Molecular Predictors of Outcome With Gefitinib and Docetaxel in Previously Treated Non–Small-Cell Lung Cancer: Data From the Randomized Phase III INTEREST Trial

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See accompanying editorial on page 713 and article on page 753

ABSTRACT

Purpose
In the phase III INTEREST trial, 1,466 pretreated patients with advanced non–small cell lung cancer (NSCLC) were randomly assigned to receive gefitinib or docetaxel. As a preplanned analysis, we prospectively analyzed available tumor biopsies to investigate the relationship between biomarkers and clinical outcomes.

Methods
Biomarkers included epidermal growth factor receptor (EGFR) copy number by fluorescent in situ hybridization (374 assessable samples), EGFR protein expression by immunohistochemistry (n = 380), and EGFR (n = 297) and KRAS (n = 275) mutations.

Results
For all biomarker subgroups analyzed, survival was similar for gefitinib and docetaxel, with no statistically significant differences between treatments and no significant treatment by biomarker status interaction tests. EGFR mutation–positive patients had longer progression-free survival (PFS; hazard ratio [HR], 0.16; 95% CI, 0.05 to 0.49; P = .001) and higher objective response rate (ORR; 42.1% v 21.1%; P = .04), and patients with high EGFR copy number had higher ORR (13.0% v 7.4%; P = .04) with gefitinib versus docetaxel.

Conclusion
These biomarkers do not appear to be predictive factors for differential survival between gefitinib and docetaxel in this setting of previously treated patients; however, subsequent treatments may have influenced the survival results. For secondary end points of PFS and ORR, some advantages for gefitinib over docetaxel were seen in EGFR mutation–positive and high EGFR copy number patients. There was no statistically significant difference between gefitinib and docetaxel in biomarker-negative patients. This suggests gefitinib can provide similar overall survival to docetaxel in patients across a broad range of clinical subgroups and that EGFR biomarkers such as mutation status may additionally identify which patients are likely to gain greatest PFS and ORR benefit from gefitinib.

INTRODUCTION

Gefitinib is an oral epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) that has been shown to be efficacious and well tolerated in patients with pretreated advanced non–small-cell lung cancer (NSCLC). The complex relationship between EGFR-related biomarkers and response to EGFR-TKIs has been investigated extensively. In single-arm studies and randomized placebo-controlled trials, higher response rates have been seen in patients whose tumors express EGFR protein, and in those with high EGFR copy number or activating mutations in exons 19 or 21 of the EGFR gene compared with those without these markers when treated with an EGFR-TKI.8–15

The terms prognostic and predictive are often used to describe tumor biomarkers or patient characteristics. A good/poor prognostic factor is a patient or tumor characteristic that is associated with
longer/shorter survival compared with patients without the factor in the absence of active treatment. It reflects the underlying aggressiveness of the patient’s disease and its effect on survival, and can only be identified from a study including a best supportive care arm, as the addition of active treatment may alter survival and makes it impossible to distinguish disease and treatment effects.

A predictive factor is defined as a patient or tumor characteristic that is associated with a greater survival difference between two treatments in the patients with the factor compared with those without. It reflects the relative effect of two treatments on a patient’s survival and can only be identified from a comparative study (a active treatment or best supportive care). The predictive value of a factor may differ with comparator; therefore, the comparator should be stated. A characteristic can be a good/poor prognostic factor, predictive factor, both, or neither.

In the Iressa Survival Evaluation in Lung Cancer (ISEL) study comparing gefitinib with placebo in pretreated patients with advanced NSCLC, both EGFR protein expression and high EGFR copy number were predictors of a gefitinib-related effect on survival, with significant interactions between biomarker status and survival outcome. There were insufficient data for survival analysis by EGFR mutation status, although gefitinib-treated patients with EGFR mutations had higher objective response rates (ORRs) than those without. In National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) BR.21 comparing erlotinib with placebo, no significant interaction was seen for EGFR protein expression; however, as in ISEL, high EGFR copy number was associated with differentially greater survival compared with low copy number. In BR.21, no significant interaction was demonstrated for patients with EGFR activating mutations in exons 19 or 21, although the hazard ratio (HR) for survival was 0.55 (95% CI, 0.25 to 1.19; P = .12) compared with 0.74 for patients with wild-type EGFR (95% CI, 0.52 to 1.05; P = .09). It is not clear whether clinical outcome in patients treated with chemotherapy is also associated with these biomakers, as there are few published prospective studies.

