possible to subdivide non–small-cell lung cancer (NSCLC) patients into genetically discrete subsets on the basis of the activating mutations that they harbor. (3) Certain subsets of patients can benefit from treatment with specific inhibitors; for example, in Caucasian patients harboring activating mutations in the epidermal growth factor receptor (EGFR) kinase domain, landmark outcomes of a 70% response rate, 14-month progression-free survival, and 27-month median

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Translational Relevance

Epidermal growth factor receptor (EGFR) mutations are considered a relevant biomarker for treatment with EGFR tyrosine kinase inhibitors. However, different genetic abnormalities can affect the duration of response. In the present study, the concomitant presence of the EGFR T790M mutation was observed in 35% of the patients and adversely affected the progression-free survival to erlotinib. In addition, high levels of BRCA1 mRNA expression also had a negative effect. These findings provide more accurate predictive information on the effect of erlotinib in patients with EGFR mutations and pave the way for clinical trials targeting either the T790M mutation or BRCA1 expression.

survival have been attained with erlotinib. (4) In 3 phase III trials of Asian patients with EGFR mutations, median progression-free survival was significantly longer in patients receiving gefitinib (9.2–10.8 months) than in those receiving chemotherapy (5.4–6.3 months), with a significant improvement in the hazard ratios for progression (0.30–0.48; $P < 0.001$; ref.. 5–7) However, current markers can only imperfectly predict the length of progression-free survival in these patients. Moreover, a secondary mutation in EGFR, the T790M "acquired resistance mutation", has been observed in 50% of cases resistant to gefitinib or erlotinib. (8–11) A critical issue is whether this T790M mutation occurs as a result of treatment or whether it exists prior to treatment and is selected during the course of therapy. Achievable plasma concentrations of gefitinib led to the development of EGFR T790M *in vitro*, (12, 13), but EGFR T790M has also been determined in EGFR-mutated cell lines at frequencies of 55% (H1975), 7% (H820), and 2% (the gefitinib-resistant H3255; ref.. 14) Moreover, EGFR T790M was detected at low levels in pretreatment tumor samples from 38% of patients with EGFR-mutant NSCLC and was associated with shorter progression-free survival (7.7 versus 16.5 months; ref. 15)

EGFR-mutated NSCLC cell lines, with or without the T790M mutation, showed enhanced radiosensitivity, related to impaired nonhomologous end-joining and the resultant altered formation of the Ku/DNA-dependent protein kinase (DNA-PK) catalytic subunit (cs) complex. (16, 17) Both erlotinib and radiation increased phosphorylated H2AX (γH2AX) foci (a marker of double-strand breaks) in human breast cancer cells, which was related to attenuated homologous recombination repair. Subsequent disruption of BRCA1 greatly enhanced erlotinib cytotoxicity. (18) Hypoxia-induced downregulation of BRCA1 expression is a functional, nonmutational mechanism of BRCA1 inactivation in sporadic cancers. (19) Moreover, decreased BRCA1 resulted in increased sensitivity to the DNA cross-linking agents mitomycin C and cisplatin but not to the antimicrotubule paclitaxel. (20) Experimental (21, 22) and clinical (22, 23) studies, including our own experience, (23–25) have also indicated a differential effect of BRCA1 expression on chemotherapy. In a phase II study of customized chemotherapy based on BRCA1 mRNA levels, the expression of the receptor-associated protein 80 (RAP80), a ubiquitin-binding protein that interacts with BRCA1, strongly modulated the effect of BRCA1, (26) confirming preclinical evidence (27) that RAP80 is essential for the BRCA1 repair function in an H2AX-dependent manner (Fig. 1).

Poly-ubiquitylated histones serve as a platform to recruit the BRCA1/RAP80 complex, directed by the ability of RAP80 to bind ubiquitin through its UIM domains. BRCA1 is then sumoylated in a protein inhibitor of activated STAT (PIAS)1- and PIAS4-dependent manner, which increases its E3 ubiquitin ligase activity. (28) In addition, CtIP forms complexes with BRCA1 and processes double-strand breaks in the initial DNA damage response. (29) Finally, the 7 *in absentia* homologue (SIAH2) and the ubiquitin-conjugating enzyme E2T (UBE2T) can cause proteasomal degradation of BRCA1.

