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From clinical trials to clinical practice: predictors of response to erlotinib in advanced non-small cell lung cancer patients pretreated with chemotherapy

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ABSTRACT

Background. Inhibition of the epidermal growth factor receptor pathway with tyrosine kinase inhibitors can improve outcome of patients with advanced non-small cell lung cancer after first-line chemotherapy. The use of clinical characteristics and molecular markers may permit the identification of patients who are more likely to benefit from erlotinib.

Patients and methods. Retrospective analysis of unselected patients with metastatic non-small cell lung cancer who had previously failed on at least one line of chemotherapy and treated at our institution with erlotinib (150 mg/day orally) until disease progression. Mutations of epidermal growth factor receptor (exon 19-21) and KRAS (codon 12-13) genes were screened with high-resolution melting analysis and identified with direct sequencing.

Results. Fifty-three patients were included in the study. The disease control rate was 38%. Median progression-free survival and median overall survival were 4 and 15 months, respectively. Skin rash, diarrhea and mucositis were the most common toxicities of erlotinib. In 19 patients, erlotinib dose was reduced for toxicity. The disease control rate and progression-free survival were significantly better in non-smokers, responders to chemotherapy and patients with epidermal growth factor receptor mutations. Overall survival was longer in patients with skin toxicity and epidermal growth factor receptor mutations.

Conclusions. In our experience, epidermal growth factor receptor mutations, response to previous chemotherapy and non-smoking status were predictors of higher disease control rate and longer progression-free survival. Overall survival was significantly longer in patients with epidermal growth factor receptor mutations and skin toxicity.

Key words: epidermal growth factor receptor, erlotinib, molecular markers, non-small cell lung cancer.

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Introduction

Lung cancer is the first cause of cancer death worldwide, accounting for 12% of all new cancers and 18% of cancer deaths[1]. Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancers, and approximately 60% of patients have advanced disease at diagnosis. Patients with advanced NSCLC have a very poor prognosis (5-year survival rates <5%), and principal end points of treatment are to improve disease-related symptoms and quality of life and to prolong overall survival. For patients with advanced disease, a good performance status, younger than 70 years and no sig-

nificant comorbidities, platinum-based doublet chemotherapy is considered the standard first-line treatment[2]. Despite recent advances in the chemotherapy approach, median survival increased only to about 8-10 months and 2-year survival probability to 10-15%. Current data suggest that chemotherapy has reached a therapeutic plateau and indicate a continuing need for new and more effective strategies.

Recently, key molecules involved in the signal transduction pathways and angiogenesis have been identified as therapeutic targets. The epidermal growth factor receptors (EGFR) are at the origin of a major signaling pathway involved in the growth of lung cancer[3]. Overexpression of EGFR is reported in 40 to 80% of NSCLC cases (84% of squamous cell carcinoma, 65% of adenocarcinoma)[4]. Inhibitors of TK phosphorylation (TKI) are small molecules that block EGFR activity by interfering with the adenosine triphosphate-binding site on the intracellular region of the receptor.

A variety of TKI has been developed for advanced NSCLC. Two of these compounds, gefitinib and erlotinib, have emerged as effective therapies for patients with advanced NSCLC resistant to chemotherapy. Erlotinib has shown improvement in survival in patients with advanced NSCLC previously treated with chemotherapy in BR.21 trial[5], a multicenter study conducted by the National Cancer Institute of Canada, and received approval in this setting by the US Food and Drug Administration in November 2004 and in Europe (EMEA) in June 2005.

A breakthrough in the understanding of NSCLC biology and in the clinical optimization of EGFR inhibitors came with the identification of somatic mutations in the TK domain of EGFR associated with clinical response to TKI[6,7]. Approximately 10-15% of Caucasian and 25-35% of east Asian patients diagnosed with NSCLC have somatic activating mutations of EGFR, and 90% of EGFR gene mutations affect a small region of the gene within exons 18-24, coding for the TK domain. The most common mutations are an in-frame deletions in exon 19 and missense mutations leading to a leucine to arginine substitution at codon 858 (L858R) in exon 21. In most of studies, activating EGFR mutations are most frequently detected in a subpopulation of NSCLC patients with characteristics associated with a better treatment outcome[8,9]. EGFR signaling pathways include downstream hydrolysis guanosine triphosphatase (GTPase) encoded by the Ras gene. Fifteen to thirty percent of lung adenocarcinomas contain activating mutations in the Ras family member, KRAS. Such mutations are most frequently found in codons 12 and 13 and may be associated with an unfavorable outcome[9].

