VOLUME 28 · NUMBER 5 · FEBRUARY 10 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

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

Jean-Yves Douillard, Frances A. Shepherd, Vera Hirsh, Tony Mok, Mark A. Socinski, Radj Gervais, Mei-Lin Liao, Helge Bischoff, Martin Reck, Mark V. Sellers, Claire L. Watkins, Georgina Speake, Alison A. Armour, and Edward S. Kim

See accompanying editorial on page 713 and article on page 753

A B S T R A C T

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.

J Clin Oncol 28:744-752. © 2009 by American Society of Clinical Oncology

From the Centre René Gauducheau, Nantes, France; Princess Margaret Hospital, University of Toronto, Toronto, Canada; McGill University Health Centre, Montreal, Canada; State Key Laboratory in Oncology in South China, Sir YK Pao Centre for Cancer, Department of Clinical Oncology, Hong Kong Cancer Institute, The Chinese University of Hong Kong, Hong Kong; Shanghai Chest Hospital, Shanghai, China; Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC; Centre Regional de Lutte Contre le Cancer Baclesse, Caen, France; Thoraxklinik Heidelberg; Hospital Grosshansdorf, Grosshansdorf, Germany; AstraZeneca, Macclesfield, United Kingdom; AstraZeneca, Wilmington, DE; and The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Submitted May 25, 2009; accepted August 24, 2009; published online ahead of print at www.jco.org on December 28, 2009.

Supported by AstraZeneca.

Presented in part at the 12th World Conference on Lung Cancer, Seoul, Korea, September 2-6, 2007, and the 14th European Cancer Conference, Barcelona, September 23-27, 2007.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Edward S. Kim, MD, M. D. Anderson Cancer Center, Thoracic/Head and Neck Medical Oncology, Box 432, 1515 Holcombe Blvd, Houston, TX 77401; e-mail: edkim@mdanderson.org.

0732-183X/10/2805-744/$20.00

DOI: 10.1200/JCO.2009.24.3030

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).[1-7] 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 (*v* 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,[7] 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.[10] 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.[10] In National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) BR.21 comparing erlotinib with placebo,[16] no significant interaction was seen for EGFR protein expression[15]; however, as in ISEL, high *EGFR* copy number was associated with differentially greater survival compared with low copy number.[17] 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 biomarkers, as there are few published prospective studies.[13,18,19]

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/m^2 intravenously every 3 weeks) for overall survival (OS) in pretreated patients with advanced NSCLC (gefitinib *v* docetaxel, HR 1.02; 96% CI, 0.90 to 1.15).[3] 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

