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ORIGINAL REPORT

Gefitinib and *EGFR* Gene Copy Number Aberrations in Esophageal Cancer

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ABSTRACT

Purpose

The Cancer Esophagus Gefitinib trial demonstrated improved progression-free survival with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib relative to placebo in patients with advanced esophageal cancer who had disease progression after chemotherapy. Rapid and durable responses were observed in a minority of patients. We hypothesized that genetic aberration of the EGFR pathway would identify patients benefitting from gefitinib.

Methods

A prespecified, blinded molecular analysis of Cancer Esophagus Gefitinib trial tumors was conducted to compare efficacy of gefitinib with that of placebo according to *EGFR* copy number gain (CNG) and *EGFR*, *KRAS*, *BRAF*, and *PIK3CA* mutation status. *EGFR* CNG was determined by fluorescent in situ hybridization (FISH) using prespecified criteria and *EGFR* FISH-positive status was defined as high polysomy or amplification.

Results

Biomarker data were available for 340 patients. In *EGFR* FISH-positive tumors (20.2%), overall survival was improved with gefitinib compared with placebo (hazard ratio [HR] for death, 0.59; 95% CI, 0.35 to 1.00; $P = .05$). In *EGFR* FISH-negative tumors, there was no difference in overall survival with gefitinib compared with placebo (HR for death, 0.90; 95% CI, 0.69 to 1.18; $P = .46$). Patients with *EGFR* amplification (7.2%) gained greatest benefit from gefitinib (HR for death, 0.21; 95% CI, 0.07 to 0.64; $P = .006$). There was no difference in overall survival for gefitinib versus placebo for patients with *EGFR*, *KRAS*, *BRAF*, and *PIK3CA* mutations, or for any mutation versus none.

Conclusion

EGFR CNG assessed by FISH appears to identify a subgroup of patients with esophageal cancer who may benefit from gefitinib as a second-line treatment. Results of this study suggest that anti-EGFR therapies should be investigated in prospective clinical trials in different settings in *EGFR* FISH-positive and, in particular, *EGFR*-amplified esophageal cancer.

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INTRODUCTION

An estimated 455,000 individuals worldwide are diagnosed annually with esophageal cancer.[1,2] In North America, Northern and Western Europe, and Oceania, the incidence of esophageal adenocarcinoma has risen in the last four decades and is now the predominant histologic subtype.[2] Squamous cell carcinoma of the esophagus remains more common globally and in southeastern and central Asia.[2]

Five-year survival is only 19%.[3] Most patients present with advanced disease not amenable to curative therapy.[4] Systemic treatment with cytotoxic chemotherapy provides palliative benefits; however, current treatment options are of more limited effectiveness following progression after first-line therapy.[4,5] Phase III randomized trials of second- or third-line treatment in gastric and/or esophagogastric junction adenocarcinomas have demonstrated benefit from apatinib, irinotecan, ramucurimab, and ramucurimab combined with paclitaxel.[6-12] Some caution is needed in extrapolating results for gastroesophageal adenocarcinomas from different sites, because although molecular analysis suggests that esophagogastric junction and more-proximal esophageal adenocarcinomas are

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biologically similar, more-distal gastric adenocarcinomas appear distinct.[13] There is more limited evidence supporting the use of second-line therapy in esophageal squamous cell carcinoma.[5,14]

The Cancer Esophagus Gefitinib (COG) trial is the only randomized phase III study of second-line therapy specifically in chemoresistant esophageal cancer, including adenocarcinoma and squamous cell carcinoma.[15] In the COG trial, 450 patients were randomly assigned to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) gefitinib or placebo. Progression-free survival (PFS) and patient-reported outcomes (PROs) were improved for gefitinib, reflecting the occurrence of rapid and durable responses to gefitinib in a minority subset of patients. Benefit from gefitinib occurred in adenocarcinomas and squamous cell carcinomas to an equal extent.