The Iressa NSCLC Trial Evaluating Response and Survival Versus Taxotere (INTEREST) trial, a randomized, phase III study, established that gefitinib (250 mg/d orally) was noninferior to docetaxel (75 mg/m² intravenously every 3 weeks) for overall survival (OS) in pretreated patients with advanced NSCLC (gefitinib vs docetaxel, HR 1.02; 96% CI, 0.90 to 1.15). Progression-free survival (PFS), ORR, and disease-related symptom improvement rates were similar for gefitinib and docetaxel in the overall population. Gefitinib was associated with improved tolerability and greater quality-of-life improvement rates. Herein, we present the results of the preplanned analyses of the relationships between
EGFR biomarkers and clinical outcome after treatment with gefitinib or docetaxel from INTEREST.

**METHODS**

**Study Design**

Full details of the INTEREST study (18390L/0721; clinicaltrials.gov identifier NCT00076388) have been published previously. Eligible patients had locally advanced or metastatic NSCLC that had progressed or recurred after at least one prior platinum-based chemotherapy regimen (up to two prior regimens allowed; Fig 1). The primary objective of the study was to compare OS between gefitinib (Iressa; AstraZeneca, Wilmington, DE) and docetaxel (Taxotere; sanofi-aventis) using two coprimary analyses: an assessment of noninferiority in the overall population, and an assessment of superiority in patients with high EGFR copy number. Investigation of the correlation of EGFR protein expression and mutation status for the EGFR and KRAS genes, with gefitinib and docetaxel efficacy in patients with assessable tumor material, was a preplanned exploratory objective.

All patients provided written informed consent and separate consent was obtained for optional provision of a tumor sample for biomarker analyses. Study approval was obtained from independent ethics committees at each institution. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice, applicable regulatory requirements, and AstraZeneca’s policy on bioethics.

**Biomarker Analyses**

Biomarker analyses were performed on paraffin-embedded, archival diagnostic tumor tissue (tissue collection was not mandatory) after an initial pathology review (Appendix, online only). Fluorescent in situ hybridization (FISH) was used to analyze EGFR copy number using previously published methodology. High copy number was defined as high polysomy (≥ 4 copies in ≥ 40% of cells) or gene amplification (presence of tight gene clusters; a gene:chromosome ratio per cell ≥ 2; or ≥ 15 copies of EGFR per cell in ≥ 10% of cells analyzed). EGFR protein expression status was assessed by immunohistochemistry using the DAKO EGFR pharmDx kit (Dako, Glostrup, Denmark). Tumors were classified as EGFR expression positive if more than 10% of cells stained. EGFR gene mutations were investigated by direct gene sequencing of exons 18 to 21 of chromosome 7. Tumors were positive if a mutation was detected in both the forward and reverse directions in at least one of the three independent polymerase chain reaction products derived from the tumor DNA. KRAS gene mutation status was assessed via the amplification refractory mutation system to detect known mutations in codons 12 and 13 of this gene. Tumors were positive if any KRAS mutation was detected. All biomarkers were determined blinded to clinical outcome and randomized treatment before any statistical analysis had been performed. EGFR and KRAS assessments were performed in approved laboratories (at Peking Union Medical College