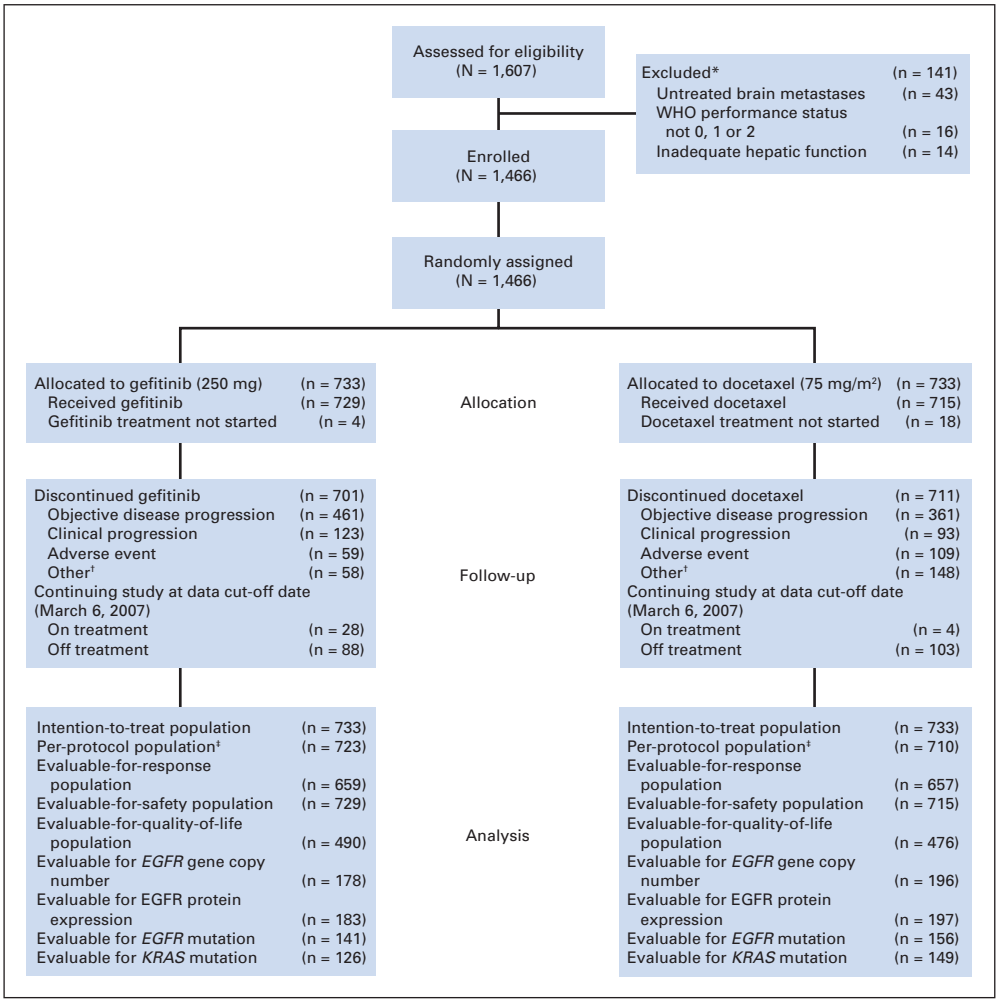

Fig 1. CONSORT diagram. Intention to treat, all randomly assigned patients; per-protocol population, patients who did not significantly deviate from the inclusion or exclusion criteria at entry or significantly deviate from the protocol; evaluable-for-response population, patients in the per-protocol population with unidimensional measurable disease as per the Response Evaluation Criteria In Solid Tumors (RECIST) criteria; evaluable-for-safety population, all patients who received one or more dose of study treatment; evaluable-for-quality-of-life population, patients in the per-protocol population with an evaluable baseline and one or more evaluable post-baseline quality-of-life assessment; evaluable for *EGFR* gene copy number/EGFR protein expression/*EGFR* mutation/*KRAS* mutation, patients in the intention-to-treat population who had a baseline tumor sample assessable. EGFR, epidermal growth factor receptor. (*) Reasons for exclusion were not mutually exclusive. Patients were also excluded for several other reasons, including evidence of significant clinical disorder and withdrawal of informed consent. (†) Other reasons for discontinuation include loss to follow-up, withdrawal of consent, noncompliance, and completing the planned number of docetaxel cycles (docetaxel group). (‡) Reasons for exclusion from per-protocol population include failure to start study treatment, newly diagnosed CNS metastases not yet treated with surgery or radiation, clinical evidence of other coexisting malignancies, previous docetaxel treatment, no histologic or cytologic confirmation of non–small-cell lung cancer, and non–small-cell lung cancer not locally advanced or metastatic or amenable to curative surgery or radiotherapy.

EGFR biomarkers and clinical outcome after treatment with gefitinib or docetaxel from INTEREST.

METHODS

Study Design

Full details of the INTEREST study (1839IL/0721; clinicaltrials.gov identifier NCT00076388) have been published previously.[3] 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.[8] High copy number was defined as high polysomy ($\geq$ 4 copies in $\geq$ 40% of cells) or gene amplification (presence of tight gene clusters; a gene:chromosome ratio per cell $\geq$ 2; or $\geq$ 15 copies of *EGFR* per cell in $\geq$ 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

