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Epidermal Growth Factor Receptor Immunohistochemistry:

Comparison of Antibodies and Cutoff Points to Predict Benefit From Gefitinib in a Phase 3 Placebo-Controlled Study in Advanced Nonsmall-Cell Lung Cancer

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Abstract

BACKGROUND—The ISEL (Iressa Survival Evaluation in Lung Cancer) clinical trial evaluated the efficacy of gefitinib versus placebo in pretreated nonsmall-cell lung cancer patients. Two different antibodies, scoring systems, and cutoff points of epidermal growth factor receptor (EGFR) protein expression were compared to predict response and survival of enrolled patients.

METHODS—EGFR expression was assessed in tumor samples by immunohistochemistry using the Dako EGFR pharmDx kit (scoring percent of tumor cells with positive staining) and Zymed monoclonal antibody clone 31G7 (scoring staining index derived from proportion of positive cells times staining intensity).

RESULTS—Data for EGFR expression were available for 379 patients for Dako and 357 patients for Zymed antibody (22% and 21%, respectively, of trial population). Objective response rates in gefitinib-treated EGFR-positive patients defined with various cutpoints with Dako antibody varied between 8% and 12%, and with Zymed antibody between 10% and 13%. Lower cutoff points with Dako antibody provided the best discrimination between EGFR-positive and EGFR-negative patients for survival hazard ratios comparing gefitinib to placebo, with a significant treatment/cutoff point interaction for 10% cutoff point ($P = .049$). A similar but less apparent trend was noted for Zymed antibody, although the discrimination between hazard ratios was not significant for any cutoff point analyzed.

CONCLUSIONS—Assessment with the Dako PharmDx kit and percentage of cells with positive staining may provide more accurate prediction of differential effect on survival with gefitinib than assessment with Zymed antibody and staining index. Using higher cutpoints to define positivity does not improve test discrimination.

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Keywords

nonsmall-cell lung cancer; epidermal growth factor receptor; immunohistochemistry; phase 3 trial; cutoff point

Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs, gefitinib and erlotinib) are active in a subset of nonsmall-cell lung cancer (NSCLC) patients. The response rates and disease control rates reported in clinical trials of EGFR TKIs in advanced pretreated NSCLC patients in Western populations are 10% to 20% and 40%, respectively,[1–3] indicating that a proportion of NSCLC patients do not derive any benefit from EGFR TKIs. A major research effort over the last decade focused on the identification of predictive biomarkers for response and survival benefit to EGFR TKIs, and many of these studies analyzed EGFR protein expression by immunohistochemistry as the most applicable method to assess the presence of molecular target in the tumor.

Results of the ISEL (Iressa Survival Evaluation in Lung cancer) phase 3 clinical trial in advanced NSCLC patients who were refractory to or intolerant of their latest chemotherapy regimen showed some improvement in survival with gefitinib (plus best supportive care), which failed to reach statistical significance compared with placebo (plus best supportive care), in the overall population and in patients with adenocarcinoma.[4] Preplanned subgroup analysis of the ISEL demonstrated a statistically significant increase in survival with gefitinib in patients of Asian ethnicity and in patients who had never smoked.

A biomarker analysis of this study demonstrated a nonsignificant 23% reduction in the risk of death for gefitinib-treated patients who expressed EGFR protein as assessed by the EGFR Dako PharmDx kit with a cutoff point of 10% of cells exhibiting the staining of at least slight intensity.[5] No benefit was observed in the subset of patients who were classified as EGFR protein-negative. The National Cancer Institute of Canada BR.21 clinical trial that demonstrated a significant improvement in survival of erlotinib versus placebo-treated advanced NSCLC patients who failed at least 1 chemotherapy regimen[6] showed a 32% reduction in the risk of death for patients with EGFR protein-positive tumor samples.[7] No survival advantage was seen among patients with EGFR protein-negative tumor samples. This study also used a cutoff point of 10% stained cells and the Dako PharmDx kit. In a retrospective evaluation of gefitinib-treated NSCLC patients, Cappuzzo et al.[8] used Zymed anti-EGFR monoclonal antibody and a staining index that takes into account the percent of positive cells and staining intensity, scored from 0 to 4. Using a cutpoint of 200 on the scale from 0 to 400, superior survival was demonstrated in EGFR protein-positive versus negative patients ($P = .01$). However, other studies performed on tumor samples from phase 3 clinical trials investigating the combination of gefitinib or erlotinib with chemotherapy failed to show any predictive value of EGFR protein expression for either clinical response or survival.[9,10] Also, there was no association with EGFR protein expression and survival for NSCLC patients who received gefitinib monotherapy in the phase 2 clinical studies IDEAL1 and 2 (Iressa Dose Evaluation in Advanced Lung cancer).[11]