We hypothesized that the gefitinib-responsive subgroup of patients were a subset for whom EGFR signaling was an important driver. A variety of different EGFR signaling abnormalities have been described in esophageal cancer, including copy number gain (CNG) of *EGFR*.[16-20] Study results suggest that chromosomal instability is an early and frequent feature of esophageal cancer pathogenesis, and somatic copy number alterations occur frequently in esophageal adenocarcinoma and squamous cell carcinoma.[19-24] Therefore, we hypothesized that EGFR signaling was a key pathogenic driver in the minority subset of esophageal cancers with *EGFR* CNG, and that these patients would benefit from gefitinib.

Accordingly, we investigated EGFR signaling pathway abnormalities in an adequately powered, prospectively collected cohort of tumor specimens from patients in the COG trial, with prespecified biomarker assays undertaken blind to treatment allocation and outcome, and a statistical analysis plan that was formulated before biomarker assay results were available.

METHODS

Study Design and Oversight

The COG trial (ISRCTN29580179) compared efficacy of 500 mg of gefitinib daily with that of placebo in patients with esophageal cancer who had disease progression after chemotherapy.[15] Participants were recruited from 48 centers in the United Kingdom and randomly assigned (1:1) to gefitinib or matching placebo by simple randomization with no stratification factors. The primary end point was overall survival (OS). Secondary end points were PFS, disease control rate (DCR; calculated as Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1 partial response plus complete response plus stable disease at 8 weeks), and PROs.[15] Formalin-fixed paraffin-embedded tumor tissues were prospectively collected for a translational substudy of the COG trial, TRANSCOG (ISRCTN32435732).

The TRANSCOG study was undertaken in accordance with the protocol and was approved by the National Research Ethics Service Committee (Reference 11/0372/AL). All handling and assays of tumor specimens were performed according to good clinical laboratory standards in diagnostically accredited (ISO15189:2012) laboratories. All molecular analysis was undertaken blind to treatment and clinical outcome data.

A reporting recommendations for tumor marker prognostic studies (REMARK)[25] compliance checklist is provided (Data Supplement).

Tumor Specimens

Archived formalin-fixed paraffin-embedded tumor specimens were collected and processed according to a prespecified standard operating procedure. Central pathology review was performed to confirm histologic diagnosis and assess tumor cellularity. Tumor tissue sections (4 μm) were prepared for *EGFR* fluorescent in situ hybridization (FISH). DNA was extracted using a standard dewaxing, tissue digestion, and phenol/chloroform methodology with macrodissection to enrich for tumor in specimens with $<$ 50% tumor cellularity.

EGFR *Gene Copy Number Analysis*

EGFR copy number analysis was by FISH and tumors were classified using the 6-point scale described previously.[26] Tumors scoring 5 (high polysomy) or 6 (amplification) were classified as having *EGFR* high CNG and defined as *EGFR* FISH positive; tumors scoring 1 to 4 were classified as having no or low CNG and defined as *EGFR* FISH negative[26] (Data Supplement). Analysis was performed by two independent scorers in a laboratory with Clinical Pathology Accreditation. Discordance led to further analysis by a third independent scorer. The testing plan and methodology were prespecified.

Mutational Analysis

Methods for each mutation were optimized for sensitivity and reliability. The final testing plan and methodology were prespecified. *KRAS* mutation was analyzed by pyrosequencing using primers and probes specifically designed for codons 12, 13, and 61. *EGFR*, *PIK3CA*, *BRAF* V600E, and mutations were detected by Sanger sequencing as a first option; failed samples were analyzed using COBAS *EGFR PIK3CA* and *BRAF* V600E mutation testing kits (Roche Molecular Systems, Branchburg, NJ). Deletions in exon 19 of *EGFR* were detected by fragment length analysis. Details are available in the Data Supplement.