	EGFR Copy Number by FISH		EGFR Protein Expression		*EGFR* Mutation		*KRAS* Mutation		Overall	
Parameter	No.	%	No.	%	No.	%	No.	%	No.	%
No. of patients	374		380		297		275		1,466	
Adenocarcinoma	207	55.3	206	54.2	169	56.9	144	52.4	830	56.6
Non-adenocarcinoma	167	44.7	174	45.8	128	43.1	131	47.6	636	43.4
Sex										
Female	116	31.0	124	32.6	93	31.3	84	30.5	512	34.9
Male	258	69.0	256	67.4	204	68.7	191	69.5	954	65.1
WHO PS										
0 or 1	336	89.8	339	89.2	264	88.9	245	89.1	1,296	88.4
2	38	10.2	41	10.8	33	11.1	30	10.9	170	11.6
Smoking status										
Never*	60	16.0	67	17.6	50	16.8	31	11.3	298	20.3
Ever	314	84.0	313	82.4	247	83.2	244	88.7	1,168	79.7
Line										
Second	316	84.5	322	84.7	253	85.2	239	86.9	1,229	83.8
Third	58	15.5	58	15.3	44	14.8	36	13.1	237	16.2
Race/ethnicity										
Asian origin†	54	14.4	58	15.3	48	16.2	5	1.8	323	22.0
Non-Asian origin	320	85.6	322	84.7	249	83.8	270	98.2	1,143	78.0
Overall survival, HR	1.00		1.02		0.97		0.99		1.01	
95% CI‡	0.80 to 1.25		0.82 to 1.27		0.76 to 1.25		0.76 to 1.28		0.90 to 1.14§	
PFS, HR	1.01		1.16		1.01		1.14		1.04	
95% CI‡	0.80 to 1.27		0.92 to 1.47		0.78 to 1.31		0.86 to 1.51		0.93 to 1.18	
ORR										
Gefitinib‖	16/157	10.2	17/160	10.6	15/125	12.0	9/114	7.9	60/659	9.1
Docetaxel‖	16/180	8.9	18/185	9.7	16/142	11.3	14/136	10.3	50/657	7.6

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).

‖In the assessable-for-response population.

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.[20]