Clinical trials of cetuximab, a monoclonal antibody targeted against the EGFR in both lung and colorectal cancer required EGFR protein expression in tumor samples for study entry in most trials. EGFR protein expression was evaluated by the EGFR PharmDx kit with cutoff points of at least 1+ (at least 1% or at least 10% of cells with weak staining according to individual study). More than 90% of screened patients were scored as EGFR protein-positive in phase 2 clinical studies with cetuximab in lung cancer[12–14] and >75% in phase 2 or 3 colorectal cancer trials.[15,16] Because these trials were performed largely in EGFR protein-positive patients, the efficacy of cetuximab in EGFR-negative patients remains

unknown, although 1 report suggests that EGFR protein-negative colorectal cancer patients may respond to cetuximab.[17] In a pivotal phase 3 clinical trial comparing cetuximab versus cetuximab and irinotecan in metastatic colorectal cancer that was refractory to treatment with irinotecan, the degree of EGFR staining did not associate with response rates in either study groups.[15]

The presence of mutations in the *EGFR* gene has also been investigated in tumor samples and is linked with increased responsiveness to EGFR TKIs in numerous NSCLC studies.[5,18–20] An additional approach of measuring the *EGFR* gene copy number in tumor samples has also demonstrated a survival advantage for patients with a high copy number in prospective placebo-controlled clinical trials.[5,7,21] These 2 gene-based biomarkers appear to outperform EGFR protein evaluation in predicting the benefit from EGFR TKIs, but, particularly for *EGFR* mutations, prospective placebo-controlled clinical studies are lacking. In contrast to the above evaluations of *EGFR* gene mutations and copy number, *EGFR* immunohistochemistry is a widely applicable and inexpensive test to conduct. HER-2 protein expression evaluation by immunohistochemistry, in conjunction with HER-2 FISH assay, is used for the selection of breast cancer patients most likely to benefit from trastuzumab therapy,[22] and *HER-2* gene copy number has been closely associated with HER-2 protein expression.[23]

This biomarker study of the placebo-controlled ISEL trial has provided us the opportunity to compare 2 antibodies (Dako and Zymed), which have previously been associated with clinical outcome for NSCLC patients treated with gefitinib, and evaluate whether different cutoff levels of protein expression could improve the prediction of response and survival benefit from gefitinib.

MATERIALS AND METHODS

Clinical Study Design

The results of the ISEL study were previously published.[4] This was a randomized, double-blind, phase 3 clinical trial comparing the efficacy of gefitinib 250 mg/day (plus best supportive care) versus placebo (plus best supportive care) in 1692 patients with advanced NSCLC who were refractory to or intolerant of their latest chemotherapy regimen. The associations between selected biomarkers (EGFR protein expression, *EGFR* gene copy number, *EGFR*, K-*ras* and B-*raf* gene mutations) and treatment outcome in the ISEL were also reported.[5] Of the 379 patients who were evaluable for EGFR protein expression by the Dako PharmDx kit in this biomarker analysis, 177 patients were also evaluable for *EGFR* gene copy number and *EGFR* mutation, and some overlap was seen between those patients who were positive for these biomarkers.[5]