**Table 1. Baseline Demographic Characteristics and Clinical Outcomes for Patients With Assessable Tissue Samples for Each Biomarker Compared With the Overall Study Population**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EGFR Copy Number by FISH</th>
<th>EGFR Protein Expression</th>
<th>EGFR Mutation</th>
<th>KRAS Mutation</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>No. of patients</td>
<td>374</td>
<td>380</td>
<td>297</td>
<td>275</td>
<td>1,466</td>
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<tr>
<td>Adenocarcinoma</td>
<td>207</td>
<td>55.3</td>
<td>206</td>
<td>54.2</td>
<td>169</td>
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<td>Non-adenocarcinoma</td>
<td>167</td>
<td>44.7</td>
<td>174</td>
<td>45.8</td>
<td>128</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<td>Female</td>
<td>116</td>
<td>31.0</td>
<td>124</td>
<td>32.6</td>
<td>93</td>
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<tr>
<td>Male</td>
<td>258</td>
<td>69.0</td>
<td>256</td>
<td>67.4</td>
<td>204</td>
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<tr>
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<tr>
<td>0 or 1</td>
<td>336</td>
<td>89.8</td>
<td>339</td>
<td>89.2</td>
<td>264</td>
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<tr>
<td>2</td>
<td>38</td>
<td>10.2</td>
<td>41</td>
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<td>33</td>
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<td>60</td>
<td>16.0</td>
<td>67</td>
<td>17.6</td>
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<tr>
<td>Ever</td>
<td>314</td>
<td>84.0</td>
<td>313</td>
<td>82.4</td>
<td>247</td>
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<tr>
<td>Line</td>
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<tr>
<td>Second</td>
<td>316</td>
<td>84.5</td>
<td>322</td>
<td>84.7</td>
<td>253</td>
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<tr>
<td>Third</td>
<td>58</td>
<td>15.5</td>
<td>58</td>
<td>15.3</td>
<td>44</td>
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<tr>
<td>Race/ethnicity</td>
<td></td>
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<tr>
<td>Asian origin†</td>
<td>54</td>
<td>14.4</td>
<td>58</td>
<td>15.3</td>
<td>48</td>
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<tr>
<td>Non-Asian origin</td>
<td>320</td>
<td>85.6</td>
<td>322</td>
<td>84.7</td>
<td>249</td>
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<tr>
<td>Overall survival, HR</td>
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<td>1.02</td>
<td>0.97</td>
<td>0.99</td>
<td>1.01</td>
</tr>
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<td>95% CI†</td>
<td>0.80 to 1.25</td>
<td>0.62 to 1.27</td>
<td>0.76 to 1.25</td>
<td>0.76 to 1.28</td>
<td>0.90 to 1.14‡</td>
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<tr>
<td>PFS, HR</td>
<td>1.01</td>
<td>1.16</td>
<td>1.00</td>
<td>1.14</td>
<td>1.04</td>
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<td>95% CI‡</td>
<td>0.80 to 1.27</td>
<td>0.92 to 1.47</td>
<td>0.78 to 1.31</td>
<td>0.86 to 1.51</td>
<td>0.93 to 1.18</td>
</tr>
<tr>
<td>ORR</td>
<td>Gefitinib]</td>
<td>16/157</td>
<td>10.2</td>
<td>17/160</td>
<td>10.6</td>
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<tr>
<td>Docetaxel[</td>
<td>16/180</td>
<td>8.9</td>
<td>18/185</td>
<td>9.7</td>
<td>16/142</td>
</tr>
</tbody>
</table>

Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; PS, performance status; HR, hazard ratio; PFS, progression-free survival; ORR, objective response rate.

*Never-smoker refers to patients who had never smoked in their lifetime.
†Refers to the racial origin of a group of patients, not necessarily the place of birth. This definition excludes those of Indian origin.
‡Using an unadjusted Cox proportional hazard model in the intent-to-treat population for overall survival and an adjusted Cox proportional hazard model in the assessable-for-response population for PFS.
§Intent-to-treat analysis shown for comparison (the per-protocol population with 96% CI was the primary analysis for overall survival in the overall population).

1. Douillard et al

2. The International Conference on Harmonization.
Gefitinib v Docetaxel in NSCLC

Hospital for samples in China; at Genzyme for samples outside China) using appropriate quality control methods.

Statistical Considerations

The coprimary analysis of OS in patients with high EGFR copy number was introduced in August 2006 via protocol amendment after emerging data that suggested high EGFR copy number was a strong predictor of gefitinib survival benefit over placebo.10 The coprimary analyses employed a modified Hochberg procedure to ensure noninflation of the overall 5% type-I error rate.50

For each biomarker, tumors were classified as positive, negative, or unknown, and for each of these groups, HRs, 95% CIs, and P values were estimated for OS and PFS (using an unadjusted Cox proportional hazards model in the intent-to-treat [ITT] population for OS and an adjusted Cox proportional hazards model in the assessable-for-response population for PFS). Odds ratios, 95% CIs, and P values were estimated for ORRs (using an adjusted logistic regression model in the assessable-for-response population). For each biomarker for OS, the biomarker status by randomized treatment interaction was also assessed in patients with assessable samples using a Cox proportional hazards model adjusted for randomized treatment, biomarker status (positive or negative), and the biomarker status by treatment interaction, using a significance level of 10% to indicate potential predictive factors for gefitinib versus docetaxel.

RESULTS

Patients

Of 1,466 patients randomly assigned (Fig 1), 453 (31%) had a tissue sample assessable for at least one biomarker analysis and 253 (17%) were assessable for all four biomarkers (Appendix). Key demographic characteristics and clinical outcomes for patients with assessable tissue samples are presented in Table 1. Patients with assessable samples were generally representative of the overall study population, but there were slightly fewer assessable samples from Asian patients (approximately 15% compared with 22% in the overall population). Among patients with a sample evaluable for KRAS mutation, only 1.8% were Asian due to unavailability of the assay in China.

Among patients with assessable samples for EGFR copy number, mutation, and protein expression (Appendix Fig A1, online only), the majority of patients with a high EGFR copy number were also positive for EGFR protein expression (97 of 117 [83%]); 24 (62%) of 39 patients positive for EGFR mutation also had high copy number and were positive for protein expression. Corresponding data for overlap of EGFR copy number, EGFR protein expression, and KRAS mutation are in Appendix Fig A2 (online only).