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*

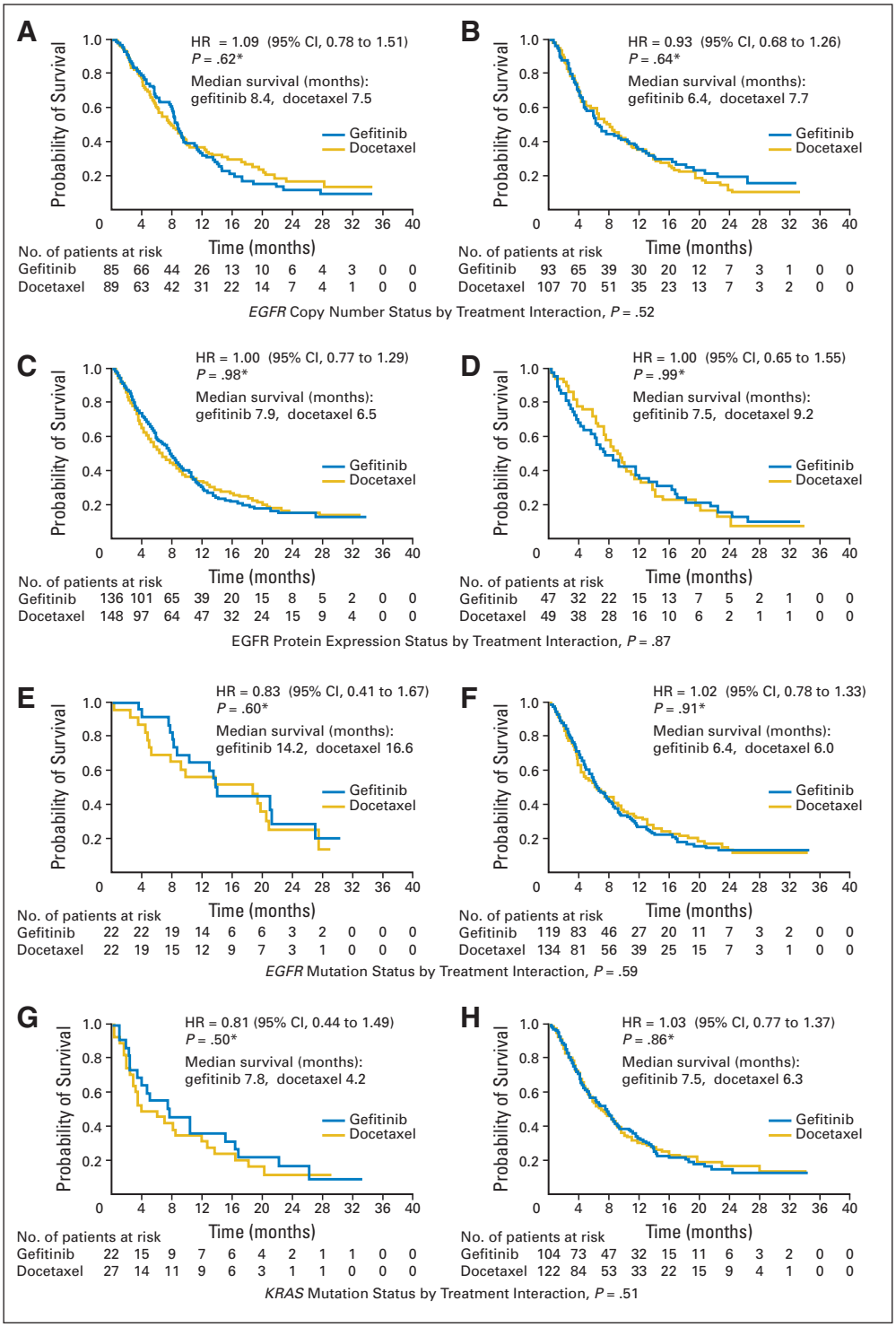

Fig 2. Overall survival in patients with (A) high epidermal growth factor receptor (*EGFR*) copy number (reprinted from Kim et al[3] with permission from Elsevier); (B) low *EGFR* copy number; (C) EGFR protein expression positive; (D) EGFR protein expression negative; (E) mutant *EGFR*; (F) wild-type *EGFR*; (G) mutant *KRAS*; and (H) wild-type *KRAS*. (*) Cox analysis without covariates. HR, hazard ratio.

mutation status, and no evidence that *KRAS* mutation is a predictive factor for a differential survival effect between gefitinib and docetaxel (mutation-positive HR 0.81 *v* 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).

DISCUSSION

Two large randomized trials[7,16] comparing EGFR-TKI therapy with placebo showed that high *EGFR* copy number was significantly associated with a differentially greater survival benefit from treatment.[10,15] However, our copy number analyses of tumors from a subset of