EGFR Protein Expression

EGFR protein expression was assessed by immunohistochemistry using the Dako EGFR PharmDx kit (Dako, Glostrup, Denmark) and Zymed mouse anti-human EGFR monoclonal antibody clone 31G7 (Zymed Laboratories, San Francisco, Calif). The staining procedures were performed according to the antibody manufacturer's recommendations as previously reported.[8,24] For scoring of samples stained with the Dako antibody, the percentage of tumor cells showing membranous staining was recorded, and a predefined cutoff point of ≥10% cells with at least weak staining intensity was applied to define protein positivity. Scoring was performed by a trained individual (D.V.P.) who was blinded to the clinical outcome data. For scoring of samples stained with the Zymed antibody, a staining index calculated as percent of stained tumor cells × average staining intensity graded from 0 to 4 was used, resulting in an index value between 0 and 400. Consistent with our previous reports,[8,24]

samples with a staining index of 200 or higher were predefined as EGFR protein-positive. The scoring was performed by 2 pathologists (F.R.H., W.A.F.) who were blinded to the clinical outcome data. Consensus scoring was performed for all specimens with staining index difference of 50 or more. Otherwise, the mean of the 2 readings was reported.

Statistical Analyses

Summary tables and scatterplots were obtained to explore the relation between percentage of tumor cells stained with the Dako antibody and the staining index with the Zymed antibody.

An evaluation of different cutoff points of EGFR expression with Dako to predict overall survival was performed using a Cox Proportional Hazards regression model. Separate models were fitted for the different cutoff points used to define EGFR protein-positive. The fitted models allowed for the effect of treatment and included terms for histology, sex, smoking history, reason for prior chemotherapy failure, number of prior chemotherapy regimens, and performance status. From the fitted models a hazard ratio and 95% confidence interval, for gefitinib versus placebo were calculated for patients with EGFR-positive and -negative tumor samples for each of the different cutoff points analyzed. A treatment by EGFR expression interaction was assessed to determine whether the treatment effect is consistent across the EGFR protein-positive and -negative tumor samples as defined by different cutoff points. This therefore reflects the degree of discrimination between positive and negative tumor samples for each cutoff point. As the analysis performed was exploratory in nature no adjustments for multiple testing were made.

The number of patients, deaths, response rate, and disease control rate for gefitinib and placebo were also calculated for EGFR-positive and -negative tumor samples, as defined by different cutoff points.

The evaluation of different cutoff points of EGFR expression with Dako was repeated to evaluate different cutoff points of EGFR expression with Zymed antibody scoring index.

A correlation coefficient between the staining index with the Zymed antibody as assessed by 2 pathologists (F.R.H., W.A.F.) was calculated. In order to estimate the inter- and intrareader components of variability a random effects model was fitted to the data with reader effects taken as random and subject effects as fixed.

RESULTS

Patient Characteristics

EGFR protein expression was assessed by the Dako PharmDx kit in 379 patients (22.4% of all study subjects) and using Zymed clone 31G7 antibody in 357 patients (21.0% of all study subjects). Data for both antibodies were available for 296 patients (17.5%). Demographic and baseline characteristics of patients evaluable for expression of EGFR with the Dako or Zymed antibody compared with overall study population are presented in Table 1. The subset of patients analyzed for protein expression was comparable to overall study population with the exception of never-smokers and patients of Asian origin, who are underrepresented in our analysis.

Comparison of Antibodies for Protein Expression Evaluation

A correlation coefficient between the staining index with the Zymed antibody as assessed by 2 investigators was calculated as 0.96 ($P < .001$).

The resulting components of variation for the interreader and intrareader (interpatient) variation in determining the staining index with the Zymed antibody were 5.4 and 876.7

respectively. The intrareader variability component is very small compared with the variability between patients, indicating good consistency between readers.

Using predefined criteria for protein positivity (≥10% of cells with any positive staining for Dako and staining index ≥200 for Zymed antibody), 70% (264 of 379) of tumor samples were scored as positive with Dako and 68% (244 of 357) were scored as positive with Zymed antibody. For the samples with available data for both antibodies the agreement between assessments was 76% (Table 2). The proportion of tumor samples showing no staining of any intensity with the Dako antibody was 26% as compared with 4% for the Zymed antibody. The relation between both indices appears to be cubic, and the majority of the best fit cubic line is in the region where both scores are positive or both are negative (data not shown).

