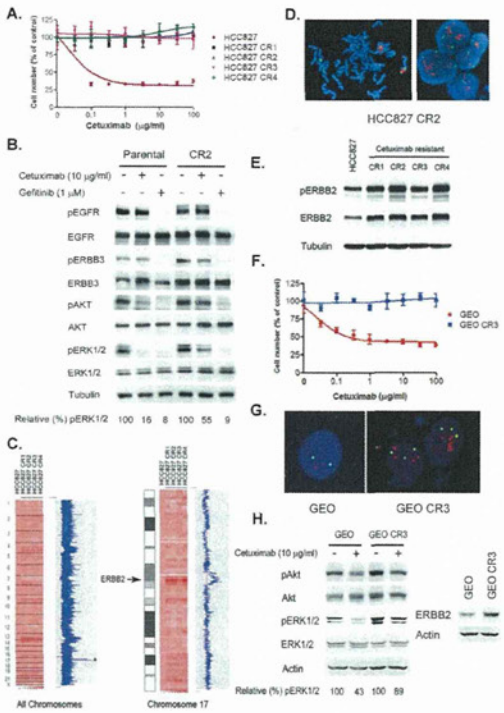

Figure 1. Cetuximab resistant NSCLC and CRC cells maintain ERK 1/2 signaling and contain an *ERBB2* amplification

A. Parental and resistant HCC827 CR cells were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **B.** Parental HCC827 and CR2 cells were treated with cetuximab (10 μg/ml) or gefitinib (1 μM) for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **C.** Amplification on chromosome 17 encompassing the *ERBB2* locus (asterisk, HCC827 CR cells). The HCC827 CR clones (right) were compared with parental HCC827 cells (first column). The blue curve on the right indicates degree of amplification of each SNP from 0 (left) to 8 (right). Left, genome wide view; right, chromosome 17. **D.** Metaphase (left) and interphase (right) fluorescence in situ hybridization (FISH) on HCC827 CR2 cells using ERBB2 (red) and CEP 17 (green) probes. The HER2/CEP17 ratio was 4.7. **E.** Expression of p-ERBB2 and ERBB2 in HCC827 and CR cells. Cell extracts were immunoblotted to detect indicated proteins. **F.** Parental and resistant GEO CR3 cells were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **G.** Interphase FISH on GEO and GEO CR3 cells using ERBB2 (red) and CEP 17 (green) probes. HER2/CEP17 ratio ≥ 2 was observed in 50% of GEO CR3 cells. **H.** (Left) Parental GEO and CR3 cells were treated with cetuximab (10 μg/ml) for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. (Right) Expression of ERBB2 in GEO and GEO CR3 cells.

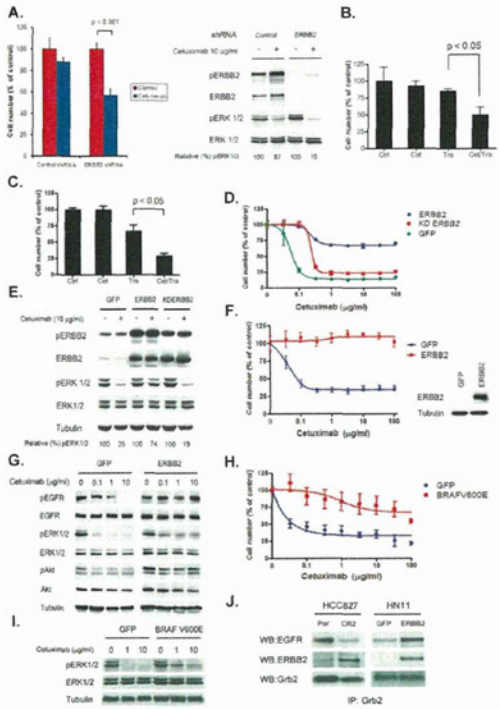

Figure 2. Inhibition of ERBB2 restores cetuximab sensitivity in cetuximab resistant cancer cell lines

A. Depletion of *ERBB2* by an ERBB2 specific shRNA restores sensitivity to cetuximab. Control and *ERBB2* shRNA treated HCC827 CR2 cells were treated with cetuximab (10 μg/ml) and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. Cell extracts were immunoblotted to detect indicated proteins. **B.** HCC827 CR2 cells were treated with cetuximab (10 μg/ml) or trastuzumab (10 μg/ml) alone or with both agents. Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **C.** GEO CR3 cells were treated with cetuximab (10 μg/ml) or trastuzumab (10 μg/ml) alone or with both agents. Viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **D.** HCC827 cells expressing either GFP, ERBB2 or kinase dead (KD) ERBB2 were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **E.** The indicated cell lines from D. were untreated or treated with cetuximab (10 μg/ml) for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **F.** HN11 cells expressing GFP or ERBB2 were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **G.** HN11 GFP and HN11 ERBB2 cells were treated with indicated concentrations of cetuximab for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **H.** HN11 cells expressing GFP or BRAFV600E were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **I.** Cells from H. were treated with indicated concentrations of cetuximab for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **J.** GRB2 co-precipitates with ERBB2 in HCC827 CR2 and HN11 ERBB2 cells. Cell extracts were immunoprecipitated with an anti-Grb2 antibody. The precipitated proteins were determined by immunoblotting with the indicated antibodies.

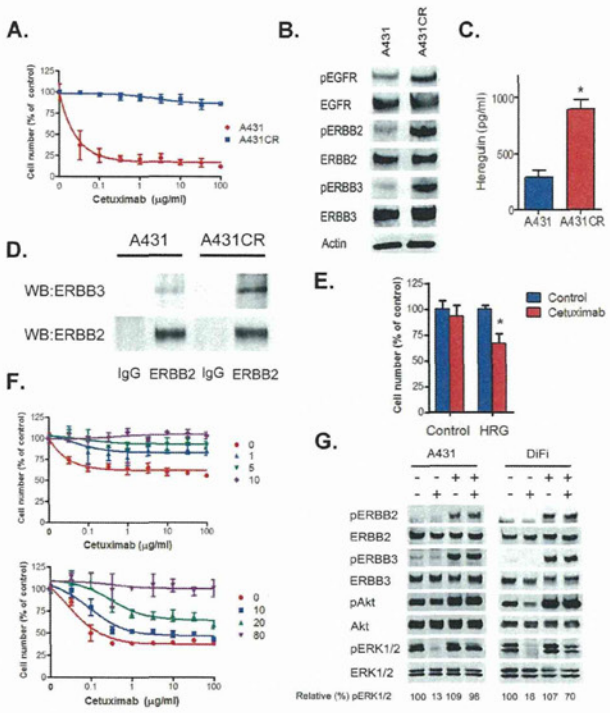

Figure 3. Heregulin causes resistance to cetuximab

A. Parental and cetuximab resistant A431 cells were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **B.** A431 CR cells have increased ERBB2 and ERBB3 phosphorylation. Cell extracts were immunoblotted to detect indicated proteins. **C.** Heregulin in cell culture medium was detected by ELISA from A431 and A431CR cells. *, p = 0.0021; t-test. **D.** A431 and A431CR cell lysates were immunoprecipitated with anti-ERBB2 antibody. ERBB2 and ERBB3 were detected by immunoblotting. **E.** Control or HRG siRNAs were transfected into A431CR cells, and cells were treated with 100 μg/ml cetuximab. The percentage of viable cells is shown (mean +/− SD) relative to untreated control. *, p = 0.0007 compared to control; t-test. **F.** A431 and DiFi cells were treated with cetuximab at the indicated concentrations in the presence of heregulin at the indicated concentrations (ng/ml). Viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. **G.** A431 and DiFi cells were treated with cetuximab (10 μg/ml) alone, heregulin alone (10 ng/ml for A431; 20 ng/ml for DiFi) or the combination. Cells were lysed, and the indicated proteins were detected by immunoblotting.

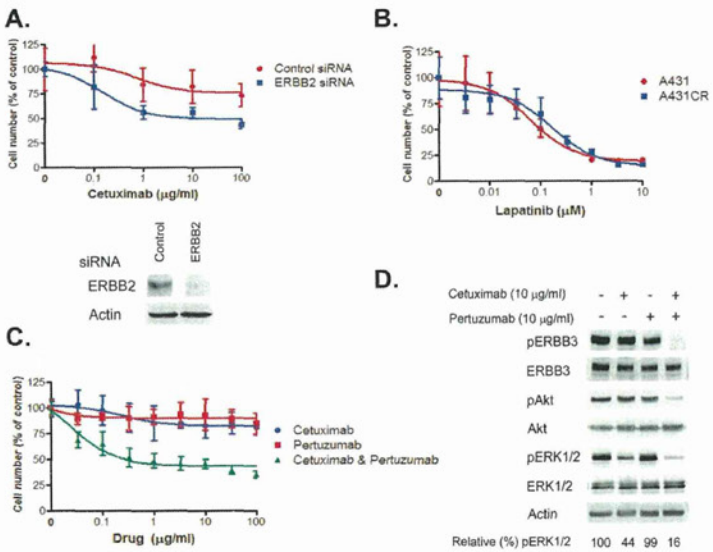

Figure 4. ERBB2 inhibition restores cetuximab sensitivity in A431 CR cells

A. Cells transfected with control or ERBB2 siRNA were treated with indicated concentrations of cetuximab. Viable cells were measured after 72 hours of treatment and plotted (mean +/− SD) relative to untreated controls. ERBB2 expression was detected by immunoblotting. **B.** A431 and A431 CR cells are equally sensitive to lapatinib. **C.** A431CR cells were treated with cetuximab alone, pertuzumab alone, or a combination of both drugs at the indicated concentrations, and viable cells were measured (mean +/− SD) after 6 days' treatment. **D.** A431CR cells were exposed to 10 μg/ml cetuximab alone, 10 μg/ml pertuzumab alone, or a combination of both drugs for 6 h. Cell extracts were immunoblotted to detect the indicated proteins.