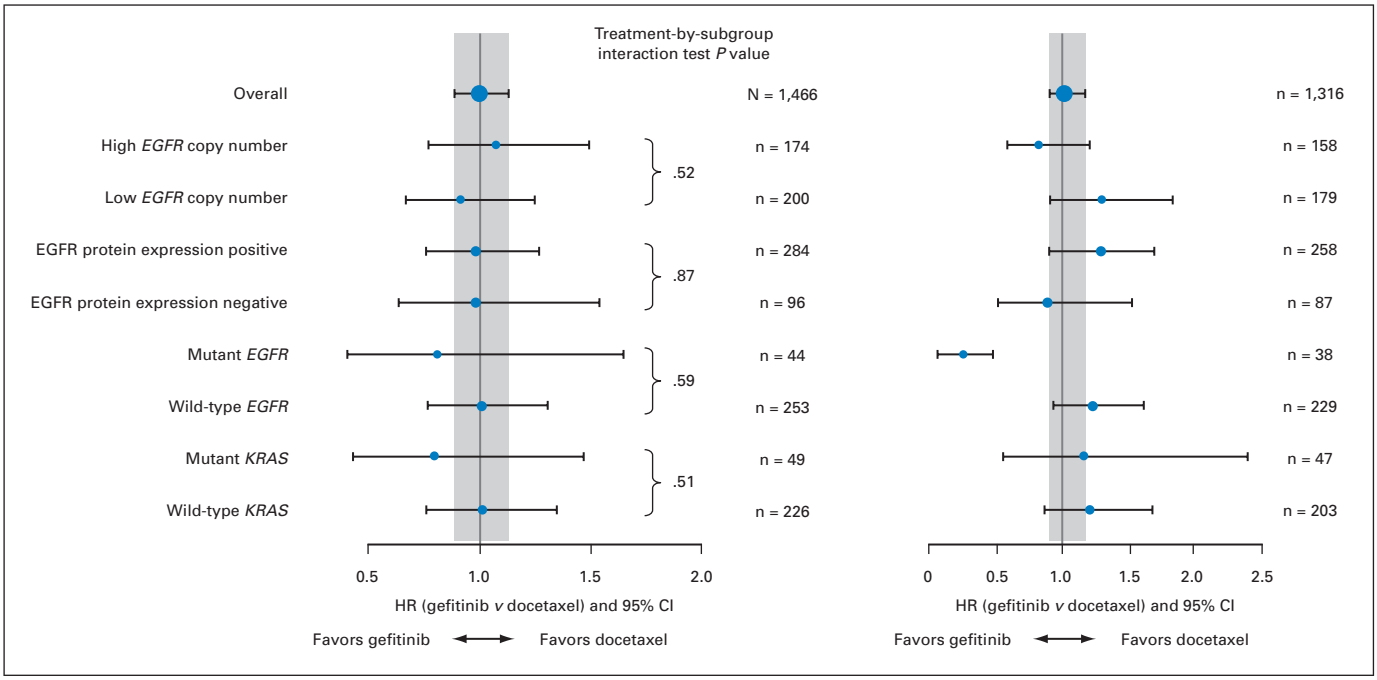

Fig 3. Forest plot of (left) overall survival by biomarkers in intention to treat population (reprinted from Kim et al[3] with permission from Elsevier); and (right) progression-free survival by biomarkers in the assessable-for-response population. Cox analysis without covariates. EGFR, epidermal growth factor receptor; HR, hazard ratio.

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 evaluated while the treatment is being administered, such as PFS and ORR. Indeed, PFS and ORR were higher for gefitinib-treated than docetaxel-treated patients with high *EGFR* copy number, with a significant difference for ORR, whereas the reverse was true for patients with low *EGFR* copy number, although the differences were not significant.

Interestingly, exploratory analyses showed no difference between patients with high and low *EGFR* copy number within the gefitinib arm (high:low HR, 1.02; 95% CI, 0.74 to 1.41; $P = .9114$), whereas previous studies have shown longer survival in gefitinib-treated patients with high *EGFR* copy number compared with those with low *EGFR* copy number. However, since high *EGFR* copy number seems 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 progression[3] 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 gefitinib[24] 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.