Assessment With Dako PharmDx Kit, Zymed Antibody, and Clinical Outcome With Gefitinib

Objective response rates to gefitinib according to various cutoff points of EGFR immunostaining with Dako and Zymed antibody are shown in Tables 3 and 4. For the Dako antibody the objective response rates for EGFR protein-positive/negative patients treated with gefitinib were 8.0%/1.6% with a cutoff point of ≥1% of positive cells and 10.0%/5.3% with a cutoff point ≥90% positive cells, respectively. For the Zymed antibody the objective response rates with gefitinib were 9.9%/3.0% with the cutoff staining index ≥50 and 12.8%/6.3% with the cutoff staining index ≥350, respectively. Disease control rates were similar for patients with EGFR-positive tumors across all cutoff points analyzed for both antibodies. The survival hazard ratios (HRs) for gefitinib versus placebo according to the cutoff points are presented in Tables 3 and 4 and Figures 1 and 2. For the Dako PharmDx kit, and percent of positive staining, the largest differences between EGFR protein-positive and -negative HRs for survival were with low cutoff levels, indicating better discrimination between positive and negative subgroups at low cutoff levels. The originally predefined cutoff point ≥10% was the only cutoff with a *P*-value of less than 5% ($P = .049$) in the interaction test. Similar tendency was noted for the Zymed antibody, although the differences between survival HRs appeared smaller and were nonsignificant in the interaction test for any cutoff point analyzed.

DISCUSSION

In this study we compared immunohistochemical assessments of EGFR protein expression with 2 different procedures, the Dako PharmDx kit and the Zymed clone 31G7 antibody, which are most frequently used in evaluation of NSCLC patients treated with EGFR tyrosine kinase inhibitors. We also assessed the predictive significance of using different cutoff points to define EGFR positivity for outcome of gefitinib therapy and we found that lower cutoff points are better predictors of survival outcomes than higher cutoff points for both antibodies studied.

To our knowledge, a comparison of EGFR immunostaining with Dako and Zymed antibodies in NSCLC tumor samples has not previously been published. Both antibodies are being used in ongoing and planned studies of EGFR inhibitors in NSCLC. The setting of the ISEL placebo-controlled randomized phase 3 clinical study enabled us to compare the procedures of EGFR assessment (with different antibodies but also different staining protocols and scoring criteria). Thus, this comparison reflects not only the properties of individual antibodies but also the predictive power of the procedures to distinguish the subsets of patients with different outcomes after gefitinib therapy.

According to previously established criteria of EGFR positivity for the Dako and Zymed antibodies,[7,24] the proportions of patients with results for both antibodies that scored as positive is almost identical (69% and 72%, respectively). The agreement in identifying negative and positive samples is 76%, indicating that these 2 procedures identify slightly different subsets of patients. The number of patients with no staining of any intensity was higher with the Dako PharmDx kit as compared with Zymed (26% vs 4%), indicating that staining with Zymed is more sensitive compared with the Dako PharmDx kit. Similar results were reported by Penault-Llorca et al.[25] in colorectal cancer specimens. The authors compared the FDA-approved Dako PharmDx kit, Zymed EGFR kit with clone 31G7 antibody, Ventana EGFR 3C6 antibody, and concluded that the Dako antibody is less sensitive than other antibodies for all cutoff points analyzed. In another study in normal colon, adenoma, and colon carcinoma samples, similar sensitivity of the EGFR PharmDx Kit and Zymed Clone 31G7 antibody was found according to manual and automatic scoring systems.[26]

The range of gefitinib response rates in patients with EGFR-positive were similar for both the Zymed and Dako antibodies (10%–13% and 8%–12% gefitinib responders with any cutoff level, respectively; Tables 3 and 4). Disease control rates appeared to be similar for patients with EGFR-positive across all cutoff levels analyzed for both antibodies. The Zymed antibody had a higher sensitivity to detect samples with any staining. The Dako antibody appeared to better predict the differential survival outcome with gefitinib, as indicated by a greater difference between EGFR protein-positive and -negative HRs. Although these HRs are overlapping over 1.00 for any cutoff point analyzed, it seems unlikely that patients that are EGFR-negative, defined as staining <10%, benefit from gefitinib. It should be stressed that our study did not directly compare the antibodies, for which the same methodology of staining procedure and the same scoring system should have been performed. We sought to compare different antibodies together with the staining procedures and scoring systems originally reported in large clinical studies.[5–7] Applying the standard Dako cutoff of 10% to both antibodies gives 72% EGFR-positive for Dako but 94% EGFR-positive for Zymed. Applying the standard Zymed 200 cutoff to both antibodies gives 69% EGFR-positive for Zymed but 15% EGFR-positive for Dako. Therefore, the scoring systems appear unique to the antibody.