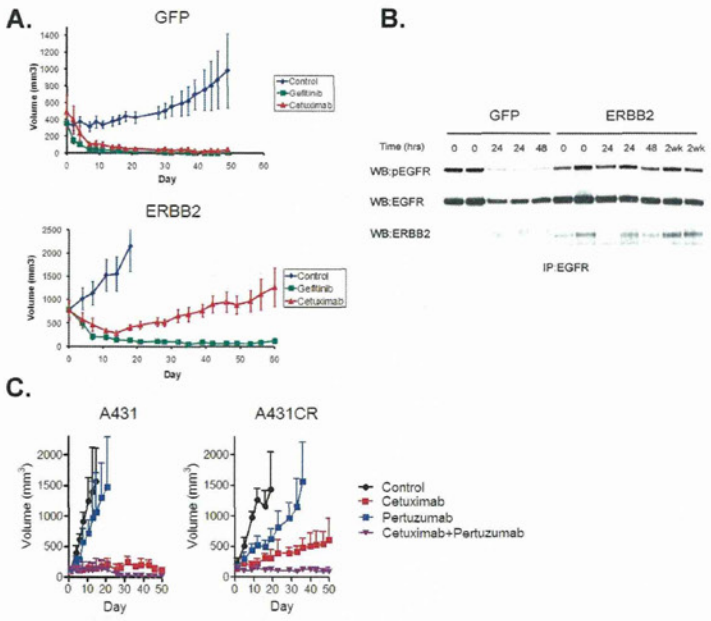

Figure 5. Both *ERBB2* amplification and heregulin cause cetuximab resistance *in vivo*

A. Xenografts generated using either HCC827 GFP or ERBB2 cells were treated with vehicle, gefitinib or cetuximab. Vehicle treated mice yielded a median tumor size of 2000 mm^3 by 15 days of treatment and were sacrificed. **B.** Cell extracts from HCC827 GFP or HCC827 ERBB2 tumors treated with cetuximab were immunoprecipitated with anti-EGFR antibody. Precipitated proteins were determined by immunoblotting with the indicated antibodies. **C.** Xenografts generated using either A431 or A431 CR cells were treated with vehicle, cetuximab alone, pertuzumab alone or the combination of cetuximab and pertuzumab.

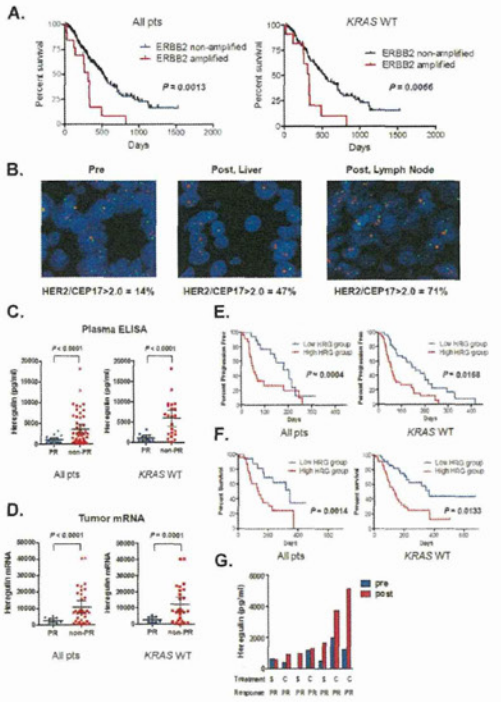

Figure 6. Both *ERBB2* amplification and heregulin cause drug resistance in cetuximab treated colorectal cancer patients

A. (Left) Overall survival for all CRC patients with (n = 13) and without *ERBB2* amplification (n = 220) treated with cetuximab based therapy. Data for *KRAS* wild type only patients (*ERBB2* amplified; n = 11; *ERBB2* non-amplified; n = 171). Comparison based on log-rank test. **B.** *ERBB2* FISH from a baseline primary tumor specimen (left) and following acquired cetuximab resistance in two independent drug resistant specimens (right). The patient was initially treated with single agent cetuximab and achieved a PR. ERBB2 (red) and CEP 17 (green). **C.** Scatter diagram of pre-treatment heregulin concentration in plasma from all (n = 65) or *KRAS* wild type only (n = 33) CRC patients achieving a PR and those not achieving a PR when treated with cetuximab based therapy. Mean ± 95% CI is shown. **D.** Scatter diagram of pre-treatment heregulin mRNA expression in tumors from all (n = 44) or *KRAS* wild type only (n = 34) CRC patients achieving a PR and those not achieving a PR when treated with cetuximab based therapy. Mean ± 95% CI is shown. **E.** (Left) Progression free survival for all CRC patients treated with cetuximab based therapy divided based on low (n = 35) or high (n = 35) plasma expression. (Right) Data for *KRAS* wild type only patients (low; n = 18; high n = 24). Comparison based on log-rank test. **F.** (Left) Overall survival for all CRC patients treated with cetuximab based therapy divided based on low (n = 35) or high (n = 35) plasma expression. (Right) Data for *KRAS* wild type only patients (low; n = 18; high n = 24). Comparison based on log-rank test. **G.** Comparisons of plasma levels of heregulin from CRC patients treated with cetuximab based therapy prior to therapy and after the development drug resistance. All patients achieved a PR. S; single agent cetuximab; C; combination with irinotecan.

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ORIGINAL REPORT

Biomarker Analyses and Final Overall Survival Results From a Phase III, Randomized, Open-Label, First-Line Study of Gefitinib Versus Carboplatin/Paclitaxel in Clinically Selected Patients With Advanced Non–Small-Cell Lung Cancer in Asia (IPASS)

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See accompanying editorial on page 2843; listen to the podcast by Dr Sequist on www.jco.org/podcast

A B S T R A C T

Purpose

The results of the Iressa Pan-Asia Study (IPASS), which compared gefitinib and carboplatin/paclitaxel in previously untreated never-smokers and light ex-smokers with advanced pulmonary adenocarcinoma were published previously. This report presents overall survival (OS) and efficacy according to epidermal growth factor receptor (EGFR) biomarker status.

Patients and Methods

In all, 1,217 patients were randomly assigned. Biomarkers analyzed were *EGFR* mutation (amplification mutation refractory system; 437 patients evaluable), *EGFR* gene copy number (fluorescent in situ hybridization; 406 patients evaluable), and EGFR protein expression (immunohistochemistry; 365 patients evaluable). OS analysis was performed at 78% maturity. A Cox proportional hazards model was used to assess biomarker status by randomly assigned treatment interactions for progression-free survival (PFS) and OS.

Results

OS (954 deaths) was similar for gefitinib and carboplatin/paclitaxel with no significant difference between treatments overall (hazard ratio [HR], 0.90; 95% CI, 0.79 to 1.02; $P = .109$) or in *EGFR* mutation–positive (HR, 1.00; 95% CI, 0.76 to 1.33; $P = .990$) or *EGFR* mutation–negative (HR, 1.18; 95% CI, 0.86 to 1.63; $P = .309$; treatment by *EGFR* mutation interaction $P = .480$) subgroups. A high proportion (64.3%) of *EGFR* mutation–positive patients randomly assigned to carboplatin/paclitaxel received subsequent EGFR tyrosine kinase inhibitors. PFS was significantly longer with gefitinib for patients whose tumors had both high *EGFR* gene copy number and *EGFR* mutation (HR, 0.48; 95% CI, 0.34 to 0.67) but significantly shorter when high *EGFR* gene copy number was not accompanied by *EGFR* mutation (HR, 3.85; 95% CI, 2.09 to 7.09).

Conclusion

EGFR mutations are the strongest predictive biomarker for PFS and tumor response to first-line gefitinib versus carboplatin/paclitaxel. The predictive value of *EGFR* gene copy number was driven by coexisting *EGFR* mutation (post hoc analysis). Treatment-related differences observed for PFS in the *EGFR* mutation–positive subgroup were not apparent for OS. OS results were likely confounded by the high proportion of patients crossing over to the alternative treatment.

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From the Kinki University School of Medicine; AstraZeneca, Osaka, Japan; Guangdong General Hospital, Guangzhou; Cancer Hospital, Chinese Academy of Medical Sciences, Beijing; State Key Laboratory in Oncology in South China, Li Ka Shing Institute of Health Science and the Sir Y.K. Pao Center for Cancer, The Chinese University of Hong Kong, Hong Kong, China; Maharaj Nakorn ChiangMai Hospital, ChiangMai University, ChiangMai; Prince of Songkla University, Songkla; Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; National Cancer Centre Singapore, Singapore; Tri-Service General Hospital; National Taiwan University Hospital and College of Medicine, Taipei, Taiwan; and AstraZeneca, Macclesfield, United Kingdom.

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

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INTRODUCTION

The epidermal growth factor receptor (EGFR) represents an important signaling pathway that regulates tumorigenesis and cell survival and is frequently overexpressed in the development and progression of non–small-cell lung cancer (NSCLC).[1-4] EGFR tyrosine kinase inhibitors (TKIs) such as gefitinib (Iressa, AstraZeneca, Macclesfield, United Kingdom) are effective in the treatment of relapsed NSCLC,[5,6] with certain clinical subgroups deriving greater clinical benefit (adenocarcinoma histology,