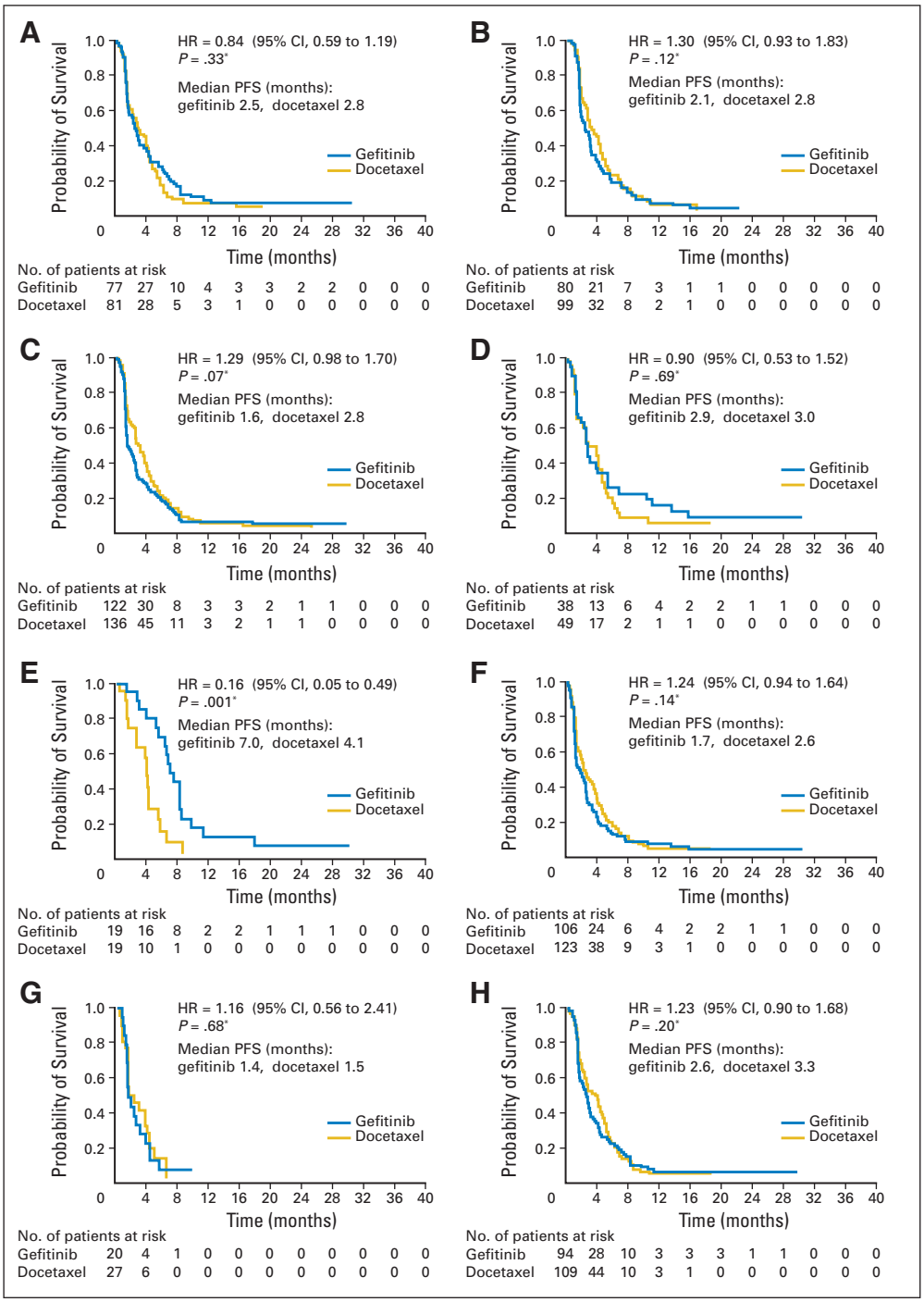

Fig 4. Progression-free survival (PFS) in patients with (A) high epidermal growth factor receptor (*EGFR)* copy number; (B) low *EGFR* copy number; (C) EGFR protein expression positive; (D) EGFR protein expression negative; (E) mutant *EGFR*; (F) wild-type *EGFR*; (G) mutant *KRAS*; and (H) wild-type *KRAS*. (*) Cox analysis without covariates. HR, hazard ratio.

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.[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

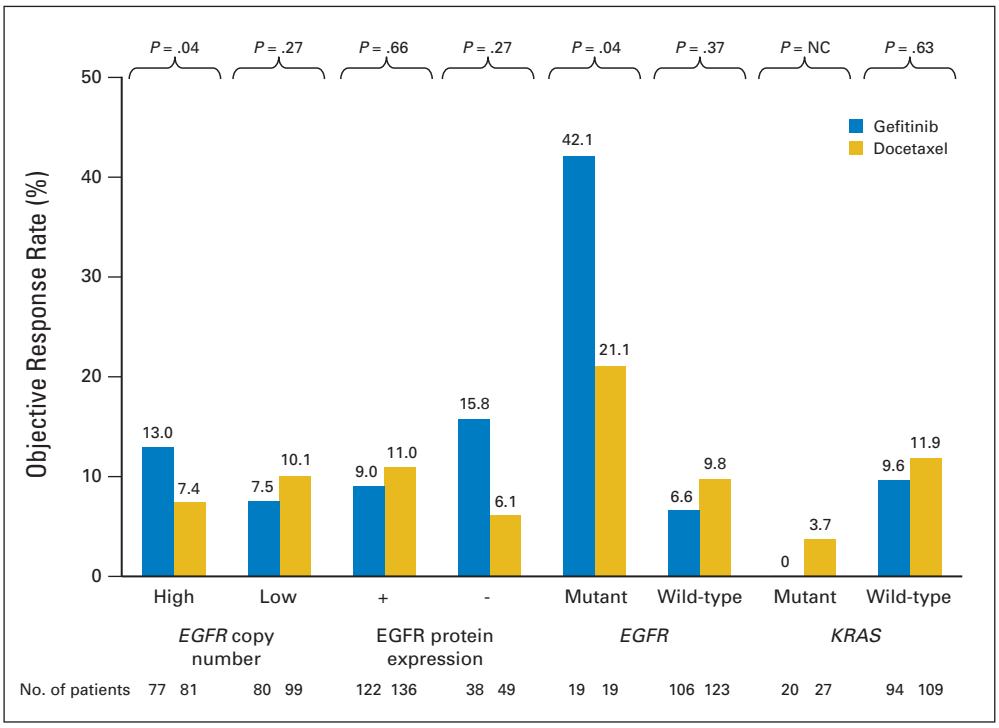

Fig 5. Objective response rate by treatment and biomarker status. *P* values from logistic regression with covariates. NC, not calculated; EGFR, epidermal growth factor receptor.