Similar observation of better prediction of survival benefit from erlotinib with lower cutoff points to define positive EGFR immunostaining was published by Clark et al.[27] In their study, based on the results of the BR.21 trial and EGFR immunohistochemical staining of tumor samples with the Dako PharmDx kit, the largest differences between EGFR protein-positive and -negative HRs for survival were observed with low cutoff points of percent of positive cells, staining intensity, and staining index. These data are in agreement with the present study and do not support the selection of patients to EGFR tyrosine kinase inhibitors based on the higher cutoff points to define EGFR positivity.

Lack of association between the degree of EGFR protein expression and the effectiveness of EGFR inhibitors was demonstrated in colorectal cancer studies.[15,16] Based on a specific ligand binding assay (Scatchard analysis), the presence of low- and high-affinity EGFRs was identified in tumor samples from colorectal cancer patients.[28] In this study, high-affinity receptors represented a smaller fraction of the total receptor pool on tumor cells in most samples. Another report suggested that a subclass of high-affinity receptors is primarily activated after EGFR binding and is responsible for signal transduction downstream of EGFR.[29] The distinction between low-and high-affinity receptors is not possible by immunohistochemistry with most anti-EGFR antibodies, including both the Dako and Zymed antibodies, which were generated from mice immunized with the purified EGFR antigen from the A-431 cell line containing both low- and high-affinity EGFRs.[28–30] Thus, it

is possible that the results of immunohistochemical staining may not adequately reveal the pool of biologically active EGFRs.

The type of biopsy (eg, lung biopsy vs resection specimen) may influence the results of EGFR protein expression analysis, as demonstrated recently by Taillade et al.[31] In this study the authors used anti-EGFR 3C6 primary antibody from Ventana Medical Systems (Illkirch, France) and scoring based on percentage of positive staining. The concordance between EGFR protein expression in lung biopsies versus surgical specimens was poor (correlation coefficient $r = 0.24$, $P = .17$, n = 41).

In summary, we have demonstrated that EGFR immunostaining with the Dako PharmDx kit according to percent of cells with positive staining appears to better predict for survival outcome with gefitinib than Zymed antibody according to staining index. Further analysis using similar methods to our study with samples from other large randomized phase 3 studies would be useful to provide additional evidence of whether 1 immunohistochemical antibody/methodology is superior. If EGFR immunohistochemistry is to be applied for patient selection, low cutoff levels to define protein positivity should be used.

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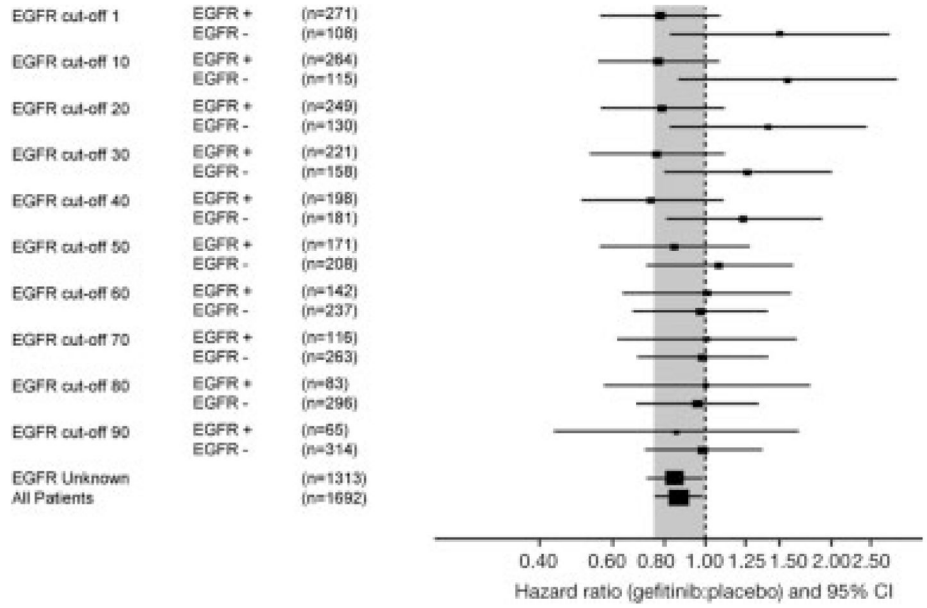