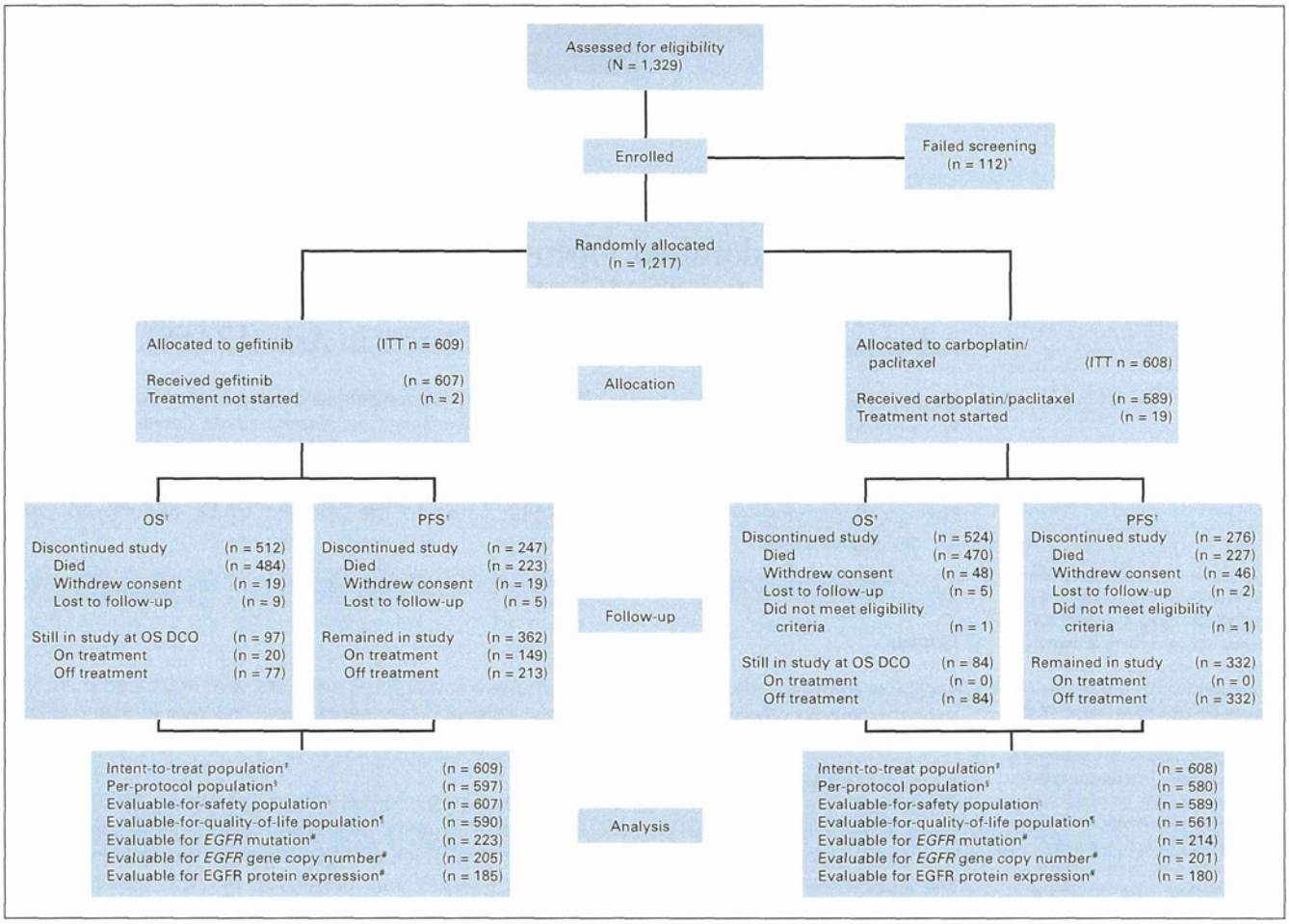

Fig 1. CONSORT diagram. (*) Among the 112 patients who failed screening, the main reasons for exclusion were abnormal serum creatinine (> 1.5 × upper limit of reference range)/creatinine clearance (≤ 60 mL/min) levels; untreated CNS metastases; or low neutrophil (< 2.0 × 10^9/L), platelet (< 100 × 10^9/L), or hemoglobin (< 10 g/dL) counts. (†) Cutoff dates: June 14, 2010, for overall survival (OS) and April 14, 2008, for progression-free survival (PFS). (‡) All patients who were randomly assigned to a study group were included in the intent-to-treat (ITT) analysis. (§) Patients who did not deviate substantially from the inclusion and exclusion criteria at entry or from the protocol were included in the per-protocol analysis. (‖) All patients who received at least one dose of study treatment were included in the safety analysis. (¶) All patients with a baseline and at least one postbaseline quality-of-life assessment that could be evaluated were included in the quality-of-life analysis. (#) All patients in the ITT population with an evaluable tumor sample. Of 683 patients (56%) who provided samples, 118 were cytology samples, and 128 were histologic samples of insufficient quality and were therefore not included in the main analysis. DCO, data cutoff; EGFR, epidermal growth factor receptor.

Asian ethnicity, female sex, and never-smoker status).[5-7] These subgroups are associated with a higher incidence of activating somatic mutations of the *EGFR* gene.[8-10] Optimization of anti-EGFR therapy depends on patient selection, and the exploration and identification of predictive biomarkers is important.

EGFR mutations, *EGFR* gene copy number, and EGFR protein expression are three EGFR-related biomarkers that have been studied in major clinical trials.[11-14] The significant overlap between EGFR biomarkers and limited availability of tumor samples in some studies made the interpretation of their individual predictive and prognostic values difficult.

Prolonged progression-free survival (PFS) and higher objective response rate (ORR) have been reported in patients with high *EGFR* gene copy number in single-arm and placebo-controlled randomized studies.[12,15-17] However, in the large phase III, randomized Iressa NSCLC Trial Evaluating Response and Survival Versus Taxotere (INTEREST) study with an active comparator, high *EGFR* gene copy number was not predictive for differential survival between gefitinib and docetaxel in patients with advanced NSCLC.[18]

The Iressa Pan-Asia Study (IPASS) is a phase III, randomized study of gefitinib versus carboplatin/paclitaxel in previously untreated never-smokers and light ex-smokers with advanced pulmonary adenocarcinoma in East Asia. As previously reported, IPASS exceeded its primary objective of noninferiority, demonstrating superiority of gefitinib relative to carboplatin/paclitaxel for PFS in this clinically selected population.[19] The treatment effect was not constant over time, driven by different outcomes according to mutation status. In the subgroup of patients with *EGFR* mutation–positive tumors, PFS was significantly longer for gefitinib versus carboplatin/paclitaxel (hazard ratio [HR], 0.48; 95% CI, 0.36 to 0.64; $P < .001$; median PFS, 9.5 *v* 6.3 months). Conversely, carboplatin/paclitaxel was superior in the *EGFR* mutation–negative subgroup (HR, 2.85; 95% CI, 2.05 to 3.98; $P < .001$; median PFS, 5.5 *v* 1.5 months); similarly, ORR significantly favored gefitinib and carboplatin/paclitaxel in the *EGFR* mutation–

Table 1. Summary of All Systemic Treatment After Discontinuation of Randomly Assigned Treatment in the Overall Population and in *EGFR* Mutation Subgroups (ITT population; data from OS data cutoff)

	Overall Population				*EGFR* Mutation Positive				*EGFR* Mutation Negative				*EGFR* Mutation Unknown			
	G (n = 609)		C/P (n = 608)		G (n = 132)		C/P (n = 129)		G (n = 91)		C/P (n = 85)		G (n = 386)		C/P (n = 394)	
Treatment	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Still on study treatment	20	3.3	0	0	3	2.3	0	0	1	1.1	0	0	16	4.1	0	0
None	190	31.2	230	37.8	29	22.0	37	28.7	21	23.1	25	29.4	140	36.3	168	42.6
Chemotherapy	393	64.5	251	41.3	99	75.0	61	47.3	69	75.8	44	51.8	225	58.3	146	37.1
Platinum-based†‡	363	59.6	55	9.0	90	68.2	13	10.1	65	71.4	10	11.8	208	53.9	32	8.1
C/P†‡	301	49.4	3	0.5	72	54.5	0	0	52	57.1	0	0	177	45.9	3	0.8
EGFR TKI	119	19.5	313	51.5	34	25.8	83	64.3	13	14.3	43	50.6	72	18.7	187	47.5
Gefitinib*†§	29	4.8	250	41.1	6	4.5	61	47.3	4	4.4	33	38.8	19	4.9	156	39.6
Erlotinib†§	71	11.7	83	13.7	16	12.1	31	24.0	9	9.9	7	8.2	46	11.9	45	11.4
Other EGFR TKI†§	33	5.4	35	5.8	15	11.4	12	9.3	2	2.2	5	5.9	16	4.1	18	4.6

NOTE. A patient may appear in more than one post-discontinuation treatment group. Patients may have received the same first- and second-line therapy. "None" is defined as patients who did not receive any form of cancer treatment after discontinuation of randomly assigned treatment. Radiotherapy, surgery, medical procedures, and other treatments were excluded.

Abbreviations: EGFR, epidermal growth factor receptor; ITT, intent-to-treat; OS, overall survival; G, gefitinib; C/P, carboplatin/paclitaxel; TKI, tyrosine kinase inhibitor.

*Non–study medication after discontinuation of randomly assigned study treatment.

†Patients may have also received other chemotherapy and/or EGFR TKIs during the study.

‡Excludes single platinum-based chemotherapy.

§Patients may have had more than one type of EGFR TKI and are counted once for each type received.

positive and *EGFR* mutation–negative subgroups, respectively.[19] A total of 1,038 of 1,217 patients consented to the preplanned exploratory biomarker analyses; 683 patients provided samples.

Early analysis of survival data (37% maturity) was presented in 2008.[19] Here we present the final results of the survival analyses and the results of the preplanned and post hoc analyses of the relationships between EGFR biomarkers (*EGFR* mutation, *EGFR* gene copy number, and EGFR protein expression) and clinical outcomes from IPASS.

PATIENTS AND METHODS

Study Design and Treatment

Full details of IPASS have been published previously.[19] Eligible patients had stage IIIB to IV pulmonary adenocarcinoma (including bronchoalveolar carcinoma), were either never-smokers (< 100 cigarettes in their lifetime) or light ex-smokers (stopped smoking ≥ 15 years previously and smoked ≤ 10 pack-years), and had received no prior chemotherapy or biologic or immunologic therapy.

Patients were randomly assigned 1:1 to gefitinib (250 mg/d) or carboplatin/paclitaxel (Paraplatin/Taxol, Bristol-Myers Squibb, Princeton, NJ; paclitaxel 200 mg/m^2 was given intravenously over 3 hours on day 1, immediately followed by carboplatin area under the serum concentration-time curve [AUC] 5.0 or 6.0 intravenously over 15 to 60 minutes in once every 3 weeks cycles for ≤ six cycles).

The primary objective of IPASS was noninferiority of gefitinib relative to carboplatin/paclitaxel in terms of PFS. ORR and overall survival (OS) were secondary end points. Evaluation of biomarker status (*EGFR* mutation, gene copy number, and protein expression) and efficacy of gefitinib versus carboplatin/paclitaxel were preplanned exploratory objectives. Post hoc analyses included clinical outcomes according to *EGFR* mutation subtype, *EGFR* gene copy number by *EGFR* mutation status, and clinical outcomes for patients with tumor *EGFR* gene high polysomy, and *EGFR* gene amplification. Correlation between *EGFR* mutation status and *EGFR* gene copy number was also investigated.

Patients provided written, informed consent with separate consent obtained for optional provision of tumor material 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 status was determined by analyzing paraffin-embedded archival tumor tissue in the following priority order: (1) *EGFR* mutation status, (2) *EGFR* gene copy number, (3) EGFR protein expression. Analyses were conducted at two central laboratories (Genzyme, Framingham, MA, and Quintiles-Lab in association with Peking Union Medical College Hospital, Beijing, China); scientists were blinded to clinical outcome and randomly assigned treatment. Samples underwent central histopathologic review; only those considered suitable for downstream biomarker analysis were progressed (on the basis of quality, sample source, and tumor content). If a patient provided more than one sample, the appropriate section was selected before database lock and analyzed on the basis of sample quality and largest area of tumor tissue.[20]

EGFR mutations were detected by using an amplification mutation refractory system with an *EGFR* mutation detection kit (DxS, Manchester, United Kingdom).[21,22] Patients were considered *EGFR* mutation positive if at least one of 29 *EGFR* mutations (Data Supplement) was detected. Additional validation for samples with T790M mutations was performed by using three methods: DNA sequencing, multithreaded electronic polymerase chain reaction sequencing, and an alternative amplification mutation refractory system assay (Data Supplement). *EGFR* gene copy number was measured by using fluorescent in situ hybridization and a previously published methodology.[15] High *EGFR* gene copy number was defined according to the University of Colorado Scoring System, which included both high polysomy (≥ four copies in ≥ 40% of cells; score 5) or gene amplification (presence of tight *EGFR* gene clusters and a ratio of gene/chromosome per cell ≥ two, or ≥ 15 copies of *EGFR* per cell in ≥ 10% of analyzed cells; score 6).[15] EGFR protein expression was assessed by immunohistochemistry by using the DAKO EGFR pharmDx kit (Dako, Glostrup, Denmark). Positive EGFR protein expression status was defined as having ≥ 10% of cells stained.