Statistical Analysis

The statistical analysis plan was prespecified before molecular results were available. The primary objective was to compare the effect of gefitinib with that of placebo in *EGFR* FISH-positive and -negative patients, and patients with and without *EGFR*, *KRAS*, *BRAF*, and *PIK3CA* mutations in the primary analysis the COG trial study population.[15] The primary end point was OS; secondary end points were PFS, DCR (calculated as RECIST version 1.1 partial response plus complete response plus stable disease at 8 weeks), and PROs. We assumed that tumor samples would be available from $>$ 300 patients. Considering $\alpha = .05$, the accrual of tumor tissues over the 30 months of the COG study, a 12-month minimum follow-up, a hazard ratio (HR) of 0.50 favoring gefitinib in biomarker subgroups, and a median survival of 3 months in placebo-treated patients, and then assuming equal representation of gefitinib or placebo in tested samples, a predictive biomarker-defined subgroup of 10% within the gefitinib arm only comparing positive with negative biomarker groups would provide a power of 0.73, 15% would provide a power of 0.88, and 20% would provide a power of 0.93.

The power to compare within a biomarker-positive group between gefitinib- and placebo-treated patients was reduced because of the small sample size expected in these groups (biomarker positive: 10%, power of 0.45; 15%, power of 0.61; and 20%, power of 0.72).The study was not powered to test the interaction between biomarkers and treatment formally.

To estimate the treatment effects of gefitinib, we used the Cox proportional hazard model to compare outcomes in gefitinib with placebo in each biomarker-positive and -negative subgroup. The proportional hazard assumption was tested by examining the log cumulative hazards plot and Schoenfeld residual plot, and no significant deviations were found. Comparisons between biomarker status and DCR, between biomarker status and PROs, and between biomarker status and clinical variables were performed using a χ^2 test or Fisher exact test, as appropriate. In the biomarker analysis, multiple testing was not adjusted for. To avoid errors for multiple testing in PRO analysis, biomarker status was investigated only for the four PROs of particular importance prespecified in the COG trial.[15]

The significance level for all statistical outcomes was prespecified as 0.05 and 95% CIs were calculated. The definitions of OS, PFS, and DCR, and methods for health-related quality of life (HRQL) assessment for PROs are detailed in the COG trial primary publication.[15]

RESULTS

Patients

Tumor specimens were available from 340 of 450 patients (76%) in the COG study. Overall, 292 patients (65%) had tumor evaluable for *EGFR* CNG by FISH and 326 patients (72%) had tumor evaluable for *EGFR, KRAS, PIK3CA*, and *BRAF* mutation (Fig 1). The mutation analysis comprised *EGFR* exon 19 deletion in 254 patients, *EGFR* exons 18 to 21 in 223 patients, *KRAS* codon 12 and 13 in 268 patients, *KRAS* codon 61 in 287 patients, *PIK3CA* exon 9 in 267 patients and exon 20 in 273 patients, and *BRAF* V600E in 267 patients.

A total of 165 patients with *EGFR* FISH results completed HRQL questionnaires at baseline and 4 weeks, and 88 completed them at baseline and 8 weeks; these were included in the PRO analysis (Data Supplement).

The cohorts of patients evaluable for *EGFR* CNG, mutations, and PROs were not different than the COG trial cohort in terms of clinical features, OS, PFS, and baseline HRQL, and clinical features were balanced in the gefitinib and placebo groups (Table 1; Data Supplement).