Upregulation of other receptor tyrosine kinases can also affect response to gefitinib or erlotinib. Amplification of the MET oncogene is observed in 20% of resistant cases. (30, 31) AXL is a member of a receptor tyrosine kinase family that contains a kinase domain closely related to MET, and AXL overexpression leads to resistance to imatinib in gastrointestinal stromal tumors. (32) IL-6ST expression leads to gefitinib resistance in EGFR-mutated cell lines, (33) and activation of IGF-1Rβ signaling drives gefitinib resistance in EGFR wild-type cancer cell lines. (34) Activation of these receptor tyrosine kinases led to the maintenance of the phosphoinositide 3-kinase-AKT and Ras-extracellular signal-regulated kinase pathways in the presence of gefitinib or erlotinib. (35)

We have examined the presence of the EGFR T790M mutation in pretreatment tumor samples of NSCLC patients who were prospectively treated with erlotinib as part of a Spanish Lung Cancer Group (SLCG) program (4) and correlated the presence of the T790M mutation with progression-free survival. We have also analyzed the potential influence on progression-free survival of the expression of several components of homologous recombination (BRCA1, RAP80, CtIP, PIAS1, PIAS4) and nonhomologous end-joining (PP2A/C, DNA-PKcs, and Ku70/Ku86), as well as SIAH2 and UBE2T. The influence of the expression levels of MET, AXL, IL-6ST, and IGF-1R was also examined.

Materials and Methods

Clinical samples and pathology evaluation

For the Spanish Lung Adenocarcinoma Data Base (SLADB; ref. 4), the SLCG had prospectively screened 2,105 NSCLC patients from 129 institutions for EGFR mutations. EGFR mutations were detected in 350 patients,

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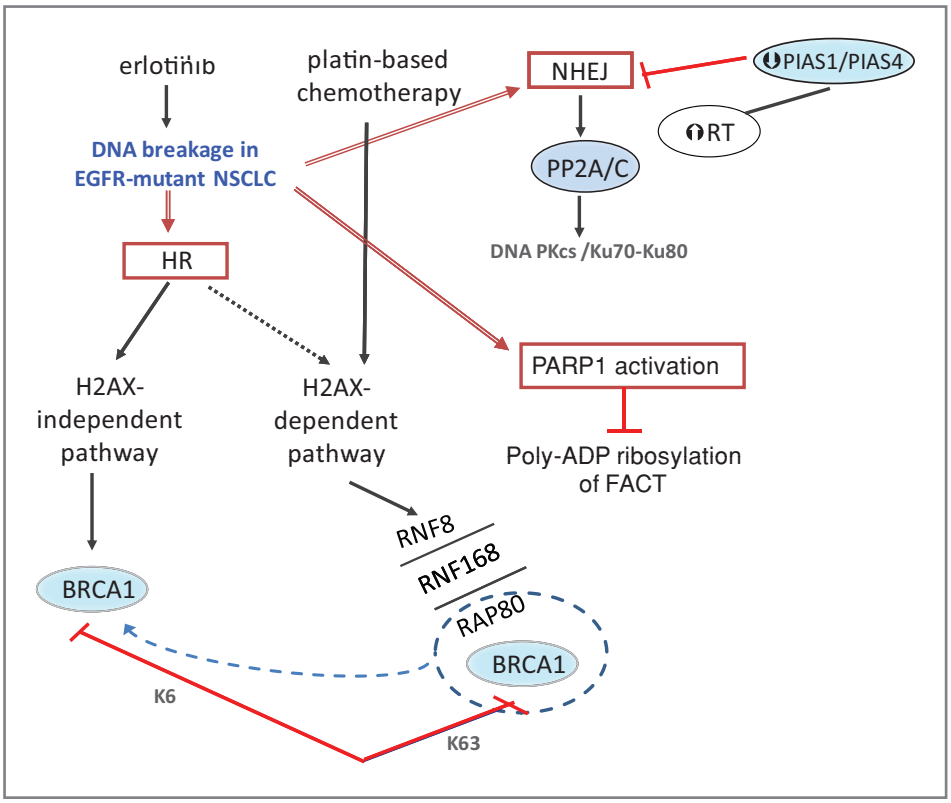

Figure 1. A proposed model for BRCA1 repair of erlotinib-induced DNA damage through an H2AX-independent pathway. DNA damage repair involves homologous recombination (HR), usually through the H2AX-dependent pathway. At sites of DNA breakage, the ubiquitin ligase ring-finger protein (RNF)8 is recruited and, together with the second ubiquitin ligase RNF168, it generates ubiquitin chains bound by receptor-associated protein 80 (RAP80), which in turn recruits the final ubiquitin ligase in the cascade, BRCA1. In addition, protein inhibitor of activated STAT (signal transducer and activator of transcription), PIAS1 and PIAS4 SUMO (small ubiquitin-related modifier) ligases, are required for complete accretion of repair proteins to the damaged sites. Depletion of PIAS1 and PIAS4 reduces the response to double-strand breaks by both HR and nonhomologous end-joining (NHEJ), increasing radiosensitivity (RT) (40, 41). Our findings support a predominant predictive role of BRCA1 in patients with EGFR mutations through an H2AX-independent pathway similar to what has been found in experimental studies (27, 29, 39). We speculate that the DNA breakage caused by erlotinib is different from that caused by radiotherapy or platinum-based chemotherapy, and BRCA1 by itself can be a relevant predictive biomarker. In addition, poly(ADP)-ribosylation of proteins by PARP1 is a rapid response to DNA lesions (45). Turning off the DNA damage checkpoint after DNA repair involves the removal of phosphorylated H2AX from the chromatin, followed by its replacement with canonical H2A. This exchange of phosphorylated H2AX for H2A is mediated by facilitates of chromatin transcription (FACT). The ability of FACT to remove phosphorylated H2AX from the chromatin is inhibited by PARP1 (46). Because PARP1 inhibitors downregulate BRCA1 expression (42), further studies could lead to their use in combination with erlotinib in patients with elevated BRCA1 expression.