Moreover, a higher probability of response to TKI therapy appears to be associated with some clinical characteristics, such as adenocarcinoma histotype, Asian ethnic origin, female sex, skin toxicity and non-smoking status[5]. Most data suggest that treatment decision regarding the use of TKI might be improved by determining the mutational status of EGFR and KRAS, but the literature on this area is often controversial.

Therefore, the aim of the present study was to assess retrospectively the predictive value of clinical characteristics (sex, smoking status, histology, response to first-line therapy, skin toxicity) and biomarkers (KRAS and EGFR mutations) in a cohort of unselected patients with advanced NSCLC treated with erlotinib at our hospital.

Patients and methods

According to internal guidelines in use at our institution, patients were considered candidate to receive erlotinib if they had histologically or cytologically confirmed NSCLC and met all the following criteria: unresectable, stage IIIB or IV disease; Eastern Cooperative Oncology Group (ECOG) performance status 0-3; adequate hematological, renal and hepatic function; had previously failed on at least one line of chemotherapy; and no prior treatment with anti-EGFR agents. To be included in the present study, all patients had to have at least one measurable lesion and tumor tissue suitable for biomarker analyses. As per institutional policy, all patients signed a written informed consent before treatment.

Therapy consisted of erlotinib, 150 mg/day orally, given until disease progression, unacceptable toxicity and patient refusal or death. Tumor response was assessed with computed tomography (CT) scan every 2-3 months according to response evaluation criteria in solid tumors[10]. Patients were assessed for toxicity according to the National Cancer Institute common toxicity criteria version 3.0. Dose reductions (erlotinib, 100 mg/day) or interruptions were permitted at the physician's discretion if treatment-related adverse events occurred. Re-escalation was not permitted, except after skin toxicity.

Biomarker analyses

Formalin-fixed, paraffin-embedded (FFPE) biopsies and cytological slides from tumor tissues were selected by microscopic examination. Five-μm-thick sections from FFPE tissues were treated with proteinase K overnight at 56 °C. DNA was extracted using the DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. DNA was extracted from cytologic slides using a QIAamp DNA Micro Kit (QIAGEN). Two cell lines harboring KRAS mutations on codons 12 or 13 — SW620 (G12V, homozygous) and HCT116 (G13D, heterozygous) — were used as positive references, respectively. For EGFR mutations on exons 19 and 21, we used H1650 (delE746-A750, heterozygous) and H1975 (L858R, heterozygous). For analysis, high-resolution melting analysis was performed as previous-

ly described performing a denaturation profile from 75-91 °C[11]. Primers were selected using Primer3 software and were as follows: EGFR exon 19, 5'-GTGCATCGCTGGTAACATCCA-3'(forward) and 5'-AAAGGTGGGCCTGAGGTTCA-3'(reverse); EGFR exon 21, 5'-CCTCACAGCAGGGTCTTCTCTG-3'(forward) and 5'-TGGCTGACCTAAAGCCACCTC-3' (reverse). Data were acquired and analyzed using the RotorGene 6000 (Corbett Research, Sydney, Australia) accompanying software. After normalization and temperature adjustment steps, melting curve shapes were compared between samples and controls. Samples revealing anomalous profiles or left-shifted curves from control samples were then sequenced for further characterization. After high-resolution melting analysis, samples were purified with a PCR Purification Kit (QIAGEN) and submitted to cycle sequencing with 2 μl of BigDye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, CA, USA) and the same primers used in high-resolution melting analysis but with 0.8 μmol/L in a final volume of 10 μl. After purification with a DyeEx 2.0 Spin Kit (QIAGEN), samples were analyzed with the ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis

For the assessment of tumor response, we considered the best response obtained during the treatment with erlotinib. The disease control rate (DCR) was calculated as the sum of overall response rate (ORR: partial response and complete response) and stable disease. The relationship between clinical (gender, smoking status, histology, response to first-line chemotherapy, skin toxicity) and biological (KRAS and EGFR mutations) characteristics and DCR were tested in univariate analyses using the standard χ^2 test or Fisher's exact test. The impact of clinical and biological characteristics on DCR was also assessed with the multivariate logistic regression model. Progression-free survival (PFS) was calculated from the date of first dose of erlotinib until date of progressive disease or death for any cause and overall survival from date of start of treatment until date of death, whatever the cause. Estimates of PFS and overall survival were calculated according to the Kaplan-Meier method. Differences in PFS and overall survival according to clinical and biological characteristics were tested with the log-rank test. Finally, to investigate the joint effect of the clinical and biological characteristics on PFS and OS, the Cox proportional hazard model was fitted to the data. Analyses were conducted using SAS system v9.1.

Results

Patient characteristics

A total of 53 patients with advanced NSCLC treated with erlotinib at our institution from May 2006 to February 2009 met all the inclusion criteria and were included in the study. Characteristics of patients are summarized in Table 1. Median age was 65 years (range, 37-81); all patients had stage IV NSCLC; 19 (36%) patients were females and 34 (64%) were males; 46 (87%) were smokers and 7 (13%) non-smokers. Twenty-nine (55%) patients had adenocarcinoma, 13 (25%) squamous cell carcinoma, and 11 (20%) included other types of NSCLC.

Erlotinib was administrated as second-, third- and ≥ fourth line in 28 (53%), 20 (38%), 5 (9%) of patients, respectively. EGFR mutations were analyzed in all patients: 39 (74%) patients presented wild-type gene and 8 (15%) presented mutations (six deletions in exon 19 and two L858R amino acid substitutions in exon 21); in 6 patients the evaluation of EGFR mutations were not possible. KRAS mutations were analyzed in all 53 patients: 37 (70%) patients were wild-type and 13 (24%) presented mutations (12 single amino acid substitutions in codon 12 and one in codon 13); in 3 patients KRAS mutations were not assessable. EGFR and KRAS mutations were detected together in 3 patients.

Efficacy and safety

At the time of analysis, the median follow-up was 28 months. Complete and partial response was observed in 2 (4%) and 7 (13%) patients, respectively; 11 (21%) patients had stable disease and 29 (55%) patients had progression of disease. Four (7%) patients died with clinical progression before disease evaluation. ORR was obtained in 9 patients (17%; 95% confidence interval [CI], 9-30) and DCR was 38% (95% CI, 25-52). Median PFS and OS were 4 and 15 months, respectively. Five patients had no tumor progression and continued the treatment with erlotinib.

In 19 (36%) patients, erlotinib dose was reduced to 100 mg/day for treatment-related adverse events. The most frequent adverse event was skin toxicity, which was observed in 27 (51%) patients (grade 1, 9 patients;

Table 1 - Patient characteristics

Characteristics	No. patients	%
Total no. of patients	53	100
Median age (yr)	65 (range, 37-81)	
Sex: male/female	34/19	64/36
Smoke: yes/no	46/7	87/13
Histology: A/AS/S/others	29/6/13/5	55/11/25/9
Response to first-line chemotherapy: CR/PR/SD/PD	2/13/10/28	2/24/19/53
Stage: IIIB/IV	0/53	0/100
Line with erlotinib: II/III/≥IV	28/20/5	53/38/9
EGFR (exon 19-21): Mutated/WT/NA	8/39/6	15/74/11
KRAS (codon 12-13): Mutated/WT/NA	13/37/3	24/70/6

A, adenocarcinoma; AS, adenosquamous carcinoma; S, squamous carcinoma; CR, complete response; PR, partial response; SD, stable disease; PD, progression of disease; WT, wild-type; NA, not assessable.

grade 2, 13 patients; grade 3, 5 patients). Other grade 2 or more adverse events were diarrhea in 3 (6%) patients (grade 3 in 1) and mucositis in 4 (8%) patients (grade 3 in 2). There were no dose interruptions, and no patient was withdrawn from the study for toxicity. There was no toxic death.