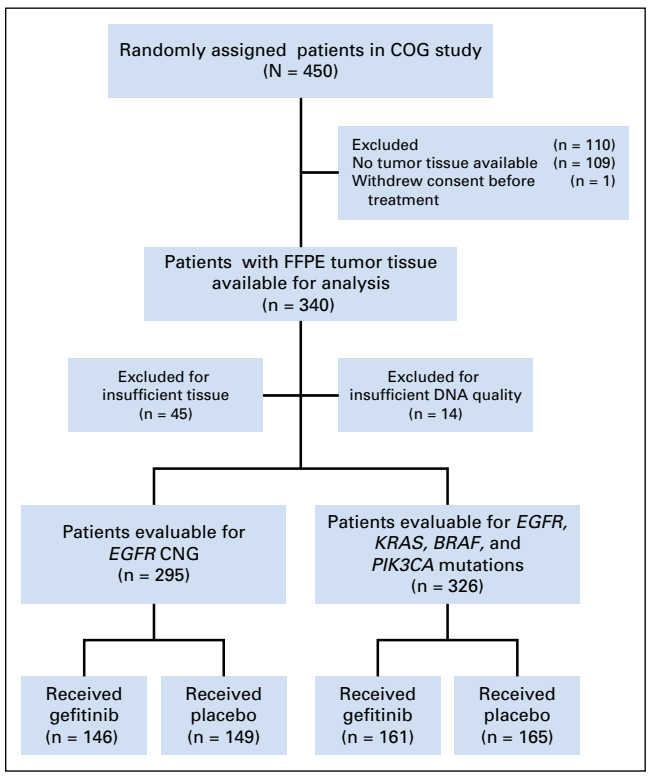

Fig 1. Patient and specimen flow in the COG and translational COG (TRANSCOG) trials. CNG, copy number gain; COG, Cancer Esophagus Gefitinib; *EGFR*, epidermal growth factor receptor; FFPE, formalin-fixed paraffin-embedded.

Tumors from 59 patients were *EGFR* FISH positive (59 of 292 patients; 20.2%), with high polysomy in 13% (38 of 292) and amplification in 7.2% (21 of 292 patients; Data Supplement). No *EGFR* mutations were detected. *KRAS* codon 12 and 13 mutations were found in 4.1% of patients (11 of 268), *KRAS* codon 61 mutations in 1.1% (three of 261), *PIK3CA* exon 9 mutations in 3.4% (nine of 267), *PIK3CA* exon 20 mutations in 0.7% (two of 273), and *BRAF* V600E in 0.4% (one of 267). There was no significant association between *EGFR* FISH (Table 2) or any mutation (Data Supplement) and clinical features.

Efficacy According to Tumor EGFR *Gene Copy Number Status*

The DCR was higher in patients with *EGFR* FISH-positive tumors who received gefitinib compared with those who received placebo: 37% (11 of 30 patients; 11 patients with stable disease) for gefitinib versus 14% (four of 29 for placebo; $P = .04$). PFS and OS were also improved in *EGFR* FISH-positive patients who received gefitinib compared with those who received placebo (PFS HR, 0.55 [95% CI, 0.32 to 0.95], $P = .03$ for gefitinib *v* placebo-treated patients; and OS HR, 0.59 [95% CI, 0.35 to 1.00], $P = .05$ for gefitinib *v* placebo-treated patients; Fig 2). OS in *EGFR* FISH-positive patients treated with gefitinib versus those treated with placebo at 3, 6, 9, and 12 months was 69% versus 64%, 38% versus 14%, 27% versus 5%, and 13% versus 0%, respectively.

A multivariate Cox proportional hazards analysis (n = 277 for PFS and n = 278 for OS) was performed (Data Supplement) adjusted for performance status, prior treatment, body mass index, histology, disease site, age, and sex. In this analysis, PFS remained significant for benefit of gefitinib compared with placebo in *EGFR* FISH-positive patients (HR, 0.42; 95% CI, 0.22 to 0.81; $P = .01$) but not OS (HR, 0.57; 95% CI, 0.30 to 1.06; $P = .08$). None of the variables were significantly associated with PFS or OS in the multivariate analysis in *EGFR* FISH-positive patients.

A post hoc analysis suggested that patients with *EGFR* amplification (7.2% of patients) gained greater benefit from gefitinib than those with high polysomy (Figs 2 and 3; Data Supplement).

DCR was also higher in *EGFR* FISH-negative patients who received gefitinib compared with those who received placebo, but DCR was greater in *EGFR* FISH-positive patients (25% [29 of 115 patients receiving gefitinib], three partial responses, and 26 stable disease) versus 14% for placebo ($P = .06$). In *EGFR* FISH-negative patients, PFS (HR, 0.87; 95% CI, 0.66 to 1.12; $P = .28$), and OS (HR, 0.90; 95% CI, 0.69 to 1.18; $P = .46$) were not different for gefitinib compared with that of placebo (Fig 2). OS in *EGFR* FISH-negative patients treated with gefitinib versus placebo at 3, 6, 9, and 12 months was 61% versus 46%, 33% versus 29%, 16% versus 22%, and 8% versus 14%, respectively. In the multivariate analysis, PFS and OS were not significantly different for gefitinib compared to placebo in *EGFR* FISH-negative patients, but performance status, prior treatment, and site of tumor were significantly associated with OS and PFS, respectively (Data Supplement).