217 of whom had advanced disease and were prospectively treated with erlotinib. Additional genetic analyses were performed in these patients if sufficient tumor tissue was available. All analyses were performed centrally at the Pangaea Biotech SA laboratory, an ISO 15189 accredited laboratory (accreditation number 750/LE1556) for the assessment of EGFR mutation sensitivity (deletion in exon 19 [del 19] and L858R) and analysis of BRCA1 mRNA expression in NSCLC (For details, see the Supplementary Data.). All patients signed a written consent form, and approval was obtained from the Institutional Review Board and the Ethics Committee of each hospital.

EGFR T790M mutation and gene expression analyses

Only 129 of 217 patients had sufficient tumor tissue for the T790M analysis, and 104 had sufficient tissue for the RNA expression analyses. The T790M mutation was assessed by TaqMan assay (Applied Biosystems) in the presence of a peptide–nucleic acid designed to inhibit the amplification of the wild-type allele. We analyzed the mRNA expression of 12 genes by real-time quantitative PCR: BRCA1, RAP80, PIAS1, PIAS4, CtIP, UBE2T, SIAH2, PP2A/C, MET, AXL, IL-6ST, and IGF-1R. Primers and probes are shown in Supplementary Table 1. (For further details, see the Supplementary Data.)

Immunohistochemistry

Sufficient tumor was available in 99 patients for immunohistochemistry analysis of the protein expression of DNA-PKcs and Ku70/Ku80. The samples were cut serially at 4 μm, placed on positively charged slides, and processed. (For details, see the Supplementary Data.)

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Statistical analyses

The primary endpoint of the study was to examine the potential effect on progression-free survival of the T790M mutation and BRCA1 mRNA expression. The secondary endpoint was the effect of the other 11 genes examined. In an ancillary analysis, we examined the protein expression of 2 additional genes. Based on our previous experience, (24–26) in addition to analyzing gene expression as a continuous variable, expression levels were divided into terciles to explore the risk trend of the gene variable and to easily identify groups of gene expression with different risk. Progression-free survival was calculated from the initiation of erlotinib therapy until either tumor progression or death. (For details, see the Supplementary Data.)

Results

EGFR T790M mutation

The T790M mutation was found in 45 of 129 patients (35%). T790M mutations were related to the presence of bone metastases ($P = 0.03$) and the type of EGFR mutation ($P = 0.05$; Table 1). No other differences in patient clinical characteristics or initial response were related to T790M mutation status (Table 1). In addition, we later validated this finding in an independent cohort of 78 NSCLC patients with EGFR mutations (December 2008–January 2010); in this cohort, the T790M mutation was present in 30 patients (38%).

In the original cohort of 129 patients, median progression-free survival was 12 months (95% confidence interval [CI], 7.6 to 16.4) in patients with the T790M mutation and 18 months (95% CI, 14.1 to 21.9; $P = 0.05$) in those without the T790M mutation (Supplementary Fig. S1). In the subgroup of 65 patients receiving erlotinib as first-line treatment, progression-free survival was 8 months (95% CI, 3.5 to 12.5) in patients with the T790M mutation and 18 months (95% CI, 13.2 to 22.7; $P = 0.04$) in those without the T790M mutation (Supplementary Fig. S2A). In the subgroup of 64 patients receiving erlotinib as second-line treatment, progression-free survival was 13 months (95% CI, 9.4 to 16.6) in patients with the T790M mutation and 18 months (95% CI, 9.9 to 26.1; $P = 0.35$) in those without the T790M mutation (Supplementary Fig. S2B).

Gene expression analyses

Terciles of each of the 12 genes were derived, and patients were subdivided into 3 groups based on low, intermediate, or high levels of gene expression to compare clinical characteristics (including type of EGFR mutation and the presence or absence of the T790M mutation) and response to erlotinib (Table 2 and Supplementary Tables S2-S12). Progression-free survival was 27 months (95% CI, 21.3 to 32.7) in patients with low BRCA1 levels, 18 months (95% CI, 6.3 to 29.7) in those with intermediate BRCA1 levels, and 10 months (95% CI, 6.7 to 13.3) in those with high BRCA1 levels ($P = 0.02$; Table 3, Fig. 2). No significant differences in median progression-free survival were observed according to the expression levels of any of the other 11 genes examined (Table 3).