Relationships between patient characteristics and clinical outcome

Relationships between patient characteristics and DCR, PFS and OS are summarized in Table 2. In the full cohort, non-smoking status and response to first-line chemotherapy were associated with a higher probability of DCR (86 *vs* 30%, $P = 0.008$ and 52 *vs* 25%, $P = 0.043$, respectively) and PFS (14 *vs* 3 months, $P = 0.026$ and 6 *vs* 3 months, $P = 0.037$, respectively). A predictive role for skin toxicity was observed: patients developing skin rash during treatment with erlotinib had a better overall survival (23 *vs* 5.5 months, $P = 0.004$).

DCR of EGFR MT patients was significantly higher than wild-type patients (100% *vs* 28%, $P < 0.001$). Median PFS and overall survival were significantly higher in patients with EGFR mutations (18 *vs* 3 months, $P = 0.007$ and not reached *vs* 14 months, $P = 0.043$; Figure 1). No statistically significant association was found between KRAS mutations and DCR ($P = 0.199$), PFS ($P = 0.432$) and overall survival ($P = 0.515$) (Figure 2).

In a logistic regression analysis including gender, smoking status, response to first-line chemotherapy and EGFR mutations, the response to first-line chemotherapy (odds ratio [OR] = 4.2; 95% CI, 1.05–20.5; $P = 0.042$) and the presence of EGFR mutations (OR = 54.1; 95% CI, 5.3-5749.4; $P < 0.001$) were confirmed to be independent predictive factors of DCR.

A Cox proportional hazard model analysis was performed including smoking status, response to first-line chemotherapy, skin toxicity and EGFR mutations. In PFS analysis, response of first-line chemotherapy (hazard ratio [HR] = 0.43; 95% CI, 0.22-0.84; $P = 0.014$) and presence of EGFR mutations (HR = 0.25; 95% CI, 0.10-0.66; $P = 0.005$) were retained as predictive factors of longer PFS. Multivariate analysis confirmed that patients who developed skin toxicity during erlotinib treatment had improvement in overall survival (HR = 0.45; 95% CI, 0.21-0.97; $P = 0.042$). Also the presence of EGFR mutations had a predictive impact (although not statistically significant) on overall survival (HR = 0.27; 95% CI: 0.06-1.15; $P = 0.077$).

Discussion

Erlotinib has shown an improvement in survival in patients with advanced NSCLC previously treated with chemotherapy[5]. The BR-21 trial, a multicenter study conducted by the National Cancer Institute of Canada, showed for the first time in a randomized trial that as a single agent erlotinib prolonged survival in advanced NSCLC patients after chemotherapy. ORR to erlotinib was 9% and overall survival was 6.7 months for erlotinib *versus* 4.7 months for placebo ($P = 0.001$). However, the activity and toxicity of erlotinib are both known to be strongly influenced by ethnicity, histology, and smoking status.

In our study, we retrospectively evaluated the association between outcome and clinical characteristics, including molecular markers, in a cohort of unselected patients treated with erlotinib for advanced NSCLC after failure of at least one line of chemotherapy.

Taking into account the limitations of a retrospective study that included only 53 cases, the ORR (17%) and DCR (38%) observed in our patients were similar to these of the BR.21 trial. Erlotinib has a favorable toxicity profile, and the percentage of patients who required erlotinib dose reductions (36%) was very similar to that of the BR.21 trial.

In the BR.21 and several other trials, patients with the highest probability of a benefit from erlotinib appeared to be females, never smokers, with Asian ethnicity and with adenocaricinoma histology. In our experience, non-smoking status and response of first-line chemotherapy were associated to a higher probability of DCR to treatment and a longer PFS. These results con-

Table 2 - Relationships between patient characteristics and disease control rate (DCR), progression-free survival (PFS), overall survival (OS)

Characteristics	DCR (%)	*P*	Median PFS (mo)	*P*	Median OS (mo)	*P*
Sex: female/male	53/29	0.094	5/3	0.334	22/12	0.181
Smoke: no/yes	86/30	0.008#	14/3	0.026	23/12	0.105
Histology: A/AS/S/other	41/67/23/20	0.245	4/6.5/3/3	0.554	19/18/6/17	0.479
Response 1st line CT: DCR/PD	52/25	0.043	6/3	0.037	17/6	0.282
Skin toxicity: G 0/any G	31/44	0.30	3/5	0.094	5.5/23	0.004
KRAS: wild-type/mutated	32/54	0.199#	4/5	0.432	15/23	0.515
EGFR: wild-type/mutated	28/100	<0.001#	3/18	0.007	14/NR	0.043

A, adenocarcinoma; AS, adeno-squamous cell carcinoma; S, squamous cell carcinoma; CT, chemotherapy; DCR, complete response + partial response + stable disease; PD, progression of disease; G, grade; NR, not reached.
#Fisher's exact test.