Because of small patient numbers, differences in the COG trial-prespecified HRQL domains at 4 weeks and 8 weeks compared with baseline were not significantly different between the gefitinib and placebo groups for *EGFR* FISH-positive and -negative patients (Data Supplement). However in *EGFR* FISH-positive patients,

Table 1. Clinical Features of the Patients Evaluable for *EGFR* Copy Number Gain by FISH

Clinical Feature	COG Trial Cohort (N = 449)*		*EGFR* FISH Cohort (N = 292)	
	Placebo (n = 225)	Gefitinib (n = 224)	Placebo (n = 147)	Gefitinib (n = 145)
Age at assignment, years, mean (SD)	64.5 (9.4)	63.7 (9.6)	64.5 (9.4)	64.1 (9.2)
Sex, No. (%)				
Male	189 (84.0)	183 (81.7)	124 (84.4)	118 (81.4)
Female	36 (16.0)	41 (18.3)	23 (15.7)	27 (18.6)
Time since diagnosis, years, median (IQR)	0.92 (0.60, 1.47)	0.96 (0.62, 1.45)	0.86 (0.57, 1.48)	0.95 (0.59, 1.38)
Original diagnosis, No. (%)				
Adenocarcinoma	168 (74.7)	173 (77.2)	102 (69.4)	112 (77.2)
Squamous	56 (24.9)	50 (22.3)	44 (29.9)	32 (22.1)
Undifferentiated	1 (0.4)	1 (0.4)	1 (0.7)	1 (0.7)
Disease site, No. (%)				
Esophageal	181 (80.4)	171 (76.3)	120 (81.6)	109 (75.2)
Type I junctional	21 (9.3)	26 (11.6)	12 (8.2)	17 (11.7)
Type II junctional	23 (10.2)	27 (12.1)	15 (10.2)	19 (13.1)
Performance status, No. (%)				
0	56 (24.9)	57 (25.5)	33 (22.5)	36 (24.8)
1	125 (55.6)	117 (52.2)	86 (58.5)	75 (51.7)
2	44 (19.6)	50 (22.3)	28 (19.1)	34 (23.5)
Previous treatments, No. (%)				
0	1 (0.4)	0	1 (0.7)	0
1	137 (60.9)	137 (61.2)	95 (64.6)	94 (64.8)
2	75 (33.3)	78 (34.8)	41 (27.9)	48 (33.1)
3	12 (5.3)	9 (4.0)	10 (6.8)	3 (2.1)
BMI, kg/m^2, mean (SD); No.	24.01 (4.77); 212	24.01 (4.94); 214	24.0 (4.18); 141	24.1 (4.4); 139
BMI grouped, No. (%)				
< 18.0	24 (10.7)	24 (10.7)	16 (10.9)	13 (9.0)
18.0-24.9	117 (52.0)	113 (50.5)	77 (52.4)	73 (50.3)
25.0-29.9	43 (19.1)	55 (24.6)	31 (21.1)	40 (27.6)
≥ 30	28 (12.4)	22 (9.8)	17 (11.6)	13 (9.0)
Missing	13 (5.8)	10 (4.5)	6 (4.1)	6 (4.1)

Abbreviations: BMI, body mass index; COG, COG, Cancer Esophagus Gefitinib; *EGFR*, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; IQR, interquartile range; SD, standard deviation.
*One patient in the gefitinib arm withdrew consent shortly after being randomly assigned and is excluded from all analyses.

all prespecified HRQL domains improved with gefitinib compared with placebo, in contrast to an observed deterioration or lesser improvement seen in *EGFR* FISH-negative patients (Fig 4). At 8 weeks, mean scores for global quality of life (+10.7) and difficulty eating (−20.8) were improved beyond the 8-point difference considered to be of clinical importance with gefitinib compared with placebo in *EGFR* FISH-positive patients. However, none of the prespecified HRQL domains were changed ≥ 8 in *EGFR* FISH-negative patients at 8 weeks (Data Supplement).