Among the 28 patients harboring the T790M mutation, median progression-free survival was 19 months

Table 1. Characteristics and treatment response of 129 patients receiving erlotinib according to the presence or absence of the T790M mutation.

	All patients	T790M present	T790M absent	
	n = 129 *n* (%)	*n* = 45 *n* (%)	*n* = 84 *n* (%)	*P*
Age, median (range)	67 (22–86)	68 (22–80)	65.5 (35–86)	0.31
Sex				0.31
Male	36 (27.9)	10 (22.2)	26 (31)	
Female	93 (72.1)	35 (77.8)	58 (69)	
Bone metastases				0.03
No	99 (76.7)	29 (64.4)	70 (83.3)	
Yes	30 (23.3)	16 (35.6)	14 (16.7)	
Brain metastases				0.99
No	116 (89.9)	41 (91.1)	75 (89.3)	
Yes	13 (10.1)	4 (8.9)	9 (10.7)	
Erlotinib therapy				0.58
First-line	65 (50.4)	21 (46.7)	44 (52.4)	
Second-line	64 (49.6)	24 (53.3)	40 (47.6)	
Type of EGFR mutation				0.05
del 19	81 (62.8)	23 (51.1)	58(69)	
L858R	48 (37.2)	22 (48.9)	26(31)	
Response				0.39
Complete or partial response	80 (68.9)	28 (63.6)	52 (72.3)	

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Table 2. Characteristics and treatment response of 81 patients receiving erlotinib, according to BRCA1 mRNA expression levels.

	BRCA1	BRCA1	BRCA1	
	≤4.92 *n* = 27 *n* (%)	4.92–10.7 *n* = 27 *n* (%)	>10.7 *n* = 27 *n* (%)	*P*
Age, median (range)	71 (44–85)	66 (48–88)	67 (22–79)	0.27
Sex				0.57
Male	7 (25.9)	8 (29.6)	11 (40.7)	
Female	20 (74.1)	19 (70.4)	16 (59.3)	
Bone metastases				0.48
No	23 (85.2)	20 (74.1)	23 (85.2)	
Yes	4 (14.8)	7 (25.9)	4 (14.8)	
Brain metastases				0.66
No	25 (80.6)	22 (71)	24 (77.4)	
Yes	6 (19.4)	9 (29)	7 (22.6)	
Erlotinib therapy				0.47
First-line	17 (63)	17(63)	13 (48.1)	
Second-line	10 (37)	10(37)	14 (51.9)	
Type of EGFR mutation				0.95
del 19	17 (63)	16 (59.3)	16 (59.3)	
L858R	10 (37)	11 (40.7)	11 (40.7)	
T790M				0.95
Present	9 (33.3)	10 (37)	9 (33.3)	
Absent	18 (66.7)	17 (63)	18 (66.7)	
Response				0.96
Complete or partial response	19 (76)	18 (72)	15 (68.2)	0.94

(95% CI, 0 to 41.2) in patients with low BRCA1 levels, 4 months (95% CI, 0 to 11.7) in those with intermediate BRCA1 levels, and 8 months (95% CI, 0 to 20.2) in those with high BRCA1 levels ($P = 0.15$; Supplementary Fig. S3A). Among the 53 patients without the T790M mutation, median progression-free survival was 27 months in patients with low BRCA1 levels, not reached in those with intermediate BRCA1 levels, and 12 months (95% CI, 5.6 to 18.4) in those with high BRCA1 levels ($P = 0.15$; Supplementary Fig. S3B). In patients receiving first-line erlotinib, progression-free survival was also 27 months for patients with the T790M mutation and low levels of BRCA1, whereas it plummeted to 3 months for those with intermediate or high levels (Supplementary Fig. S3C). In patients receiving first-line erlotinib without the T790M mutation, progression-free survival was also 27 months for patients with low BRCA1 levels and 18 months for those with intermediate or high levels (Supplementary Fig. S3D).

Immunohistochemical analysis

Supplementary Tables S13 and S14 show patient characteristics (including type of EGFR mutation and presence or absence of the T790M mutation) and response to erlotinib according to the protein expression of DNA-PKcs and Ku70/Ku80. No differences in median progression-free survival were observed (Table 3).