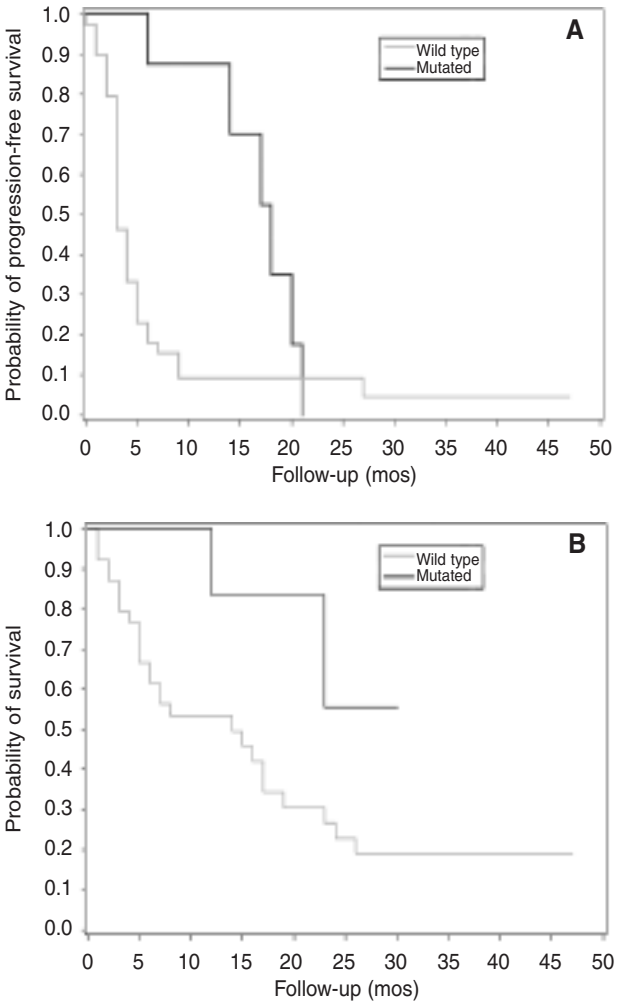

Figure 1 - Kaplan-Meier curves of correlations between EGFR mutations and progression-free survival (A) or overall survival (B). Median PFS and overall survival were significantly higher in patients with EGFR mutations (18 *vs* 3 months, *P* = 0.007 [A] and not reached *versus* 14 months, *P* = 0.043 [B]).