Efficacy According to Tumor Mutation Status

There was no significant difference in DCR, PFS, OS, or PROs for *KRAS* codon 12 and 13, *KRAS* codon 61, *PIK3CA* exon 9 or 20, or *BRAF* V600E mutations, or the presence of any mutation versus none (Data Supplement).

DISCUSSION

In the COG trial, 450 patients with esophageal adenocarcinoma or squamous cell carcinoma progressive after previous chemotherapy were randomly assigned to treatment with gefitinib or placebo. Improved DCR, PFS, and PROs were observed for gefitinib compared with placebo, reflecting rapid and durable benefits occurring in a minority subgroup.[15] Gefitinib was well tolerated and, although objective responses were rare, when observed, they invariably occurred rapidly within 4 weeks of starting gefitinib. However, it is clear that most patients do not benefit from gefitinib. Identification of a predictive biomarker for patients who receive benefit from gefitinib would enable a more accurate selection of patients for treatments and prevent futile treatment in those patients who are unlikely to benefit.

Based on the outcome of the COG trial, we hypothesized that there was a subgroup of patients whose tumors were driven by EGFR signaling and who, accordingly, benefitted from treatment with gefitinib. This is analogous to non–small-cell lung cancer and colorectal adenocarcinoma in which *EGFR* mutation and *KRAS* mutation, respectively, have provided useful predictive biomarker tests and allowed subgroups to be defined as responsive to anti-EGFR therapies.[27,28] We aimed to determine if analysis of EGFR signaling pathway abnormalities in esophageal carcinoma would similarly predict benefit from gefitinib.

EGFR FISH-positive patients whose esophageal cancers had *EGFR* CNG defined as high polysomy or amplification by FISH, had improved DCR, PFS, OS, and PROs when treated with gefitinib compared with placebo. In contrast, *EGFR* FISH-negative patients had improved DCR, but this did not translate into improved PFS, OS, or PROs. This suggests that patients with *EGFR* FISH positive tumors have increased survival, as well as improved HRQL, with gefitinib, which is important in this clinical setting of limited life expectancy. Our post-hoc analysis suggests that the

Table 2. Association of Clinical Features and *EGFR* Copy Number Gain Status

Clinical Feature	*EGFR* Copy Number Gain (N = 59)	*EGFR* No Copy Number Gain (N = 233)	*P*
Age at assignment, years, mean (SD)	63.9 (8.3)	64.4 (9.5)	.85
Sex, No. (%)			.97
Male	49 (83.1)	193 (82.8)	
Female	10 (17.0)	40 (17.2)	
Time since diagnosis, years median (IQR); No.	0.95 (0.51, 1.27); 57	0.90 (0.60, 1.45); 231	.10
Original diagnosis, No. (%)			.69
Adenocarcinoma	44 (74.6)*	170 (73.0)	
Squamous	14 (23.7)†	62 (26.6)	
Undifferentiated	1 (1.7)‡	1 (0.4)	
Disease site, No. (%)			.50
Esophageal	46 (78.0)	183 (78.5)	
Type I junctional	4 (6.8)	25 (10.7)	
Type II junctional	9 (15.3)	25 (10.7)	
Performance status, No. (%)			.27
0	12 (20.3)	57 (24.5)	
1	30 (50.9)	131 (56.2)	
2	17 (28.8)	45 (19.3)	
Previous treatments, No. (%)			.90
0	0	1 (0.4)	
1	39 (66.1)	150 (64.4)	
2	18 (30.5)	71 (30.5)	
3	2 (3.4)	11 (4.7)	
BMI, kg/m^2, mean (SD); No.	23.7 (4.5); 55	24.1 (4.6); 225	.55
BMI grouped, No. (%)			.74
< 18.0	4 (6.8)	25 (10.7)	
18.0-24.9	33 (55.9)	117 (50.2)	
25.0-29.9	14 (23.7)	57 (24.5)	
≥ 30	4 (6.8)	26 (11.2)	
Missing	4 (6.8)	8 (3.4)	