Multivariate analysis of progression-free survival

In the multivariate analysis [including the expression levels of the 12 genes by terciles, clinical and demographic characteristics, type of EGFR mutation (del 19 or L858R), the presence or absence of the EGFR T790M mutation, first- versus second-line erlotinib therapy, and the protein expression of DNA-PKcs and Ku70/Ku80], an association was observed between shortened progression-free survival and male sex (HR, 3.06; 95% CI, 1.15 to 8.11; $P = 0.02$), current smoker (HR, 6.07; 95% CI, 1.63 to 22.61; $P = 0.007$), the presence of the EGFR T790M mutation (HR, 4.35; 95% CI, 1.85 to 10.17; $P = 0.001$), intermediate BRCA1 levels (HR, 8.19; 95% CI, 2.69 to 26.89; $P < 0.0001$), high BRCA1 levels (HR, 8.46; 95% CI, 2.63 to 27.22; $P < 0.0001$), intermediate PIAS4 levels (HR, 4.49; 95% CI, 1.49 to 13.55; $P = 0.008$), intermediate PP2A/C levels (HR, 3.63; 95% CI, 1.19 to 11.05; $P = 0.02$), and high PP2A/C levels (HR, 6.71; 95% CI, 2.09 to 21.58; $P = 0.001$; Table 4). In addition, an association was observed between longer progression-free survival and second-line erlotinib therapy (HR, 0.27; 95% CI, 0.09 to 0.77; $P = 0.01$; Table 4).

Discussion

The T790M mutant allele has generally been considered to be an acquired mutation, found in 40% to 50% of cases at the time of clinical progression to gefitinib or erlotinib.

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Table 3. Univariate analysis of median progression-free survival according to terciles of mRNA expression levels of 12 genes and the protein expression of DNA-PKcs and Ku70/80.

	n	Progression-free survival Months (95% CI)	*P*
BRCA1			0.02
≤4.92	27	27 (21.3–32.7)	
4.92–10.7	27	18 (6.3–29.7)	
>10.7	27	10 (6.7–13.3)	
RAP80			0.97
≤1.67	31	20 (3.7–36.2)	
1.67–3.68	31	13 (5.4–20.6)	
>3.68	31	16 (7.5–24.5)	
CtIP			0.26
≤1.21	27	16 (2.3–29.7)	
1.21–2.1	25	23 (16–29.9)	
>2.1	25	23 (3.1–26.9)	
PIAS1			0.67
≤2.01	33	16 (7.1–24.9)	
2.01–3.32	33	16 (5.8–26.2)	
>3.32	33	22 (13.4–30.6)	
PIAS4			0.17
≤1.45	27	12 (7.9–16.1)	
1.45–2.38	27	24 (15.1–32.9)	
>2.38	26	16 (11.4–20.6)	
SIAH2			0.71
≤0.52	30	16 (6–25.9)	
0.52–0.98	26	16 (6.2–25.7)	
>0.98	27	18 (8.1–27.9)	
UBE2T			0.14
≤12.64	20	18 (-)	
12.64–24.59	20	23 (13.9–32)	
>24.59	20	11 (6.1–15.8)	
PP2A/C			0.22
≤1.4	32	18 (13.28–22.71)	
1.4–2.33	33	19 (9.76–28.32)	
>2.33	31	11 (7.48–14.51)	
DNA-PKcs			0.91
Negative	12	19(5.3–32.7)	
Positive	86	16(10.9–21.1)	
Ku70/80			0.61
Negative	14	19 (14.9–23.1)	
Positive	85	16 (11–20.9)	
MET			0.89
≤1.83	32	19 (6.42–31.58)	
1.83–3.46	32	19 (7.58–30.14)	
>3.46	32	15 (7.78–22.21)	
AXL			0.78
≤1.61	33	13 (1.01–24.98)	
1.61–2.83	33	16 (8.33–23.66)	
>2.83	32	19 (10.83–27.16)	
IL-6ST			0.12
≤2.17	36	13 (5.52–20.47)	
2.17–4.17	33	15 (9.19–20.81)	
>4.17	34	31 (15.61–46.39)	
IGF-1R			0.38
≤2.36	30	16 (5.9–26.1)	
2.36–4.06	30	12 (9.5–14.5)	
>4.06	29	24 (10.7–37.2)	

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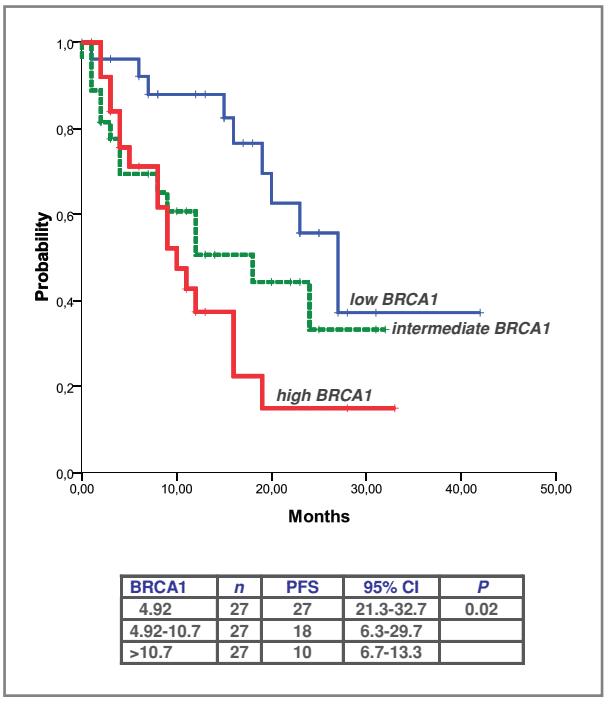

BRCA1	*n*	PFS	95% CI	*P*
4.92	27	27	21.3-32.7	0.02
4.92-10.7	27	18	6.3-29.7	
>10.7	27	10	6.7-13.3	

Figure 2. Kaplan–Meier curves of progression-free survival in 81 NSCLC patients with EGFR mutations, according to BRCA1 mRNA levels.