Statistical Analyses

The study statistician performed the statistical analyses at AstraZeneca. In the overall population and clinical subgroups, OS was analyzed by using a Cox proportional hazards model adjusted for the same covariates as for the primary PFS analysis (WHO performance status, 0 to 1 *v* 2; smoking history, never-smoker *v* light ex-smoker; and sex, female *v* male). The HR (gefitinib: carboplatin/paclitaxel) was estimated with 95% CIs and *P* values. Final analysis of OS was planned for when 944 deaths (78%) had occurred in the intent-to-treat (ITT) population, the same level of maturity as for PFS.

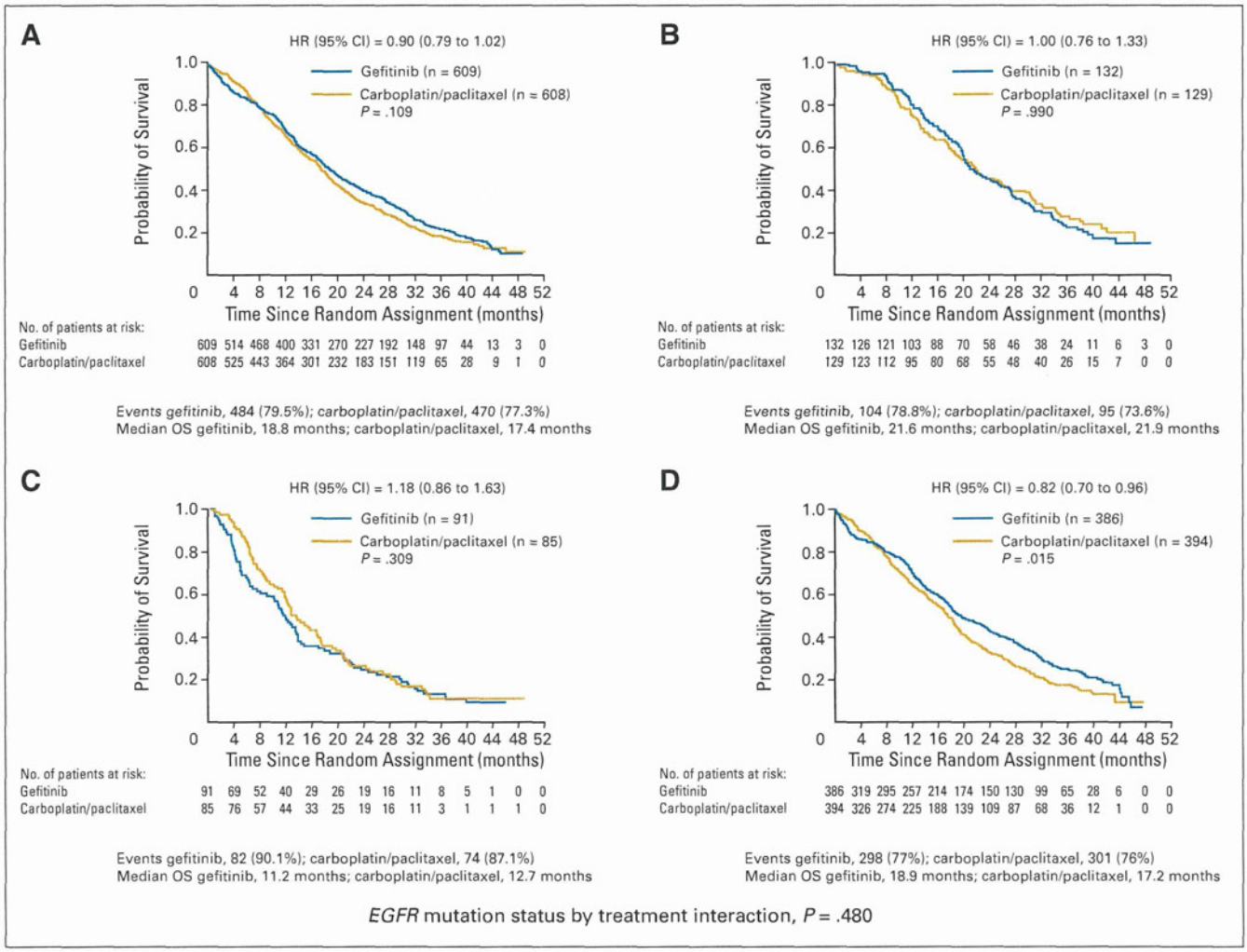

Fig 2. Kaplan-Meier curves for overall survival (OS) in the overall population and by epidermal growth factor receptor (*EGFR*) mutation status (intent-to-treat population). Hazard ratio (HR) < 1 implies a lower risk of death for patients treated with gefitinib. Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex). (A) Overall population. (B) Patients with *EGFR* mutation–positive tumors. (C) Patients with *EGFR* mutation–negative tumors. (D) Patients with *EGFR* mutation status unknown tumors.

For each biomarker, patients were classified as positive, negative, or unknown. For each of these groups, HRs, 95% CIs, and *P* values were estimated for PFS and OS (by using a Cox proportional hazards model adjusted for the same covariates as for the primary PFS analysis in the ITT population). The biomarker status by randomly assigned treatment interaction was assessed individually for each biomarker for PFS and OS by using a Cox proportional hazards model adjusted for randomly assigned treatment, biomarker status (positive or negative), and the biomarker status by treatment interaction by using a 10% significance level to indicate potential predictive factors for gefitinib versus carboplatin/paclitaxel. When there were fewer than 20 events in a subgroup for PFS or OS, only descriptive summaries were produced. Odds ratios, 95% CIs, and *P* values were estimated for ORRs by using a logistic regression model adjusted for the same covariates as those used in the analysis of PFS in the ITT population.

RESULTS

Patients

Patient disposition is presented in Figure 1. Therapies received postdiscontinuation of randomly assigned treatment are listed in Table 1. Specifically, 83 (64.3%) of 129 patients with *EGFR* mutation–positive tumors randomly assigned to carboplatin/paclitaxel received subsequent EGFR TKIs.

OS (ITT Population)

The median duration of follow-up for OS was 17.0 months. At the time of data cutoff for OS (June 14, 2010), 954 patients (78%) had died (Fig 2A). In the overall population, OS was similar for gefitinib and carboplatin/paclitaxel with no significant difference between treatments (484 and 470 events, respectively; HR, 0.90; 95% CI, 0.79 to 1.02; $P = .109$; median OS for gefitinib, 18.8 months *v* 17.4 months for carboplatin/paclitaxel; Fig 2A). A consistent treatment effect was seen across all clinical subgroups (Fig 3C).

Biomarker Evaluations

Of 683 randomly assigned patients (56.1%) who provided samples for biomarker analysis, 118 were cytology samples, which were not included in the main analysis. The number of patients with an evaluable status was 437 (35.9%) for *EGFR* mutation, 406 (33.4%) for

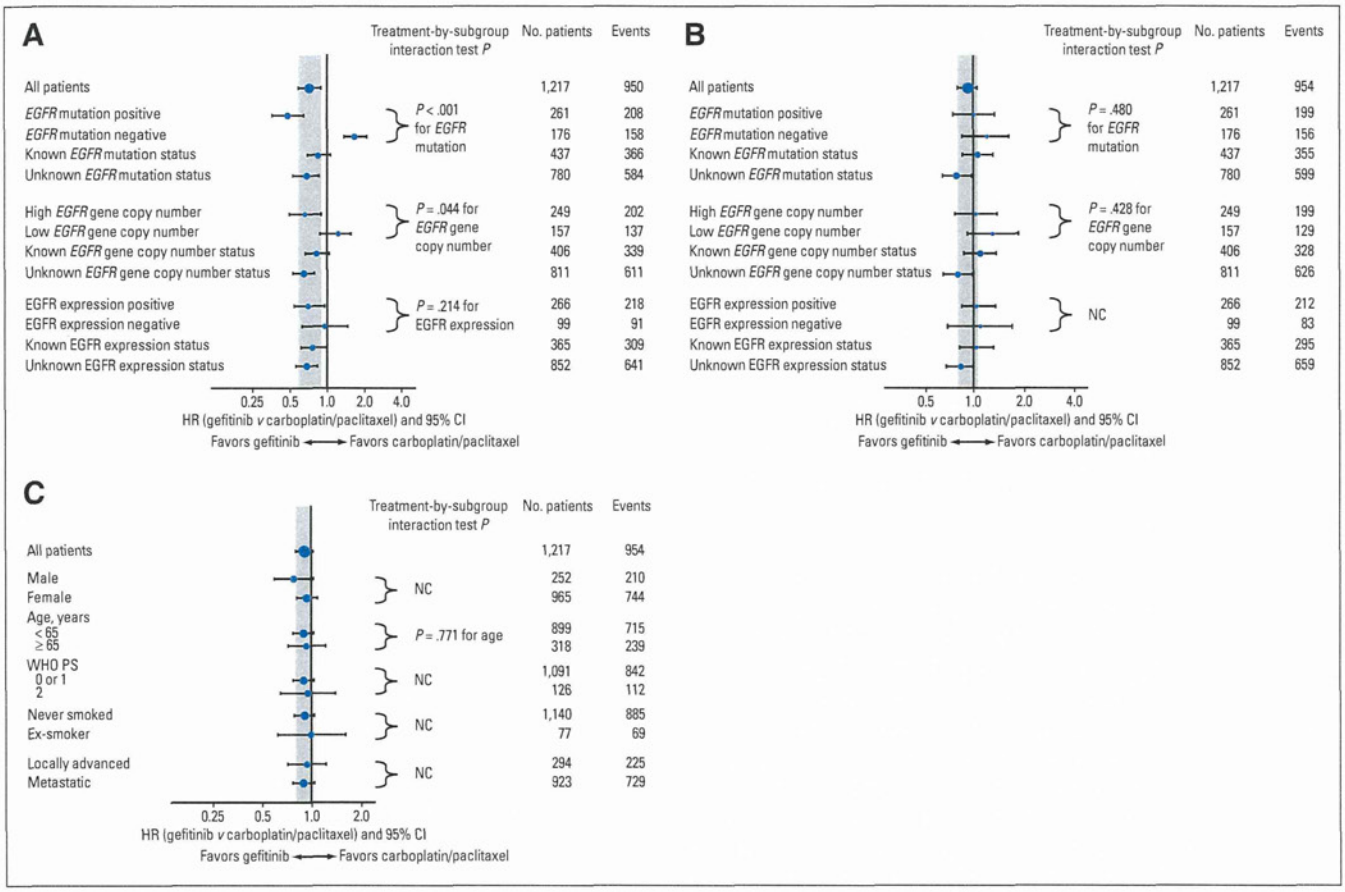

Fig 3. Forest plot of progression-free survival (PFS) and overall survival (OS) by epidermal growth factor receptor (EGFR) mutation status, gene copy number, and protein expression status (intent-to-treat population). (A) PFS by biomarker status. (B) OS by biomarker status. (C) OS by clinical subgroup. Hazard ratio < 1 implies a lower risk of progression or death for patients treated with gefitinib. The size of the point estimate reflects the number of events in the subgroup, with a larger circle indicating more events. Cox analysis with covariates (performance status [PS], 0 to 1 or 2; smoking history, never-smoker, light ex-smoker; and sex). OS by biomarker status; no formal adjustment for multiple testing was made, therefore, statistical significance at the traditional 5% level (95% CI < 1) cannot be claimed. Protocoled interaction tests were calculated only for OS and clinical subgroups if there was a significant interaction test for PFS. NC, not calculated.