Abbreviations: BMI, body mass index; *EGFR*, epidermal growth factor receptor; IQR, interquartile range; SD, standard deviation.
*Twenty-nine of 44 *EGFR* fluorescent in situ hybridization (FISH) –positive adenocarcinomas (65.9%) had *EGFR* high polysomy and 15 of 44 (34.1%) had *EGFR* amplification.
†Fourteen of 14 *EGFR* FISH-positive squamous cell carcinomas (64.2%) had *EGFR* high polysomy and five of 14 (35.8%) had *EGFR* amplification.
‡The *EGFR* FISH-positive undifferentiated carcinoma was *EGFR* amplified.

benefit of gefitinib is greater in those with *EGFR*-amplified tumors than with high polysomy tumors. Our study was not powered to investigate these subgroups, and additional investigation is needed to validate this observation. However, this finding is consistent with results for anti-EGFR and other targeted therapies in other tumor types.[29-31] Overall, our results suggest it is likely there is a greater benefit from gefitinib in *EGFR*-amplified esophageal cancers compared with those with high polysomy.

Our findings are supported by the report of high sensitivity to gefitinib in a primary cell line derived from a patient with esophageal adenocarcinoma with *EGFR* high polysomy.[32] In addition, a single-arm phase II trial of the EGFR TKI icotinib in esophageal squamous cell carcinoma with *EGFR* CNG determined by FISH or strongly positive EGFR immunohistochemistry reported a DCR of 46%.[33]

This suggests that *EGFR* FISH identifies those patients with esophageal cancer whose tumors are driven by EGFR signaling and for whom inhibition of EGFR confers benefit. Investigating the impact of *EGFR* FISH positivity on sensitivity to *EGFR* inhibitors other than gefitinib would test this hypothesis. Similar to HER2 in gastroesophageal adenocarcinoma, EGFR TKIs and monoclonal antibodies may have different impacts in EGFR FISH-positive patients.[34,35] Not all *EGFR* FISH-positive patients benefit from gefitinib and coamplification of other receptor tyrosine kinases (RTKs) and/or downstream signaling pathways may also be important determinants of clinical benefit.[36,37] RTK pathway copy number profiling could improve predictive accuracy and guide personalized use of *EGFR* and other RTK inhibitors.

To our knowledge, there have been no previous randomized trials of second-line therapy in esophageal cancer including adenocarcinomas and squamous cell carcinomas. However, the PFS and OS benefits from gefitinib compared with placebo in *EGFR* FISH-positive patients that we observed is of a similar proportion to those in randomized studies versus placebo or supportive care only for other second-line therapies in gastric and gastroesophageal junction adenocarcinoma, including apatinib, regorafenib, docetaxel, irinotecan, and ramucurimab.[7-12,38] In comparison with docetaxel and irinotecan, the toxicity of gefitinib is preferable. The toxicity of gefitinib is similar overall to that of ramucurimab, apatinib, or regorafenib, but because there are no predictive biomarkers for these agents, the use of gefitinib in patients selected by *EGFR* FISH status represents an alternative with increased clinical and cost effectiveness.

The use of next-generation sequencing would have provided higher sensitivity for subclonal mutations. However, the low frequency of mutations detected in our study, in contrast to *EGFR* CNG, which predicts gefitinib benefit, is consistent with other reports and genome landscaping studies that demonstrate predominant copy number changes.[18-20,36] In adenocarcinoma,