(10, 11, 36) However, in the present study, we have found the T790M mutation in 35% of 129 patients at baseline, which is similar to the 38% previously reported in 26 patients (15) and the 40% observed in plasma of 10 patients with activating EGFR mutations in a phase I-II study of advanced NSCLC patients treated with docetaxel plus intercalated erlotinib. (37) The lower frequency of pretreatment T790M mutations (2.7%) found in Asian patients (5) could be due to multiple factors, including the use of a different assay, which may be less sensitive at detecting low levels of mutations. EGFR mutations can be very heterogeneous in a single tumor sample, and some mutations are only present in less than 10% of total sequences. (38)

In the present study, double EGFR mutations (T790M plus del 19 or L858R) prior to erlotinib treatment were associated with shortened progression-free survival (HR, 4.35; $P = 0.001$). Taken together with earlier findings, (15) our results indicate that identification of the T790M mutation at diagnosis is feasible and can be an important tool for personalizing therapy. In contrast to wild-type EGFR cell lines, NSCLC cell lines carrying mutant EGFR, including those containing the T790M mutation, exhibited sensitivity to irradiation, (16) as a result of impaired nonhomologous end-joining. (17) Erlotinib cytotoxicity has been related to attenuated homologous recombination, with abrogated BRCA1 expression. (18) These observations prompted us to examine several components of both the nonhomologous end-joining and the homologous recombination pathways that could influence the effect of erlotinib.

Low levels of BRCA1 mRNA correlated with a prolonged progression-free survival of 27 months, while a risk of shortened progression-free survival was associated with intermediate (HR, 8.19; $P < 0.0001$) and high (HR, 8.46; $P < 0.0001$) levels of BRCA1. Unexpectedly, RAP80 had no influence on progression-free survival, although several studies had indicated that RAP80 recruits BRCA1 for DNA damage repair, (27) and we had observed that RAP80 strongly influenced progression-free survival in advanced NSCLC patients with low BRCA1 levels receiving cisplatin plus gemcitabine. (26) However, experimental studies have shown that BRCA1-dependent DNA damage repair can occur in the absence of phosphorylated H2AX (29, 39) or of RAP80. (27) In cell lines, incomplete BRCA1 localization at laser-induced double-strand breaks still occurs after knockdown of RAP80, although BRCA1 is not recruited to DNA lesions caused by irradiation. (27) Mild defects in homologous recombination repair can activate an H2AX-independent pathway in which BRCA1 has an essential role. (29) Based on our findings, we propose a model in which erlotinib can activate an H2AX-independent pathway, where the effect of BRCA1 is not linked to RAP80, in contrast to the more common H2AX-dependent pathway activated by DNA damage caused by irradiation or platinum-based chemotherapy (Fig. 1). In addition to the enhanced effect of erlotinib in wild-type EGFR breast cancer cell lines lacking BRCA1, (18) recent experiments have shown that knockdown of BRCA2 by gene-specific shRNAs and siRNAs restored erlotinib sensitivity in H1650 cells that express EGFR del 19 (delE746-A750) but are otherwise insensitive to EGFR TKIs (TGB and CLS, unpublished data).

The analysis of the expression of PIAS1 and PIAS4, which catalyze the sumoylation of BRCA1, (40, 41) did not provide additional information on the effect of BRCA1 in the present study (Fig. 1). The influence of PP2A/C observed in the multivariate analysis is along the lines of findings in EGFR-mutant cell lines, in which defects in nonhomologous end-joining were found. (17) Taking into account the predictive role of BRCA1 in erlotinib-treated patients with EGFR mutations, novel strategies could include the addition of inhibitors of poly (ADP-ribose) polymerase (PARP), which induces BRCA1 downregulation via induction of E2F4/p130 binding to the BRCA1 promoter (42).

Nonmutational mechanisms of resistance to EGFR tyrosine kinase inhibitors have also been described, such as "drug-tolerant" cells, (43) which have been identified in the erlotinib-sensitive PC9 cell line and which were selectively ablated by treatment with IGF-1R kinase inhibitors. (43) In the present study, however, neither IGF-1R expression nor that of MET, AXL, or IL-6ST influenced progression-free survival. Other mechanisms warrant further clinical research, including low pretreatment MET amplification or elevated expression of the hepatic growth factor ligand, which have been experimentally associated with gefitinib and erlotinib resistance. (35)

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Table 4. Multivariate analysis of progression-free survival including gene expression values by terciles.