FIGURE 1.

Comparison of hazard ratios for gefitinib versus placebo in patients with epidermal growth factor receptor (EGFR)-positive versus -negative tumor samples with various cutoff points to define positivity with the Dako EGFR PharmDx kit and percentage of cells with positive staining.

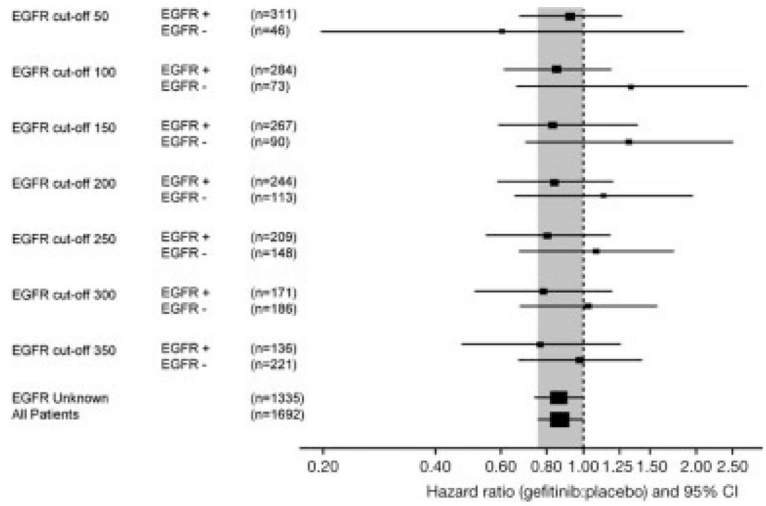

FIGURE 2.

Comparison of hazard ratios for gefitinib versus placebo in patients with epidermal growth factor receptor (EGFR)-positive versus -negative tumor samples with various cutoff points to define positivity with the Zymed antibody and scoring index.

TABLE 1

Demographics and Clinical Outcome With Gefitinib for Patients With Evaluable Tissue Samples for EGFR Protein Expression Studies Versus the Overall Study Population

Characteristic	EGFR protein expression by dako pharm Dx kit, n = 379 No. (%)	EGFR protein expression by Zymed clone 31G7 antibody, n = 357 No. (%)	Overall study population, n = 1692 No. (%)
Adenocarcinoma	168 (44.3)	150 (42.0)	812 (48.0)
Female	122 (32.2)	111 (31.1)	553 (32.7)
WHO PS 0 or 1	233 (61.5)	218 (61.1)	1126 (66.5)
Never smoker	51 (13.5)	49 (13.7)	375 (22.2)
Second-line *	177 (46.7)	175 (49.0)	823 (48.6)
Asian origin †	21 (5.5)	13 (3.6)	342 (20.2)
Refractory ‡	335 (88.4)	314 (88.0)	1523 (90.0)
HR for survival [95% CI] §	0.94 [0.71. 1.25]	0.91 [0.68. 1.23]	0.86 [0.76. 0.99]
Response rate on gefitinib	(6.2)	(8.9)	(8.0)

EGFR indicates epidermal growth factor receptor; WHO, world health organization; HR, hazard ratio; CI, confidence interval.

* Second-line refers to patients who received 1 previous line of chemotherapy.

† The definition of Asian racial origin excludes those of Indian origin and refers to the racial origin of a patient group and not necessarily their place of birth.

‡ Refractory defined as recurrent or progressive disease (clinical or radiological) while receiving or within 90 days of last dose of chemotherapy.