EGFR gene copy number, and 365 (30.0%) for EGFR protein expression (Fig 1); the percentage of patients with a positive EGFR biomarker status was 59.7% (261 of 437), 61.3% (249 of 406), and 72.9% (266 of 365), respectively. A summary of EGFR biomarker status is presented in the Data Supplement.

The demographics, baseline characteristics, and efficacy results of patients with evaluable samples for assessment of *EGFR* mutation status, gene copy number, and protein expression were generally comparable with the ITT population (Table 2). There was a high degree of overlap between patients who were positive for all three biomarkers; 190 patients (78.5%) with high *EGFR* gene copy number also harbored an *EGFR* mutation; 132 patients were positive for all three biomarkers.

EGFR *Mutation Status*

Demographic and baseline characteristics by *EGFR* mutation status are shown in the Data Supplement. PFS results by *EGFR* mutation status have been previously published[19] (Fig 3A).

There was no differential treatment effect for OS by *EGFR* mutation (treatment by *EGFR* mutation interaction test $P = .480$). There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in the subgroups of patients with *EGFR* mutation–positive tumors (104 and 95 events, respectively; HR, 1.00; 95% CI, 0.76 to 1.33; $P = .990$; median OS, 21.6 *v* 21.9 months); *EGFR* mutation–negative tumors (82 and 74 events, respectively; HR, 1.18; 95% CI, 0.86 to 1.63; $P = .309$; median OS, 11.2 *v* 12.7 months), or mutation status unknown tumors (298 and 301 events, respectively; HR, 0.82; 95% CI, 0.70 to 0.96; $P = .015$; Figs 2B, 2C, 2D, and 3B). Postdiscontinuation treatments by *EGFR* mutation status are listed in Table 1.

EGFR *Gene Copy Number*

EGFR gene copy number was a predictive biomarker for the effect of gefitinib compared with carboplatin/paclitaxel on PFS (treatment by *EGFR* gene copy number interaction test $P = .044$; Fig 3A). In patients with high *EGFR* gene copy number (fluorescent in situ hybridization scores 5 and 6; n = 249), PFS was significantly longer with gefitinib versus carboplatin/paclitaxel (HR, 0.66; 95% CI, 0.50 to 0.88; $P = .005$). ORR also significantly favored gefitinib in these patients (58.9% *v* 44.8% for gefitinib *v* carboplatin/paclitaxel, respectively; odds ratio [OR], 1.79; 95% CI, 1.08 to 2.96; $P = .024$). Conversely, in

Table 2. Demographics, Baseline Characteristics, and Analysis Outcomes for Patients with Evaluable Tissue Samples for Each Biomarker Compared With the ITT Population

	Evaluable for *EGFR* Mutation Status* (n = 437)					Evaluable for *EGFR* Gene Copy Number Status* (n = 406)					Evaluable for EGFR Protein Expression Status* (n = 365)					ITT Population (n = 1,217)				
Variable	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI	No.	%	HR	OR	95% CI
Demographic characteristic																				
Female	335	76.7				313	77.1				285	78.1				965	79.3			
Age < 65 years	326	74.6				303	74.6				262	71.8				899	73.9			
WHO PS 0 or 1	402	92.0				375	92.4				334	91.5				1,091	89.6			
Never-smoker	405	92.7				375	92.4				334	91.5				1,140	93.7			
Locally advanced	83	19.0				77	19.0				67	18.4				295	24.2			
Efficacy																				
PFS			0.85		0.69 to 1.06			0.83		0.66 to 1.03			0.79		0.62 to 0.99			0.74		0.65 to 0.85
ORR				1.21	0.83 to 1.78				1.31	0.88 to 1.95				1.43	0.94 to 2.18				1.59	1.25 to 2.01
OS			1.05		0.85 to 1.29			1.10		0.89 to 1.37			1.04		0.82 to 1.30			0.90		0.79 to 1.02

NOTE. Hazard ratio (HR) < 1 implies a lower risk of progression or death on gefitinib; odds ratio (OR) > 1 implies a greater chance of response on gefitinib.
Abbreviations: ITT, intent to treat; EGFR, epidermal growth factor receptor; PS, performance status; PFS, progression-free survival; ORR, objective response rate; OS, overall survival.
*Irrespective of whether positive or negative for each biomarker.

patients with low *EGFR* gene copy number (n = 157), PFS was numerically longer (HR, 1.24; 95% CI, 0.87 to 1.76; $P = .237$) and ORR was numerically higher (26.3% *v* 22.2%; OR, 0.80; 95% CI, 0.38 to 1.68; $P = .558$) with carboplatin/paclitaxel versus gefitinib.

A total of 190 patients (78%) with high *EGFR* gene copy number also harbored *EGFR* mutations. Of the 153 patients with low *EGFR* gene copy number, only 51 (33%) were also *EGFR* mutation positive. Post hoc analyses found that PFS was significantly shorter with gefitinib versus carboplatin/paclitaxel in patients with high *EGFR* gene copy number in the absence of a coexisting *EGFR* mutation (n = 55; HR, 3.85; 95% CI, 2.09 to 7.09), although patients with *EGFR* mutation achieved significantly longer PFS with gefitinib versus carboplatin/paclitaxel irrespective of whether they had high (HR, 0.48; 95% CI, 0.34 to 0.67; n = 190) or low (HR, 0.51; 95% CI, 0.25 to 1.04; n = 51) *EGFR* gene copy number (Figs 4A to 4D).

There was no differential treatment effect for OS by *EGFR* gene copy number (treatment by *EGFR* gene copy number interaction test $P = .428$). There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in patients with high *EGFR* gene copy number (104 and 95 events, respectively; HR, 1.03; 95% CI, 0.78 to 1.37; $P = .816$) or low *EGFR* gene copy number (67 and 62 events, respectively; HR, 1.30; 95% CI, 0.92 to 1.85; $P = .137$; Fig 3B).

EGFR Protein Expression

There was no differential treatment effect for PFS by EGFR protein expression (treatment by EGFR protein expression status interaction test $P = .214$; Fig 3A). PFS was significantly longer for gefitinib versus carboplatin/paclitaxel in patients with EGFR protein expression–positive tumors (HR, 0.73; 95% CI, 0.55 to 0.96; $P = .024$; n = 266). There was no significant difference in PFS between treatments in patients with EGFR protein expression–negative tumors (HR, 0.97; 95% CI, 0.64 to 1.48; $P = .893$; n = 99).

ORRs were similar between the gefitinib and carboplatin/paclitaxel groups for patients with either EGFR protein expression–positive (51.5% *v* 41.8%; OR, 1.49; 95% CI, 0.92 to 2.42; $P = .109$) or EGFR protein expression–negative (34.0% *v* 26.1%; OR, 1.44; 95% CI, 0.60 to 3.47; $P = .415$) tumors.

There was no significant difference in OS for gefitinib versus carboplatin/paclitaxel in patients with EGFR protein expression–positive (107 and 105 events, respectively; HR, 1.05; 95% CI, 0.80 to 1.37; $P = .731$) or EGFR protein expression–negative (46 and 37 events, respectively; HR, 1.09; 95% CI, 0.70 to 1.70; $P = .692$) tumors.

Activating EGFR *Mutation Type*

Of the 261 patients with *EGFR* mutation–positive tumors, 53.6% (n = 140) had tumors with exon 19 deletions, and 42.5% (n = 111) had exon 21 L858R mutations (Data Supplement); demography was generally similar between these groups (Data Supplement).

In post hoc analyses, PFS was significantly longer for gefitinib versus carboplatin/paclitaxel in both the exon 19 deletions (HR, 0.38; 95% CI, 0.26 to 0.56) and the exon 21 L858R mutation (HR, 0.55; 95% CI, 0.35 to 0.87; Figs 5A and 5B) subgroups. Within-treatment analysis indicated no significant difference in PFS with gefitinib in the exon 19 deletions versus exon 21 L858R mutation subgroup (HR, 0.78; 95% CI, 0.51 to 1.19). ORR was significantly higher with gefitinib (84.8%) versus carboplatin/paclitaxel (43.2%; OR, 7.23; 95% CI, 3.19 to 16.37) in the exon 19 deletions subgroup and higher (but not statistically significant) in the L858R subgroup (60.9% *v* 53.2%; OR, 1.41; 95% CI, 0.65 to 3.05).