EGFR Copy Number

Of the 374 assessable patients, 174 (47%) had a high EGFR copy number: 121 (32%) due to high polysomy and 53 (14%) due to gene amplification. The proportion of patients with high copy number was similar across different demographic characteristics (Appendix Fig A3, online only).

Superior OS for gefitinib versus docetaxel in patients with high copy number, whether due to high polysomy or gene amplification, was not seen: the HR (gefitinib v docetaxel) was 1.09 (95% CI, 0.78 to 1.51; P = .62) in all patients with high copy number (n = 174; Figs 2A and 3A), 1.19 (0.80 to 1.76; P = .39) in those with high polysomy (n = 121), and 0.88 (0.48 to 1.62; P = .68) in those with gene amplification (n = 53). Survival outcomes in patients with low copy number were also similar for both treatments (HR, 0.93; 95% CI, 0.68 to 1.26; P = .64; Figs 2B and 3A) and similar to the overall population. There was no significant difference in OS treatment effect between high and low copy number (high HR 1.09 v low HR 0.93, EGFR copy number status-by-treatment interaction test; P = .52).

Gefitinib was similar to docetaxel in terms of PFS in patients with high (HR, 0.84; 95% CI, 0.59 to 1.19; P = .33) and low (HR, 1.30; 95% CI, 0.93 to 1.83; P = .12) copy number (Figs 4A, 4B, and 3B).

ORRs were higher in patients with high copy number receiving gefitinib than docetaxel (13.0% [10 of 77] v 7.4% [six of 81]; P = .04; Fig 5).

EGFR Protein Expression

EGFR protein expression was present in 75% of the assessable patients (284 of 380). There was no evidence of a difference in OS (Figs 2C, 2D, and 3A), PFS (Figs 3B, 4C, and 4D), or ORRs (Fig 5) between treatments in patients with EGFR protein expression positive or negative tumors, and no significant difference in OS treatment effect between patients with positive or negative tumors (positive HR 1.00 v negative HR 1.00, EGFR protein expression status-by-treatment interaction test; P = .87).

EGFR Mutation

Overall, 15% of assessable patients (44 of 297) were EGFR mutation–positive: 22 patients had an exon 19 deletion, 16 had an exon 21 L858R mutation (one also had an exon 20 T790M mutation), two had an exon 18 G719A mutation, and four had other mutations (Appendix Table A1, online only). There was a higher frequency of mutation–positive tumors (of those assessable) in females (27% v 9% in males), adenocarcinoma histology (20% v 8% for nonadenocarcinoma), never smokers (56% v 6% for smokers), and Asian origin (35% v 11% in non-Asian), although mutations were observed in all subgroups examined (Appendix Fig A4, online only).

In patients with EGFR mutation–positive tumors, survival was longer in both gefitinib and docetaxel groups (median survival 14.2 and 16.6 months, respectively) than in the overall population (7.6 and 8.0 months, respectively) and in the population with wild-type (6.4 and 6.0 months, respectively), but there was no difference between treatments (Figs 2E and 3A). There was no significant difference in OS treatment effect between mutation–positive and wild-type (mutation–positive HR 0.83 v wild-type HR 1.02, EGFR mutation status-by-treatment interaction test; P = .59).

Among patients with EGFR mutation, PFS was longer for gefitinib compared with docetaxel (HR, 0.16; 95% CI, 0.05 to 0.49; P = .001) but not in wild-type (HR, 1.24; 95% CI, 0.94 to 1.64; P = .14; Figs 3B and 4E). ORRs were also higher for gefitinib than docetaxel in patients with EGFR mutation (42.1% [eight of 19] v 21.1% [four of 19]; P = .04; Fig 5).

There appeared to be a greater response for EGFR mutation positive versus wild-type patients within both treatment groups (mutation v wild-type response rates 42.1% [eight of 19] v 6.6% [seven of 106] with gefitinib and 21.1% [four of 19] v 9.8% [12 of 123] with docetaxel).