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.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Mark V. Sellers, AstraZeneca (C); Claire L. Watkins, AstraZeneca (C); Georgina Speake, AstraZeneca (C); Alison A. Armour, AstraZeneca (C) **Consultant or Advisory Role:** Jean-Yves Douillard, AstraZeneca (C), sanofi-aventis (C); Frances A. Shepherd, AstraZeneca (C); Vera Hirsh, AstraZeneca (C); Tony Mok, AstraZeneca (C), Roche (C), Eli Lilly (C), Merck Serono (C); Mark A. Socinski, AstraZeneca (C), sanofi-aventis (C); Martin Reck, Eli Lilly (C), AstraZeneca (C), Hoffmann-La Roche (C), Merck (C); Edward S. Kim, AstraZeneca (C), sanofi-aventis (C) **Stock Ownership:** Frances A. Shepherd, AstraZeneca; Mark V. Sellers, AstraZeneca; Claire L. Watkins, AstraZeneca; Georgina Speake, AstraZeneca; Alison A. Armour, AstraZeneca **Honoraria:** Jean-Yves Douillard, AstraZeneca, sanofi-aventis; Frances A. Shepherd, AstraZeneca; Vera Hirsh, AstraZeneca; Tony Mok, AstraZeneca, Roche, Eli Lilly, Pfizer; Mark A. Socinski, AstraZeneca, sanofi-aventis; Martin Reck, Eli Lilly, Hoffmann-La Roche, Merck, AstraZeneca; Edward S. Kim, AstraZeneca, sanofi-aventis **Research Funding:** Mark A. Socinski, AstraZeneca, sanofi-aventis; Edward S. Kim, AstraZeneca, sanofi-aventis **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Helge Bischoff, Claire L. Watkins, Edward S. Kim
Provision of study materials or patients: Jean-Yves Douillard, Frances A. Shepherd, Vera Hirsh, Tony Mok, Mark A. Socinski, Radj Gervais, Mei-Lin Liao, Helge Bischoff, Martin Reck, Edward S. Kim
Collection and assembly of data: Alison A. Armour, Edward S. Kim
Data analysis and interpretation: Jean-Yves Douillard, Frances A. Shepherd, Vera Hirsh, Tony Mok, Mark A. Socinski, Radj Gervais, Helge Bischoff, Martin Reck, Mark V. Sellers, Claire L. Watkins, Georgina Speake, Alison A. Armour, Edward S. Kim
Manuscript writing: Jean-Yves Douillard, Frances A. Shepherd, Vera Hirsh, Tony Mok, Mark A. Socinski, Radj Gervais, Mei-Lin Liao, Martin Reck, Alison A. Armour, Edward S. Kim
Final approval of manuscript: Jean-Yves Douillard, Frances A. Shepherd, Vera Hirsh, Tony Mok, Mark A. Socinski, Radj Gervais, Mei-Lin Liao, Helge Bischoff, Martin Reck, Mark V. Sellers, Claire L. Watkins, Georgina Speake, Alison A. Armour, Edward S. Kim

REFERENCES

1. Cufer T, Vrdoljak E, Gaafar R, et al: Phase II, open-label, randomized study (SIGN) of single-agent gefitinib (IRESSA) or docetaxel as second-line therapy in patients with advanced (stage IIIb or IV) non-small-cell lung cancer. Anticancer Drugs 17: 401-409, 2006

2. Fukuoka M, Yano S, Giaccone G, et al: Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237-2246, 2003

3. Kim ES, Hirsch V, Mok T, et al: Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): A randomised phase III trial. Lancet 372:1809-1818, 2008

4. Kris MG, Natale RB, Herbst RS, et al: Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 290:2149-2158, 2003

5. Lee D, Kim S, Park K, et al: A randomized open-label study of gefitinib versus docetaxel in patients with advanced/metastatic non-small cell lung cancer (NSCLC) who have previously received platinum-based chemotherapy. J Clin Oncol 26:430s, 2008 (suppl; abstr 8025)