DISCUSSION

Gefitinib showed similar OS to doublet chemotherapy with no significant difference in the overall population or in patients with *EGFR* mutation–positive or *EGFR* mutation–negative status. The significant treatment-related differences for PFS and ORR according to *EGFR* mutation status were not observed for OS. Although there may be other contributing factors, the subsequent treatments that patients received are likely to have confounded the true effect of the initial, randomized first-line treatment on OS. Of the *EGFR* mutation–positive subgroup randomly assigned to carboplatin/paclitaxel, 64.3% received EGFR TKIs postdiscontinuation. Fewer patients with unknown mutation status randomly assigned to carboplatin/paclitaxel received EGFR TKIs (47.5%) compared with patients with *EGFR* mutation–positive status (64.3%), which may potentially contribute to the numerical trend in favor of gefitinib in this subgroup; statistical significance at the traditional 5% level ($P < .05$) cannot be claimed because no adjustment was made for multiple testing. The First-SIGNAL study had a study design similar to that of IPASS[23] and

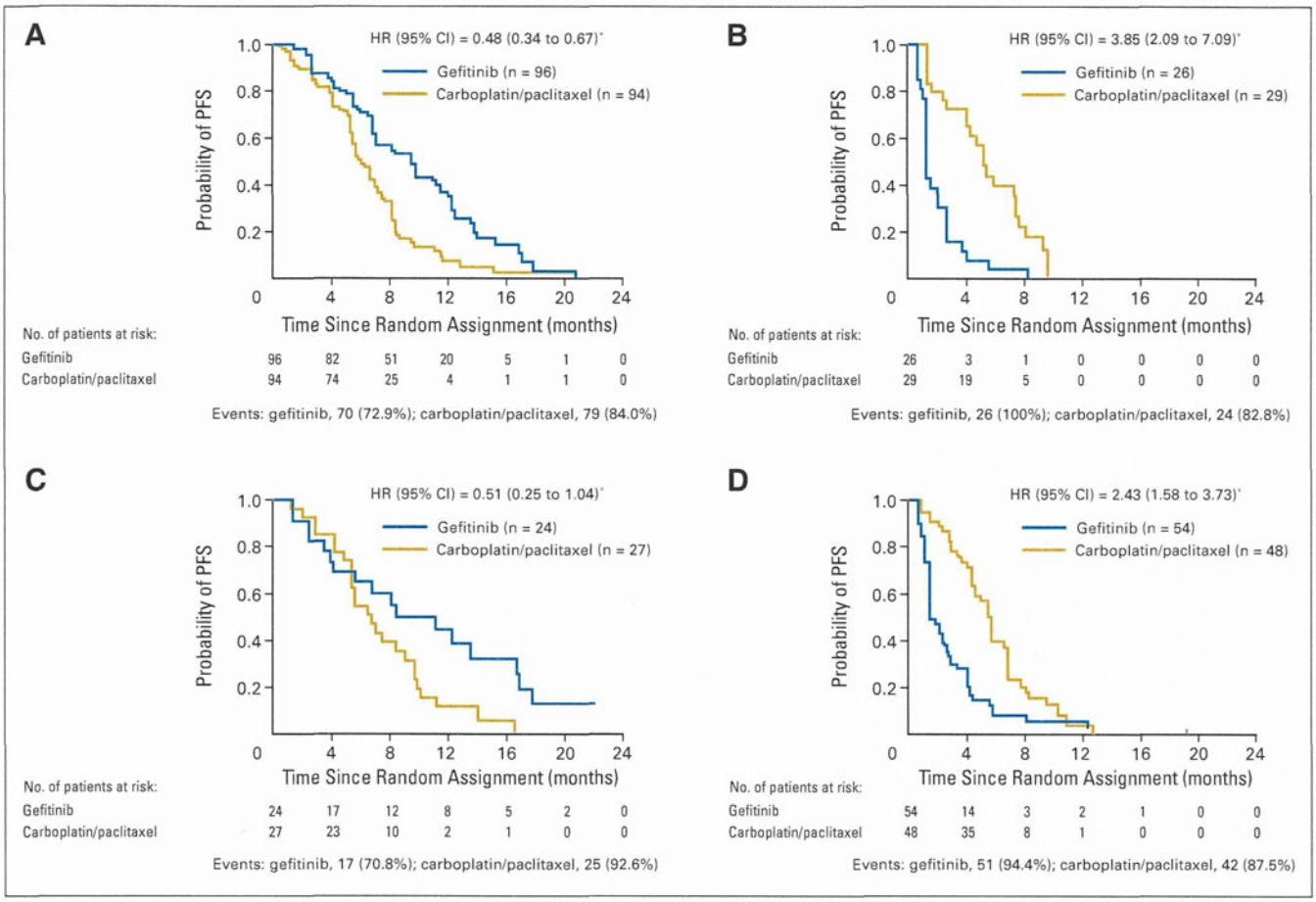

Fig 4. Kaplan-Meier curves for progression-free survival (PFS) by epidermal growth factor receptor (*EGFR*) mutation status and *EGFR* gene copy number. Hazard ratio (HR) < 1 implies a lower risk of progression/death for patients treated with gefitinib. (A) High *EGFR* gene copy number *EGFR* mutation–positive. (B) High *EGFR* gene copy number *EGFR* mutation–negative. (C) Low *EGFR* gene copy number *EGFR* mutation–positive. (D) Low *EGFR* gene copy number *EGFR* mutation–negative. (*) Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex).

reported no significant difference in OS (primary end point) between gefitinib versus gemcitabine/cisplatin (overall population, 182 events; 59% maturity; mutation-positive HR, 0.82; 95% CI, 0.35 to 1.92; $P = .648$; median survival, 30.6 *v* 26.5 months, respectively). The randomized Japanese NEJ002 study also reported that OS did not differ significantly between gefitinib and carboplatin/paclitaxel in patients selected by *EGFR* mutation status (median survival, 30.5 *v* 23.6 months, respectively; $P = .31$), likely explained by treatment crossover.[24]

Although collection of tumor material was not mandatory or feasible in all patients, IPASS has the largest group of patients with *EGFR* mutation–positive tumors studied in a randomized controlled trial in NSCLC and has confirmed *EGFR* mutation to be the strongest predictive biomarker for the effect of gefitinib with a statistically significant interaction test for PFS. Patients with mutation-negative tumors have a poorer outcome in terms of PFS and ORR with gefitinib compared with carboplatin/paclitaxel, indicating that in the first-line setting, gefitinib should not be used in preference to doublet chemotherapy in patients with a negative mutation status.

Our findings were broadly consistent with those of previous first-line, single-arm studies of gefitinib in patients with *EGFR* mutation–positive tumors.[25-32] Recently, outcomes similar to those of IPASS among patients with *EGFR* mutation–positive tumors have been reported in two randomized phase III studies[24,33] comparing first-line gefitinib with doublet chemotherapy, with PFS as the primary end point. The NEJ002 study prospectively randomly assigned 230 patients with *EGFR* mutation–positive tumors to gefitinib or carboplatin/paclitaxel. PFS favored gefitinib over carboplatin/paclitaxel (PFS HR, 0.30; 95% CI, 0.22 to 0.41; $P < .001$; median PFS, 10.8 *v* 5.4 months; tumor response rate, 73.7% *v* 30.7%, respectively; $P < .001$).[24] The similarly designed West Japan Thoracic Oncology Group 3405 (WJTOG3405) study reported increased PFS with gefitinib over cisplatin/docetaxel in 172 patients with *EGFR* mutation–positive tumors (PFS HR, 0.49; 95% CI, 0.34 to 0.70; $P < .001$; median PFS, 9.2 *v* 6.3 months; 295 events; 95% maturity).[33] Tumor response rates (n = 117) were 62.1% and 32.2%. In the First-SIGNAL study, PFS (secondary end point) increased with gefitinib compared with gemcitabine/cisplatin in 42 patients with *EGFR* mutation–positive tumors (PFS HR, 0.61; 95% CI, 0.31 to 1.22; $P = .084$; median PFS, 8.4 *v* 6.7 months).[23] The OPTIMAL study compared erlotinib with gemcitabine/cisplatin in 154 patients with *EGFR* mutation–positive tumors and also reported a significant difference in PFS (HR, 0.16; 95% CI, 0.10 to 0.26; $P = .001$).[34] The similarly designed European Tarceva

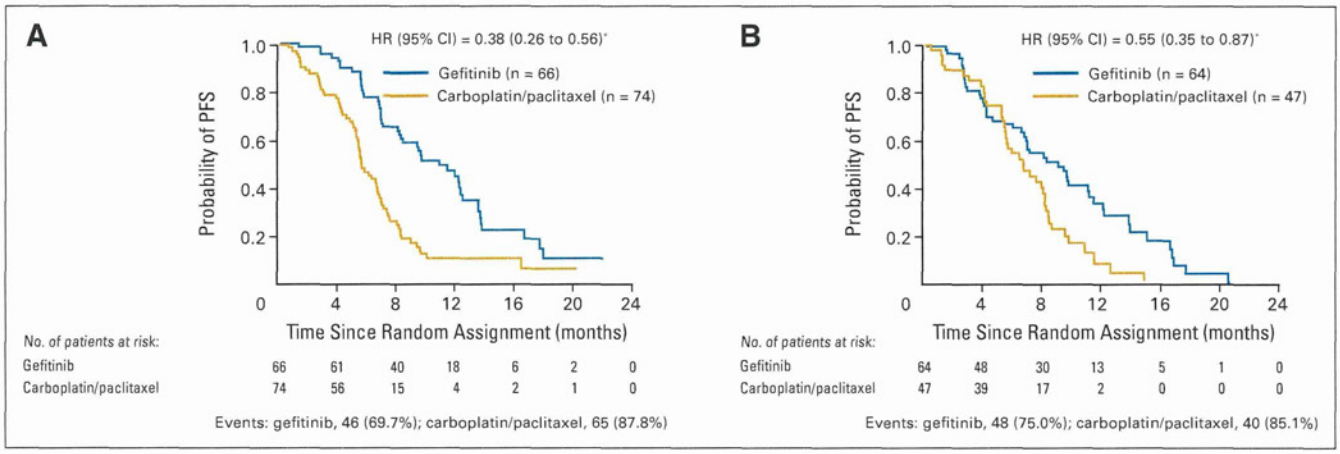

Fig 5. Kaplan-Meier curves for progression-free survival (PFS) by epidermal growth factor receptor (*EGFR*) mutation type (intent-to-treat population). Hazard ratio (HR) < 1 implies a lower risk of progression/death for patients treated with gefitinib. (A) Exon 19 deletion. (B) L858R. (*) Cox analysis with covariates (performance status [0-1, 2], smoking history [never, light ex-smoker], and sex).

versus Chemotherapy (EURTAC) study is ongoing. Therefore to date, including IPASS, five randomized studies have shown that EGFR TKIs offer significant benefits over standard chemotherapy in patients with *EGFR* mutation–positive tumors.

In IPASS, high *EGFR* gene copy number was predictive for the effect of gefitinib versus carboplatin/paclitaxel on PFS. The significantly longer PFS with gefitinib in patients with both high *EGFR* gene copy number and *EGFR* mutation–positive tumors was not observed in patients with high *EGFR* gene copy number without an accompanying mutation, suggesting that the apparent PFS benefit was driven by overlap with a coexisting *EGFR* mutation (77.6% of patients with high *EGFR* gene copy number also had *EGFR* mutation–positive tumors). Patients with *EGFR* mutation–positive tumors without accompanying high *EGFR* gene copy number showed longer PFS with gefitinib than with carboplatin/paclitaxel, suggesting that *EGFR* mutations determine the treatment outcomes independent of the status of *EGFR* gene copy number.