KRAS Mutation

Of the 275 patients assessable for KRAS mutation status, 49 (18%) were positive for KRAS mutation. There were no differences between treatments in OS, PFS, or response rates according to KRAS
Two large randomized trials comparing EGFR-TKI therapy with placebo showed that high EGFR copy number was significantly associated with a differentially greater survival benefit from treatment. However, our copy number analyses of tumors from a subset of patients showed no evidence that KRAS mutation is a predictive factor for a differential survival effect between gefitinib and docetaxel (mutation-positive HR 0.81 versus wild-type HR 1.03, KRAS mutation status-by-treatment interaction test; P = .51; Figs 2G, 2H, 3, 4G, and 4H). Response rates associated with KRAS mutations versus wild-type were 0% (0 of 20) versus 9.6% (nine of 94) for gefitinib and 3.7% (one of 27) versus 11.9% (13 of 109) for docetaxel (Fig 4).
patients in the INTEREST trial do not support the hypothesis that patients with high copy number have superior OS on gefitinib compared with docetaxel in this pretreated population. This finding does not invalidate previous findings from placebo-controlled trials as there are several possible explanations for the different results. One is that gefitinib and docetaxel have similar activity in tumors with high and low copy number (ie, high EGFR copy number is predictive of a greater survival benefit over placebo for both treatments). Another is that cross-over to the alternative therapy at disease progression rendered it impossible to detect an OS benefit because many patients received both treatments, but in a different sequence. It might be argued that a more-accurate determination of the effect of a treatment in various subgroups would be an assessment of outcomes that are to be a poor prognostic factor in the absence of treatment,21,22 it is possible that gefitinib improved survival in patients with high EGFR copy number. This highlights the benefit of randomized controlled trials over single-arm trials in distinguishing between prognostic (tumor or patient specific) and predictive (treatment specific) factors.

Our prospectively defined analyses show that patients with EGFR gene mutations, compared with patients with wild-type EGFR, had increased OS and ORRs in the gefitinib group, but also in the docetaxel group. PFS and ORR were higher for gefitinib-treated than docetaxel-treated mutation-positive patients, although this did not translate into statistically significant differences in OS in this small subgroup. Also, many patients received subsequent treatments after progression23 which may have diluted the direct randomized treatment effect observed for PFS and ORR so that it is no longer observed for OS. It is noteworthy that few if any studies describe the effect of these biomarkers in patients treated with standard chemotherapy.

Our findings in a Western pretreated population are consistent with those from IPASS in a East Asian chemotherapy-naïve population.23 In both studies, EGFR mutation-positive patients (identified by direct gene sequencing in our study and by the more sensitive amplification refractory mutation system in IPASS) had superior PFS and ORR with gefitinib compared with comparator; no statistically significant differences in OS were observed, but many patients received subsequent treatments in both studies. However, in patients with wild-type EGFR, PFS, and ORR were inferior for gefitinib versus carboplatin/paclitaxel in chemotherapy-naïve patients, but there was no statistically significant difference between gefitinib and docetaxel in pretreated patients. These results likely reflect the differing efficacy of the comparators used in the first- and second-line settings.

We also showed that few patients with KRAS mutations had responses in either the gefitinib or docetaxel groups, consistent with previous data for gefitinib24 and with studies of other chemotherapy regimens in NSCLC.25-27 There were no significant differences in survival outcome between the study arms according to KRAS mutation status, contrary to reports that patients with KRAS mutations have resistance to EGFR-TKI therapy.17,27,28 However, these findings must be interpreted in the context of subgroup analyses conducted in small patient numbers.
In our study, EGFR biomarkers were determined in the primary tumor from archived diagnostic samples. Therefore, there is a gap between when the diagnostic sample was taken and when patients entered the study. It is unknown whether biomarker status changed on progression or after exposure to first-line chemotherapy. It has recently been reported that EGFR mutation status may change during the course of disease.\textsuperscript{29-33}

In INTEREST, survival was similar for gefitinib and docetaxel in almost all subgroups; no EGFR-related biomarker or any clinical factor (including female sex, adenocarcinoma histology, never-smoker, and Asian ethnicity) appeared to be predictive of a greater survival benefit for gefitinib versus docetaxel. However, these factors may still be predictive of a greater survival benefit for gefitinib and/or docetaxel versus best supportive care; alternatively, they may just be good prognostic factors. For secondary end points of PFS and ORR, some greater advantages for gefitinib over docetaxel were seen in EGFR mutation–positive and high EGFR copy number patients; there was no statistically significant difference between gefitinib and docetaxel in EGFR mutation–negative or low EGFR copy number patients. This suggests that gefitinib can provide OS similar to docetaxel.
in patients across a broad range of clinical subgroups, with added advantages of improved tolerability and quality-of-life against a chemotherapy agent, and ease of use, with oral administration. The EGFR biomarkers such as mutation status may be used to identify which patients are likely to gain the greatest PFS and ORR benefit from gefitinib.

Fig 5. Objective response rate by treatment and biomarker status. *P*-values from logistic regression with covariates. NC, not calculated; EGFR, epidermal growth factor receptor.

**REFERENCES**


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