	Hazard ratio	95% CI	*P*
T790M			
Absent	1 ref.	1.85–10.17	0.001
Present	4.35		
Sex			
Female	1 ref.	1.15–8.11	0.02
Male	3.06		
ECOG performance status			
0	1 ref.	0.92–7.14	0.07
1	2.56	0.21–2.87	0.72
≥2	0.79		
Smoking history			
Former smoker	0.98	0.34–2.58	0.97
Current smoker	6.07	1.63–22.61	0.007
Never smoked	1 ref.		
Erlotinib therapy			
First-line	1 ref.	0.09–0.77	0.01
Second-line	0.27		
BRCA1 mRNA levels			
≤4.92	1 ref.	2.69–26.89	<0.0001
4.92–10.7	8.19	2.63–27.22	<0.0001
>10.7	8.46		
PP2A/C mRNA levels			
≤1.4	1 ref.	1.19–11.05	0.02
1.4–2.33	3.63	2.09–21.58	0.001
2.33>	6.71		
PIAS4 mRNA levels			
≤1.4	1 ref.	1.49–13.55	0.008
1.4–2.33	4.49	0.21–2.19	0.51
>2.33	0.67		

Our identification of the T790M mutation in a high proportion of pretreatment samples from patients with EGFR mutations indicates that the use of selective EGFR T790M kinase inhibitors (44) may improve outcome in these patients. In contrast, if BRCA1 levels were low, the presence of the T790M mutation did not hamper the effect of erlotinib, whereas progression-free survival plummeted in patients with both the T790M mutation and higher levels of BRCA1. Based on these findings, we strongly advocate the use of a diagnostic toolkit for patients with EGFR mutations—to include baseline assessment of the EGFR T790M mutation and BRCA1 mRNA expression—in order to accurately predict outcome to gefitinib or erlotinib and to provide alternative treatment, including customized chemotherapy, based on BRCA1 mRNA expression or the addition of PARP inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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References

1. Oliver TG, Mercer KL, Sayles LC, Burke JR, Mendus D, Lovejoy KS, et al. Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer. Genes Dev 2010;24:837–52.
2. Rottenberg S, Nygren AO, Pajic M, van Leeuwen FW, Van Der Heijden I, van de Wetering K, et al. Selective induction of chemotherapy resistance of mammary tumors in a conditional mouse model for hereditary breast cancer. Proc Natl Acad Sci U S A 2007;104:12117–22.
3. Sharma SV, Haber DA, Settleman J. Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents. Nat Rev Cancer 2010;10:241–53.
4. Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958–67.
5. Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947–57.