Post hoc analyses of PFS by *EGFR* mutation type showed that PFS was significantly longer for gefitinib than for carboplatin/paclitaxel in both the exon 19 deletions and exon 21 L858R subgroups, with a slightly greater advantage in the exon 19 deletions subgroup. First-line, single-arm studies[35,36] have reported an increased response to EGFR TKIs in patients with exon 19 deletions *v* exon 21 L858R mutation. However, IPASS (HR, 0.78; 95% CI, 0.51 to 1.19), WJTOG3405 (HR, 1.13; 95% CI, 0.63 to 2.03; $P = .681$), and NEJ002 (11.5 *v* 10.8 months; $P = .90$) randomized phase III studies and the prospective phase II iTARGET study ($P = .600$) showed no significant difference in PFS for gefitinib between the exon 19 deletions and exon 21 L858R mutation subgroups.[24,25,33]

In summary, *EGFR* mutation was the strongest predictive biomarker for benefit of gefitinib over carboplatin/paclitaxel on PFS and ORR. Post hoc analyses suggested that the predictive value of *EGFR* gene copy number for PFS benefit with gefitinib was driven by the overlap of high *EGFR* gene copy number with a positive *EGFR* mutation status. Treatment-related differences for PFS seen in patients with a positive *EGFR* mutation status were not apparent for OS. The OS results were likely confounded by the high proportion of patients receiving different types of subsequent therapies and, in particular, crossing over to the alternative treatment.

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.

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EPIDEMIOLOGY

Economic evaluation of the 21-gene signature (Oncotype DX®) in lymph node-negative/positive, hormone receptor-positive early-stage breast cancer based on Japanese validation study (JBCRG-TR03)

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Abstract The 21-gene signature is validated as a good predictor of recurrence for lymph node-negative/positive, hormone receptor-positive, early-stage breast cancer in Japanese patient population. This study evaluates the cost-effectiveness of two scenarios designed to include the assay into Japan's social health insurance benefit package: one for LN−, ER+, ESBC and another for LN−/+, ER+, ESBC. An economic decision tree and Markov model under Japan's health system from the societal perspective is constructed with new evidence from the Japanese validation study. Incremental cost-effectiveness ratios are estimated as ¥384,828 (US$3,848) per QALY for the indication for LN− scenario and ¥568,533 (US$5,685) per QALY for the indication for LN−/+ scenario. Both are not more than the suggested social willingness-to-pay for one QALY gain from an innovative medical intervention in Japan, ¥5,000,000/QALY (US$50,000/QALY). Sensitivity analyses show that this result is plausibly robust, since ICERs do not exceed the threshold by various changes of assumptions made and values employed. In conclusion, the inclusion of the assay in Japan's social health insurance benefit package for not only LN− diseases but also LN+ diseases is cost-effective. Such a decision can be justifiable as an efficient use of finite resources for health care.

Keywords Adjuvant therapy · Breast cancer · Cost-effectiveness · Gene diagnosis · 21-Gene signature

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Introduction

Hormone receptor-positive diseases have a large share in breast cancer, which amount to 75.6% in Japan [1]. And among those, approximately two thirds of them are node-negative diseases [1]. After the primary surgery on these cases, a difficult clinical decision must be made about whether to combine adjuvant chemotherapy with endocrine therapy. Whereas the effectiveness of adjuvant endocrine therapy has been established [2], the use of adjuvant chemotherapy in node-negative, hormone receptor-positive disease is still under debate [3].

The 21-gene reverse transcriptase-polymerase chain reaction assay with a patented algorithm (Oncotype DX® Breast Cancer Assay) was developed to individualise adjuvant therapy for lymph node-negative (LN−), estrogen receptor-positive (ER+), early stage breast cancer (ESBC) patients. Studies evaluating the ability of the assay to predict the risk of recurrence [4], the benefit of chemotherapy [5] and the risk of death [6] suggest that patients with LN−, ER+, ESBC and a low recurrence score (RS) produced by the assay may need adjuvant endocrine therapy only, while those with an intermediate or a high RS may require additional treatment with chemotherapy. The

assay has been included in the 2007 American Society of Clinical Oncology (ASCO) Guidelines on Use of Tumor Markers in Breast Cancer [7]. It has also been included in the National Comprehensive Cancer Network (NCCN) 2008 Breast Cancer Treatment Guidelines [8]. Additionally, further studies suggest its clinical usefulness for lymph node-positive (LN+) diseases [9, 10].

One of the notable attributes of the assay is its high cost: ¥450,000 (US$4,500; US$1=¥100). Coupled with its potential cost-saving effect by avoiding expensive but ineffective and highly toxic chemotherapy, the economic evaluation of the assay have aroused great interests among health managers and oncologists. Cost-effectiveness analyses regarding LN− diseases have been reported from the U.S. [11, 12], Israel [13] and Canada [14]. We reported the cost-effectiveness of the assay for LN−, ER+, ESBC under Japan's health system elsewhere [15], in which modelling was based on evidences found in the U.S. validation studies [4, 5]. This was because they were the best available evidences at the time of the study. Recently, clinical significance of the assay has been reported in Japanese population [16], which is also the first evidence regarding Asian population. The study analysed not only LN− diseases but also LN+ diseases. In this study, we make a revision of our economic model with that new evidence reported by the Japanese validation study, which should improve the accuracy of cost-effectiveness of the assay among LN− diseases in Japan judged by the incremental cost-effectiveness ratio (ICER), while presenting the first report of cost-effectiveness to include LN+ diseases. The results would be of help in considering the inclusion of the assay in the benefit package of Japan's social health insurance, as well as interesting to health managers in considering expanding the assay's indication to LN+ diseases.

Methods

We conduct a cost-effectiveness analysis by applying the results of the Japanese validation study of the 21-gene RT-PCR assay to our economic model [15], including sensitivity analysis from the societal perspective.

Key results of Japanese validation study

Table 1 shows the results of the Japanese validation study (JBCRG-TR03) [16]. Among 200 node-negative cases, 38 cases classified as having an indication for chemotherapy according to St Gallen 2009 criteria without any multigene assays, which is assumed to be a common practice in Japan [17], are further classified into 21 cases with high RS and 17 cases with intermediate or low RS by the assay, of which 10-year recurrence-free survival rates (RFS10) are 0.888 and 1.000, respectively; 61 cases classified as having no definitive indication for chemoendocrine therapy are further classified into 21 cases with high RS and 40 cases with intermediate or low RS by the assay, of which RFS10 are 0.694 and 0.949, respectively; and 101 cases classified as having an indication for endocrine therapy alone are

Table 1 Results of Japanese validation study

Lymph node	Chemoendocrine therapy indication by St Gallen 2009 criteria without multigene assays	Recurrence score criteria	No. of case	RFS5[a] (95% CI[b])	RFS10[c] (95% CI)
Negative ($N = 200$)	Chemotherapy	High scores	21	0.947 (0.847–1.047)	0.888 (0.741–1.035)
		Intermediate/Low scores	17	1.000 (–)	1.000 (–)
	No definitive indication	High scores	21	0.801 (0.627–0.975)	0.694 (0.488–0.900)[d]
		Intermediate/Low scores	40	0.949 (0.880–1.018)	0.949 (0.880–1.018)[d]
	Endocrine therapy alone	High scores	23	0.826 (0.671–0.981)	0.687 (0.495–0.879)[d]
		Intermediate/Low scores	78	1.000 (–)	0.986 (0.959–1.013)[d]
Negative + Positive ($N = 280$)	Chemotherapy	High scores	33	0.872 (0.754–0.990)	0.734 (0.575–0.893)
		Intermediate/Low scores	29	0.964 (0.895–1.033)	0.843 (0.700–0.986)
	No definitive indication	High scores	32	0.840 (0.711–0.969)	0.773 (0.624–0.922)
		Intermediate/Low scores	72	0.958 (0.911–1.005)	0.870 (0.792–0.948)
	Endocrine therapy alone	High scores	26	0.846 (0.707–0.985)	0.717 (0.539–0.895)[d]
		Intermediate/Low scores	88	0.977 (0.946–1.008)	0.965 (0.926–1.004)[d]

[a] 5-Year recurrence-free survival rate

[b] Confidence interval

[c] 10-Year recurrence-free survival rate

[d] Statistically significant by log rank test

further classified into 23 cases with high RS and 78 cases with intermediate or low RS by the assay, of which RFS10 are 0.687 and 0.986, respectively. Similar results are found among 280 node-negative/positive cases.

Assuming that a half of cases with no definitive indication undergoes adjuvant chemotherapy and that only cases with high RS undergo chemotherapy after the use of the assay based on the results of Japanese validation study, the use of adjuvant chemotherapy changes from 39.3 to 32.5% among 200 node-negative cases and from 40.7 to 32.5% among 280 node-negative/positive cases, respectively.

Scenarios

Based on these results of Japanese validation study, we analyse two scenarios of including the assay into Japan's social health insurance benefit package. The first scenario is that the assay is made available for LN−, ER+, ESBC (Indication for LN−). And another scenario is that the use of the assay is also approved for LN+ cases (Indication for LN−/+).

Since the use of trastuzumab for human epidermal growth factor receptor type2-positive (HER2+) diseases in the adjuvant setting has become established [18], it is foreseeable that Japan's social health insurance benefit package may include the assay for HER2− cases only. However, the study design of the Japanese validation study (JBCRG-TR03), which includes both HER2+ and HER2− cases, does not allow us any straightforward modelling of this indication. We, therefore, examine this issue not in our base-case analysis but in our sensitivity analysis.

Patient cohort

ER+, ESBC patient cohort at the age of 55 is targeted in our base-case analysis. The age, 55, is chosen according to the average age of equivalent patient population in a nationwide cancer registry [1].

Decision tree and Markov model

Our economic model shown in Fig. 1 incorporates clinical courses followed by ER+, ESBC patients.

The decision tree corresponds to the comparison between St Gallen 2009 criteria-guided treatment versus RS criteria-guided treatment. The decision node of this tree is a decision whether to use the assay or not. Following chance nodes portion out the cohort to different adjuvant therapies depending on the risk classification and HER2 status. We set up three types of adjuvant therapies: endocrine therapy (ET), ET plus chemotherapy (CT) and ET plus CT plus trastuzumab. Branches with CT lead to a subtree via chance nodes, which portion out the cohort to different toxicities.

The Markov model corresponds to the clinical course followed after the completion of adjuvant therapy. Five stages are modelled here: (1) ER+, ESBC after adjuvant therapy, (2) Distant recurrence with response to treatment, (3) Distant recurrence with no response to treatment, (4) Progression of disease after distant recurrence and (5) Death. Transitions between stages are indicated with arrows. Patients follow various courses after recurrence, and situations other than these five stages and transitions described here may be possible. However, we model the course in this way based on the available reports of prognosis model of metastatic breast cancer, which is calibrated with the results of several randomised trials [11, 19]. So here, patients with recurrence undergo drug treatment with ET, CT or/and trastuzumab depending on their status.