§ From Cox regression analysis with stratification factors; HR<1 are in favor of gefitinib.

TABLE 2

Agreement Between EGFR Assessments With Dako and Zymed Antibodies Using Predefined Criteria for Protein Expression Positivity

	Zymed+ No. (%)	Zymed− No. (%)	Total No. (%)
Dako+N (%)	173 (58)	39 (13)	212 (72)
Dako−N (%)	32 (11)	52 (18)	84 (28)
Total N (%)	205 (69)	91 (31)	296 (100)

EGFR indicates epidermal growth factor receptor.

TABLE 3

Objective Response Rates, Disease Control Rates, and Survival Hazard Ratios (HRs) With Gefitinib Versus Placebo for EGFR Protein Expression (Percent of Positive Staining) With Dako PharmDx Kit

EGFR staining cutoff level	EGFR subgroup	No. of patients	No. of deaths	Survival HR (95% CI)*	Interaction test *P*	Gefitinib responders No. (%)	Placebo responders No. (%)	Gefitinib disease control rate No. (%)	Placebo disease control rate No. (%)
EGFR cutoff 1	EGFR+	271	167	0.78 (0.56–1.08)	.067	13 (8)	1 (1.5)	60 (36.8)	26 (38.8)
EGFR cutoff 1	EGFR−	108	65	1.51 (0.82–2.77)		1 (1.6)	0 (0)	18 (28.1)	10 (34.5)
EGFR cutoff 10	EGFR+	264	166	0.77 (0.56–1.08)	.049	13 (8.2)	1 (1.5)	57 (36.1)	25 (37.9)
EGFR cutoff 10	EGFR−	115	66	1.57 (0.86–2.87)		1 (1.4)	0 (0)	21 (30.4)	11 (36.7)
EGFR cutoff 20	EGFR+	249	156	0.79 (0.56–1.11)	.114	13 (8.7)	1 (1.7)	55 (36.7)	21 (35)
EGFR cutoff 20	EGFR−	130	76	1.42 (0.82–2.44)		1 (1.3)	0 (0)	23 (29.9)	15 (41.7)
EGFR cutoff 30	EGFR+	221	138	0.77 (0.53–1.11)	.129	13 (9.4)	1 (2)	54 (38.8)	16 (31.4)
EGFR cutoff 30	EGFR−	158	94	1.27 (0.8–2)		1 (1.1)	0 (0)	24 (27.3)	20 (44.4)
EGFR cutoff 40	EGFR+	198	124	0.75 (0.51–1.1)	.135	12 (9.6)	1 (2.2)	53 (42.4)	14 (30.4)
EGFR cutoff 40	EGFR−	181	108	1.24 (0.81–1.9)		2 (2)	0 (0)	25 (24.5)	22 (44)
EGFR cutoff 50	EGFR+	171	111	0.84 (0.56–1.28)	.555	10 (9.3)	1 (2.4)	43 (39.8)	14 (33.3)
EGFR cutoff 50	EGFR−	208	121	1.08 (0.72–1.63)		4 (3.4)	0 (0)	35 (29.4)	22 (40.7)
EGFR cutoff 60	EGFR+	142	94	1.01 (0.63–1.61)	.756	8 (8.7)	1 (3.1)	37 (40.2)	10 (31.3)
EGFR cutoff 60	EGFR−	237	138	0.97 (0.67–1.41)		6 (4.4)	0 (0)	41 (30.4)	26 (40.6)
EGFR cutoff 70	EGFR+	116	81	1.01 (0.62–1.65)	.802	7 (9.5)	1 (3.4)	30 (40.5)	9 (31)
EGFR cutoff 70	EGFR−	263	151	0.99 (0.69–1.41)		7 (4.6)	0 (0)	48 (31.4)	27 (40.3)
EGFR cutoff 80	EGFR+	83	60	1.01 (0.57–1.78)	.857	6 (11.5)	0 (0)	20 (38.5)	4 (21.1)
EGFR cutoff 80	EGFR−	296	172	0.96 (0.69–1.34)		8 (4.6)	1 (1.3)	58 (33.1)	32 (41.6)
EGFR cutoff 90	EGFR+	65	44	0.85 (0.43–1.68)	.760	4 (10)	0 (0)	17 (42.5)	1 (7.1)
EGFR cutoff 90	EGFR−	314	188	0.99 (0.72–1.37)		10 (5.3)	1 (1.2)	61 (32.6)	35 (42.7)
EGFR unknown	—	1313	744	0.84 (0.73–0.98)	—	63 (8.6)	5 (1.3)	303 (41.4)	118 (30.7)
All patients	—	1692	976	0.86 (0.76–0.99)	—	77 (8)	6 (1.3)	381 (39.7)	154 (32.1)