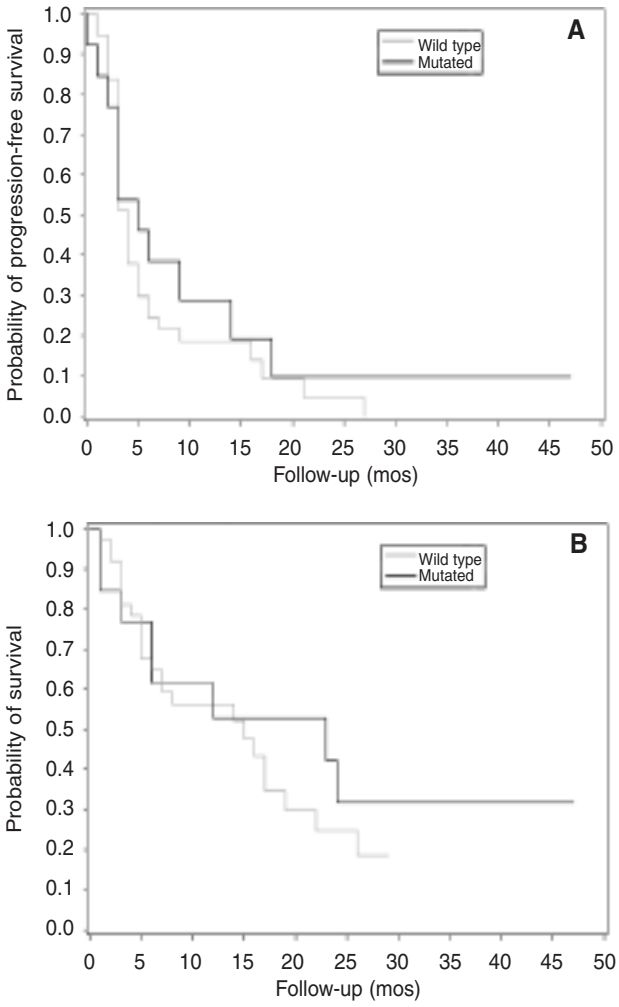

Figure 2 - Kaplan-Meier curves of correlations between KRAS mutations and progression-free survival (A) or overall survival (B). No statistically significant association was found between KRAS mutations and PFS (5 *vs* 4 months, *P* = 0.432 [A]) or overall survival (23 *vs* 15 months, *P* = 0.515 [B]).

firm that there is no scientific rationale to exclude patients from erlotinib treatment based simply on demographics or clinical factors. We also found that the presence of skin toxicity was correlated with longer overall survival in patients treated with anti-EGFR therapy, as already reported in the literature[12]. It is conceivable that effects in the skin reflect the extent of EGFR blockade achieved in the tumor, in which case the rash would correlate with EGFR saturation or with a relevant drug concentration within the tumor. Further studies to specifically investigate this issue are warranted.

In order to identify the best candidates for erlotinib treatment, we analyzed KRAS and EGFR mutations. In all published phase II trials in which patients were selected for treatment with EGFR TKI based on the presence of EGFR mutations, the ORR exceeded 50% and the PFS approximated one year. Rosell *et al.*[13] analyzed the association between EGFR mutations and the outcome of erlotinib treatment, reporting a PFS of 14 months and overall survival of 27 months in EGFR MT patients treated with erlotinib. In a phase III trial, gefitinib was compared with chemotherapy (carboplatin plus paclitaxel) in Asian, naïve, non-smokers with advanced pulmonary adenocarcinoma; in patients positive for EGFR mutations, PFS was significantly longer in those who received gefitinib than those who received chemotherapy (*P* <0.001)[14]. In our study, DCR, PFS and overall survival were significantly higher in EGFR MT patients than wild-type patients (*P* <0.001, *P* = 0.007 and *P* = 0.043, respectively). These data show that EGFR mutations (exon 19 and 21) have a great impact on clinical benefit among patients treated with erlotinib.

The KRAS pathway links the EGFR pathway to cell proliferation and survival, transducing the EGFR activation signal to multiple downstream pathways. KRAS mutations on codons 12 and 13 result in inhibition of

GTPase activity, thus leading to the constitutive activation of RAS protein, which may render tumor cells independent of EGFR signaling and thereby resistant to EGFR TKI therapy. The activity of EGFR TKI in patients with KRAS mutations appears minimal, but the effect of these agents on survival in KRAS mutations needs further studies[15]. In our study, no statistically significant association was found between KRAS mutations and DCR, PFS or OS. EGFR and KRAS mutations are considered mutually exclusive, suggesting that they have functionally equivalent roles in lung tumorigenesis[15]. EGFR mutations are highly associated with a non-smoking history, whereas KRAS mutations commonly occur in individuals with a history of substantial cigarette use. However, we identified in our study coexisting EGFR and KRAS mutations in 3 patients.

In conclusion, although limited by small numbers and its retrospective design, our study confirmed the important role of EGFR mutations as predictive factors of response to erlotinib in unselected patients with advanced NSCLC pretreated with chemotherapy. In addition to EGFR mutational status, we identified some clinical characteristics associated with response to erlotinib that could be useful in ordinary management of patients. Large-scale screening of patients with NSCLC for EGFR mutations is feasible and may have an important role in the decision-making process about treatment because of the potential impact on the outcome of erlotinib treatment, also in first-line therapy. Future prospective studies should include these molecular markers together with other biologic parameters to further improve selection of patients treated with EGFR TKI.

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