6. Maruyama R, Nishiwaki Y, Tamura T, et al: Phase III study, V-15-32, of gefitinib versus docetaxel in previously treated Japanese patients with non-small-cell lung cancer. J Clin Oncol 26:4244-4252, 2008

7. Thatcher N, Chang A, Parikh P, et al: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: Results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 366:1527-1537, 2005

8. Cappuzzo F, Hirsch FR, Rossi E, et al: Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non–small-cell lung cancer. J Natl Cancer Inst 97:643-655, 2005

9. Hirsch FR, Varella-Garcia M, McCoy J, et al: Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: A Southwest Oncology Group study. J Clin Oncol 23:6838-6845, 2005

10. Hirsch FR, Varella-Garcia M, Bunn Jr PA, et al: Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol 24:5034-5042, 2006

11. 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 350:2129-2139, 2004

12. Paez JG, Jänne PA, Lee JC, et al: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004

13. Sequist LV, Joshi VA, Janne PA, et al: Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic EGFR mutation testing. Oncologist 12:90-98, 2007

14. Sequist LV, Bell DW, Lynch TJ, et al: Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. J Clin Oncol 25:587-595, 2007

15. Tsao M-S, Sakurada A, Cutz J-C, et al: Erlotinib in lung cancer: Molecular and clinical predictors of outcome. N Engl J Med 353:133-144, 2005

16. Shepherd FA, Rodrigues PJ, Ciuleanu T, et al: Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353:123-132, 2005

17. Zhu CQ, da Cunha SG, Ding K, et al: Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR. 21. J Clin Oncol 26:4268-4275, 2008

18. Dziadziuszko R, Holm B, Skov B, et al: Epidermal growth factor receptor gene copy number and protein level are not associated with outcome of non-small-cell lung cancer patients treated with chemotherapy. Ann Oncol 18:447-452, 2007

19. Toschi L, Metro G, Magrini E, et al: EGFR, HER2, and Phospho-AKt are not predictive factors for sensitivity to first-line chemotherapy in patients with advanced non-small-cell lung cancer (NSCLC). J Clin Oncol 24:391s, 2006 (abstr 7111)

20. Hochberg Y: A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75:800-802, 1988

21. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al: Epidermal growth factor receptor in non-small-cell lung carcinomas: Correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21:3798-3807, 2003

22. Cappuzzo F, Marchetti A, Skokan M, et al: Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol 27:1667-1674, 2009

23. Mok T, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361:947-957, 2009

24. Han SW, Kim TY, Jeon YK, et al: Optimization of patient selection for gefitinib in non-small cell lung cancer by combined analysis of epidermal growth factor receptor mutation, K-ras mutation, and Akt phosphorylation. Clin Cancer Res 12:2538-2544, 2006

25. Eberhard DA, Johnson BE, Amler LC, et al: Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 23:5900-5909, 2005

26. Winton T, Livingston R, Johnson D, et al: Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 352:2589-2597, 2005

27. Pao W, Wang TY, Riely GJ, et al: KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2(1):e17, 2005

28. Massarelli E, Varella-Garcia M, Tang X, et al: KRAS mutation is an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small-cell lung cancer. Clin Cancer Res 13:2890-2896, 2007

29. Gow CH, Chang YL, Hsu YC, et al: Comparison of epidermal growth factor receptor mutations between primary and corresponding metastatic tumors in tyrosine kinase inhibitor-naive non-small-cell lung cancer. Ann Oncol 20:696-702, 2008

30. Bozzetti C, Tiseo M, Lagrasta C, et al: Comparison between epidermal growth factor receptor (EGFR) gene expression in primary non-small cell lung cancer (NSCLC) and in fine-needle aspirates from distant metastatic sites. J Thorac Oncol 3:18-22, 2008

31. Italiano A, Vandenbos FB, Otto J, et al: Comparison of the epidermal growth factor receptor gene and protein in primary non-small-cell-lung cancer and metastatic sites: Implications for treatment with EGFR-inhibitors. Ann Oncol 17:981-985, 2006

32. Kobayashi S, Boggon TJ, Dayaram T, et al: EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352:786-792, 2005

33. Petersen S, Aninat-Meyer M, Schlüns K, et al: Chromosomal alterations in the clonal evolution to the metastatic stage of squamous cell carcinomas of the lung. Br J Cancer 82:65-73, 2000