EGFR indicates epidermal growth factor receptor; CI, confidence interval.

*From Cox regression analysis with stratification factors; HR < 1 are in favor of gefitinib.

TABLE 4

Objective Response Rates, Disease Control Rates, and Survival Hazard Ratios (HRs) With Gefitinib Versus Placebo for EGFR Protein Expression (Staining Index) With Zymed Clone 31G7 Antibody

EGFR staining cutoff level	EGFR subgroup	No. of patients	No. of deaths	Survival HR (95% CI)*	Interaction test *P*	Gefitinib responders No. (%) of patients	Placebo responders No. (%) of patients	Gefitinib disease control rate No. (%) of patients	Placebo disease control rate No. (%) of patients
EGFR cutoff 50	EGFR+	311	182	0.92 (0.67–1.26)	.715	18 (9.9)	1 (1.6)	72 (39.8)	28 (32.6)
EGFR cutoff 50	EGFR−	46	25	0.61 (0.2–1.85)		1 (3)	0 (0)	15 (45.5)	3 (37.5)
EGFR cutoff 100	EGFR+	284	164	0.85 (0.61–1.18)	.483	18 (10.8)	1 (1.9)	68 (40.7)	24 (31.6)
EGFR cutoff 100	EGFR−	73	43	1.34 (0.66–2.73)		1 (2.1)	0 (0)	19 (40.4)	7 (38.9)
EGFR cutoff 150	EGFR+	267	157	0.83 (0.59–1.16)	.310	17 (10.8)	1 (2.5)	64 (40.5)	24 (34.3)
EGFR cutoff 150	EGFR−	90	50	1.32 (0.7–2.5)		2 (3.6)	0 (0)	23 (41.1)	7 (29.2)
EGFR cutoff 200	EGFR+	244	144	0.84 (0.59–1.2)	.502	16 (11)	1 (3.1)	60 (41.1)	22 (34.4)
EGFR cutoff 200	EGFR−	113	63	1.13 (0.66–1.96)		3 (4.4)	0 (0)	27 (39.7)	9 (30)
EGFR cutoff 250	EGFR+	209	123	0.8 (0.55–1.17)	.483	16 (12.6)	5 (1.3)	53 (41.7)	16 (29.6)
EGFR cutoff 250	EGFR−	148	84	1.09 (0.68–1.75)		3 (3.4%)	6 (1.3)	34 (39.1)	15 (37.5%)
EGFR cutoff 300	EGFR+	171	103	0.78 (0.51–1.19)	.582	13 (12.1)	0 (0)	46 (43)	13 (32.5)
EGFR cutoff 300	EGFR−	186	104	1.03 (0.68–1.57)		6 (5.6%)	0 (0)	41 (38.3)	18 (33.3)
EGFR cutoff 350	EGFR+	136	81	0.77 (0.47–1.26)	.659	11 (12.8)	0 (0)	37 (43)	10 (31.3)
EGFR cutoff 350	EGFR−	221	126	0.98 (0.67–1.43)		8 (6.3)	0 (0)	50 (39.1)	21 (33.9)
EGFR unknown	—	1,335	769	0.86 (0.74–1)	—	58 (7.8)	0 (0)	294 (39.5)	123 (31.9)
All patients	—	1,692	976	0.86 (0.76–0.99)	—	77 (8)	0 (0)	381 (39.7)	154 (32.1)

EGFR indicates epidermal growth factor receptor; CI, confidence interval.

*From Cox regression analysis with stratification factors; HR < 1 are in favor of gefitinib.