The span of each stage is set up at 1 year. Markov process is repeated up to 10 years, since the transition probabilities of recurrence are calculated from 5-year recurrence-free survival rates (RFS5) and RFS10, and most of the recurrences are known to occur within this time horizon. After 10 years, patients survived without recurrence are assumed to have life expectancy of 65 years old Japanese female population [20], and those with recurrence are assumed to have life expectancy of 2 years [21].

Outcomes estimation

Outcomes of each scenario in terms of quality adjusted life years (QALYs) are estimated by assigning probabilities and utility weights to the decision tree and Markov model from the literature.

Probabilities of risk classification, attached to the 1st chance nodes of each branch, are adopted from the results of Japanese validation study shown in Table 1. Table 2 shows other probabilities and utility weights used. A probability of HER2+, 9.3%, attached to the 2nd chance nodes, is adopted from a nationwide breast cancer registry [1, 15]. Probabilities of adjuvant chemotherapy toxicity, attached to the chance node in the subtree, are assumed to be 60% for minor toxicity, 5% for major toxicity and 0.5% for fatal toxicity from a report of the efficacy and cost-effectiveness of adjuvant chemotherapy in breast cancer [15, 22].

In regards to the Markov model, transition probabilities of recurrence with adjuvant ET are calculated from RFS5 and RFS10 in Table 1. The effectiveness of adding adjuvant CT and trastuzumab are incorporated as risk reduction of recurrence. Relative risk reductions resulted from CT among patients classified as high RS and intermediate RS are fixed at 74 and 39%, respectively, which are adopted from one of the validation studies of the assay in the U.S. [5, 15], since no comparative Japanese validation study has been implemented. A relative risk reduction resulted from

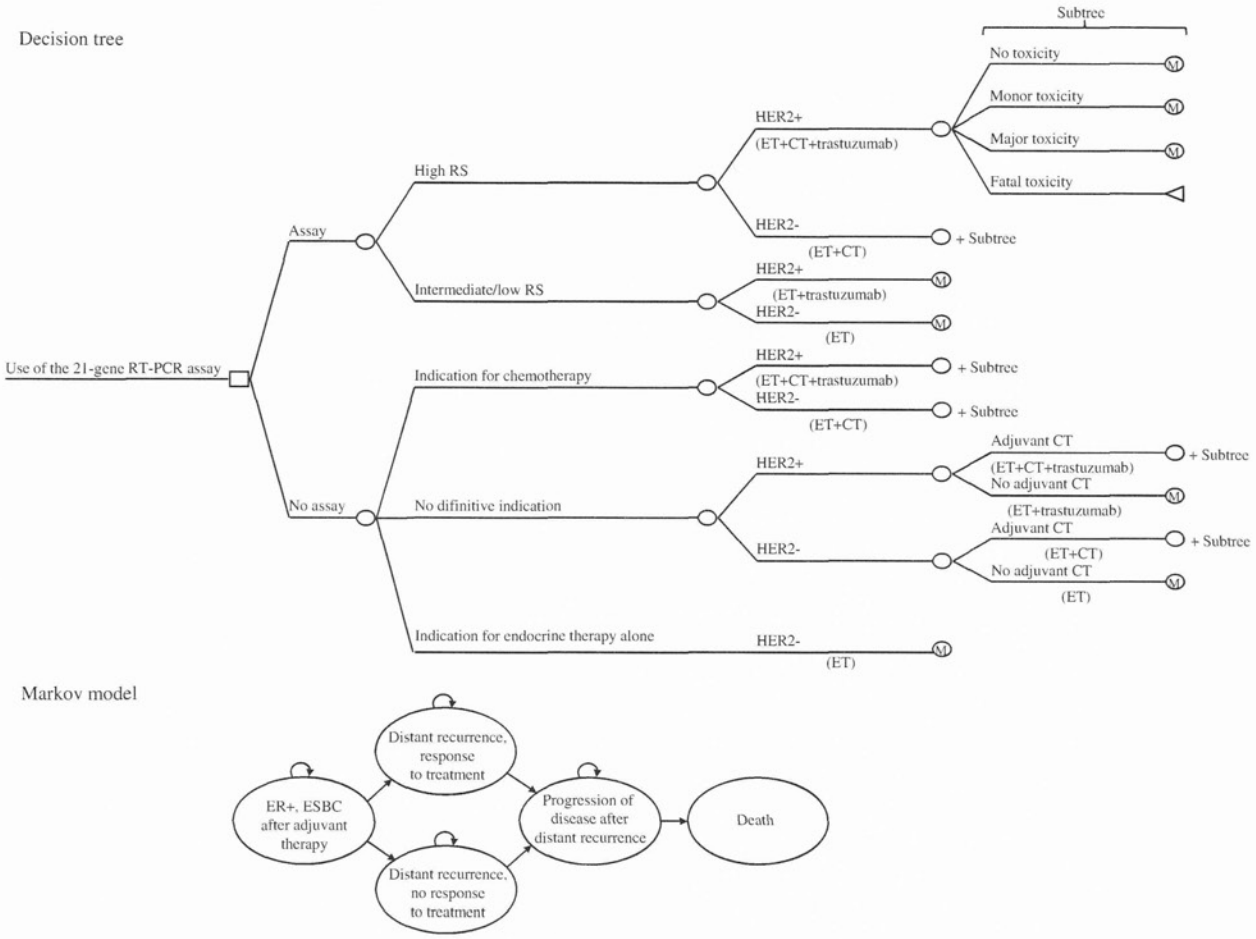

Fig. 1 Decision tree and Markov model. *RT-PCR* reverse transcriptase-polymerase reaction, *RS* recurrence score, *HER2* human epidermal growth factor type 2, *ET* endocrine therapy, *CT* chemotherapy, *ER+, ESBC* estrogen-receptor-positive, early stage breast cancer

trastuzumab among HER2+ patients are assumed to be 36% up to 2 years according to the result of clinical trial [15, 23]. As mentioned above, transition probabilities between stages after recurrence are adopted from the prognosis model of metastatic breast cancer [11, 15, 19]. It is assumed that the response to treatment and the prognosis after recurrence differ depending on HER2 status. Probabilities of the response to treatment for recurrence are fixed at 38.0% among HER2− patients and 54.0% among HER2+ patients [15, 19]. Probabilities of the progression of disease after recurrence are also fixed at: 59.7% if HER2− and having responded to treatment, 98.3% if HER2− and not having responded to treatment, 53.7% if HER2+ and having responded to treatment and 88.5% if HER2+ and not having responded to treatment [11, 15]. Probabilities of death after the progression of disease are fixed at 40.0% among HER2− patients and 37.2% among HER2+ patients [11, 15].

In order to estimate the outcomes in terms of QALYs, utility weights are chosen for various health states during the clinical course that patients follow. A weight for a health states after adjuvant therapy without any toxicity or distant recurrence is chosen to be 0.98 [15, 24]. Weights for toxicities are 0.90 for minor toxicity, and 0.80 for major toxicity [15, 22], of which duration is assumed at 6 months. The health states during chemotherapy in preventing distant recurrence or the progression of disease weighs 0.50 [15, 25], of which duration is assumed at 6 months. Health states after the chemotherapy weigh 0.84 if responded to treatment, 0.70 if stable and 0.49 if the disease progressed [15, 19].

Outcomes are discounted at a rate of 3% [26].

Costing

From the societal perspective, costing should cover the opportunity cost borne by various economic entities in the society. In the context of this study, costs borne by social insurers and patients are considered, since these two entities are the major payers to health care providers in Japan's

Table 2 Probabilities and utility weights

	Base-case value	Range tested in sensitivity analyses	Source
Probabilities			
Patient status			
HER2+[a]	9.3%	Change by ±50%	[1, 15]
Adjuvant chemotherapy toxicity			
Minor	60.0%	Change by ±50%	[15, 22]
Major	5.0%	Change by ±50%	[15, 22]
Fatal	0.5%	Change by ±50%	[15, 22]
Risk reduction of distant recurrence			
Chemotherapy in relative risk reduction			
Intermediate scores classified by RS[b] criteria	39.0%	Change 0 to 76%	[5, 15]
High scores classified by RS criteria	74.0%	Change 47 to 87%	[5, 15]
Trastuzumab in absolute risk reduction (Duration)	36.0% (2 years)	Change 24 to 46% Change to 5 years	[15, 23]
Response to treatment for distant recurrence			
HER2−	38.0%	Change by ±50%	[15, 19]
HER2+	54.0%	Change by ±50%	[15, 19]
Progression of disease after distant recurrence			
HER2−, response to treatment	59.7%	Change by ±50%	[11, 15]
HER2−, no response to treatment	98.3%	Change by ±50%	[11, 15]
HER2+, response to treatment	53.7%	Change by ±50%	[11, 15]
HER2+, no response to treatment	88.5%	Change by ±50%	[11, 15]
Death after progression of disease			
HER2−	40.0%	Change by ±50%	[11, 15]
HER2+	37.2%	Change by ±50%	[11, 15]
Utility weights			
After adjuvant therapy without distant recurrence	0.98	Change by ±20%	[15, 24]
Toxicity			
Minor	0.90	Change by ±20%	[15, 22]
Major	0.80	Change by ±20%	[15, 22]
Distant recurrence			
Chemotherapy, 6 months only	0.50	Change by ±20%	[15, 25]
If respond to treatment	0.84	Change by ±20%	[15, 19]
Stable	0.70	Change by ±20%	[15, 19]
Progression of disease	0.49	Change by ±20%	[15, 19]

[a] Human epidermal growth factor receptor type2

[b] Recurrence score

social health insurance system. The amount of direct payments by these entities, according to the national medical care fee schedule, is estimated as costs, while costs of sector other than health and productivity losses are left uncounted in this study.

Cost items are identified along the decision trees and Markov model: the assay, adjuvant therapies, treatments for toxicity, monitoring, treatments for distant recurrence and end-of-life treatments as shown in Table 3. As already mentioned, the cost of the assay is ¥450,000 (US$4,500) according to the price offered by the Japanese supplier of Onco*type* DX® Breast Cancer Assay. Costs of treatments except the end-of-life treatments are estimated by combining a model of breast cancer care and the national medical care fee schedule. The care model is developed based on both a nationwide survey of Japanese expert practice and consensus guidelines [15, 17, 27, 28].

Adjuvant endocrine therapy includes outpatient care with tamoxifen, aromatase inhibitors and LH-RH analogues depending on patient's status, and it is assumed to continue up to 5 years, which costs ¥534,610 (US$5,346) per year. Adjuvant chemotherapy includes various regimens. Anthracycline-based combination chemotherapy is used for about half of the cases, and oral fluorinated pyrimidine and CMF (cyclophosphamide, methotrexate and 5-fluorouracil) therapy are frequently used among