Published OnlineFirst January 13, 2011; DOI: 10.1158/1078-0432.CCR-10-2158

6. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121–8.
7. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
8. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, et al. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. PLoS Med 2005;2:e73.
9. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.
10. Balak MN, Gong Y, Riely GJ, Somwar R, Li AR, Zakowski MF, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494–501.
11. Kosaka T, Yatabe Y, Endoh H, Yoshida K, Hida T, Tsuboi M, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. Clin Cancer Res 2006;12:5764–9.
12. Ogino A, Kitao H, Hirano S, Uchida A, Ishiai M, Kozuki T, et al. Emergence of epidermal growth factor receptor T790M mutation during chronic exposure to gefitinib in a non small cell lung cancer cell line. Cancer Res 2007;67:7807–14.
13. Engelman JA, Mukohara T, Zejnullahu K, Lifshits E, Borras AM, Gale CM, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest 2006;116:2695–706.
14. Kuang Y, Rogers A, Yeap BY, Wang L, Makrigiorgos M, Vetrand K, et al. Noninvasive detection of EGFR T790M in gefitinib or erlotinib resistant non-small cell lung cancer. Clin Cancer Res 2009;15:2630–6.
15. Maheswaran S, Sequist LV, Nagrath S, Ulkus L, Brannigan B, Collura CV, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 2008;359:366–77.
16. Das AK, Sato M, Story MD, Peyton M, Graves R, Redpath S, et al. Non-small-cell lung cancers with kinase domain mutations in the epidermal growth factor receptor are sensitive to ionizing radiation. Cancer Res 2006;66:9601–8.
17. Das AK, Chen BP, Story MD, Sato M, Minna JD, Chen DJ, et al. Somatic mutations in the tyrosine kinase domain of epidermal growth factor receptor (EGFR) abrogate EGFR-mediated radioprotection in non-small cell lung carcinoma. Cancer Res 2007;67:5267–74.
18. Li L, Wang H, Yang ES, Arteaga CL, Xia F. Erlotinib attenuates homologous recombinational repair of chromosomal breaks in human breast cancer cells. Cancer Res 2008;68:9141–6.
19. Bindra RS, Gibson SL, Meng A, Westermark U, Jasin M, Pierce AJ, et al. Hypoxia-induced down-regulation of BRCA1 expression by E2Fs. Cancer Res 2005;65:11597–604.
20. Chan N, Koritzinsky M, Zhao H, Bindra R, Glazer PM, Powell S, et al. Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radioresistance. Cancer Res 2008;68:605–14.
21. Quinn JE, Kennedy RD, Mullan PB, Gilmore PM, Carty M, Johnston PG, et al. BRCA1 functions as a differential modulator of chemotherapy-induced apoptosis. Cancer Res 2003;63:6221–8.
22. Quinn JE, James CR, Stewart GE, Mulligan JM, White P, Chang GK, et al. BRCA1 mRNA Expression Levels Predict for Overall Survival in Ovarian Cancer after Chemotherapy. Clin Cancer Res 2007;13:7413–20.
23. Taron M, Rosell R, Felip E, Mendez P, Souglakos J, Ronco MS, et al. BRCA1 mRNA expression levels as an indicator of chemoresistance in lung cancer. Hum Mol Genet 2004;13:2443–9.
24. Boukovinas I, Papadaki C, Mendez P, Taron M, Mavroudis D, Koutsopoulos A, et al. Tumor BRCA1, RRM1 and RRM2 mRNA expression levels and clinical response to first-line gemcitabine plus docetaxel in non-small-cell lung cancer patients. PLoS ONE 2008;3:e3695.
25. Margeli M, Cirauqui B, Castella E, Tapia G, Costa C, Gimenez-Capitan A, et al. The prognostic value of BRCA1 mRNA expression levels following neoadjuvant chemotherapy in breast cancer. PLoS ONE 2010;5:e9499.
26. Rosell R, Perez-Roca L, Sanchez JJ, Cobo M, Moran T, Chaib I, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS ONE 2009;4:e5133.
27. Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science 2007;316:1198–202.
28. Zlatanou A, Stewart GS. A PIAS-ed view of DNA double strand break repair focuses on SUMO. DNA Repair (Amst) 2010;9:588–92.
29. Yuan J, Chen J. MRE11-RAD50-NBS1 complex dictates DNA repair independent of H2AX. J Biol Chem 2009;285:1097–104.
30. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.
31. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932–7.
32. Mahadevan D, Cooke L, Riley C, Swart R, Simons B, Della Croce K, et al. A novel tyrosine kinase switch is a mechanism of imatinib resistance in gastrointestinal stromal tumors. Oncogene 2007;26:3909–19.
33. Gao SP, Mark KG, Leslie K, Pao W, Motoi N, Gerald WL, et al. Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest 2007;117:3846–56.
34. Guix M, Faber AC, Wang SE, Olivares MG, Song Y, Qu S, et al. Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins. J Clin Invest 2008;118:2609–19.
35. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell 17;77–88.
36. Molina-Vila MA, Bertran-Alamillo J, Reguart N, Taron M, Castella E, Llatjos M, et al. A sensitive method for detecting EGFR mutations in non-small cell lung cancer samples with few tumor cells. J Thorac Oncol 2008;3:1224–35.
37. Mack PC, Holland WS, Burich RA, Sangha R, Solis LJ, Li Y, et al. EGFR mutations detected in plasma are associated with patient outcomes in erlotinib plus docetaxel-treated non-small cell lung cancer. J Thorac Oncol 2009;4:1466–72.
38. Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, et al. Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. Cancer Res 2005;65:7276–82.
39. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, et al. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 2003;5:675–9.
40. Morris JR, Boutell C, Keppler M, Densham R, Weekes D, Alamshah A, et al. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009;462:886–90.
41. Galanty Y, Belotserkovskaya R, Coates J, Polo S, Miller KM, Jackson SP. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009;462:935–9.
42. Hegan DC, Lu Y, Stachelek GC, Crosby ME, Bindra RS, Glazer PM. Inhibition of poly(ADP-ribose) polymerase down-regulates BRCA1 and RAD51 in a pathway mediated by E2F4 and p130. Proc Natl Acad Sci U S A 2010;107:2201–6.
43. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141:69–80.
44. Zhou W, Ercan D, Chen L, Yun CH, Li D, Capelletti M, et al. Novel mutant-selective EGFR kinase inhibitors against EGFR T790M. Nature 2009;462:1070–4.
45. Harper JW, Elledge SJ. The DNA damage response: ten years after. Mol Cell 2007;28:739–45.
46. Ransom M, Dennehey BK, Tyler JK. Chaperoning histones during DNA replication and repair. Cell 2010;140:183–95.

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Pretreatment EGFR T790M Mutation and BRCA1 mRNA Expression in Erlotinib-Treated Advanced Non–Small-Cell Lung Cancer Patients with EGFR Mutations

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