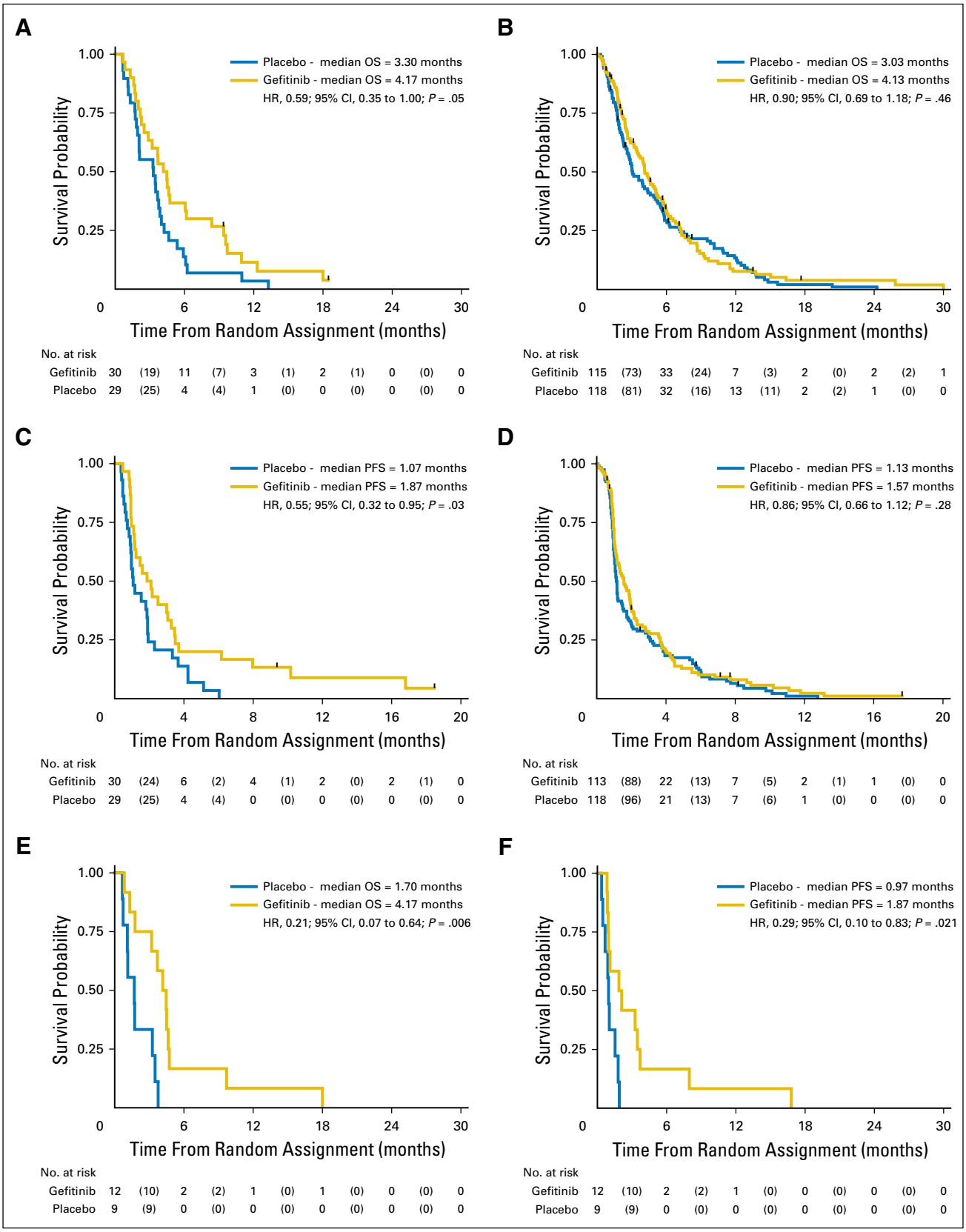

Fig 2. Kaplan-Meier estimates of PFS and OS according to treatment group. (A) OS in patients positive for *EGFR* by fluorescent in situ hybridization (FISH). (B) OS in *EGFR* FISH-negative patients. (C) PFS in *EGFR* FISH-positive patients. (D) PFS in *EGFR* FISH-negative patients. (E) OS in *EGFR*-amplified patients (FISH category 6). (F) PFS in *EGFR*-amplified patients (FISH category 6). *EGFR*, epidermal growth factor receptor; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

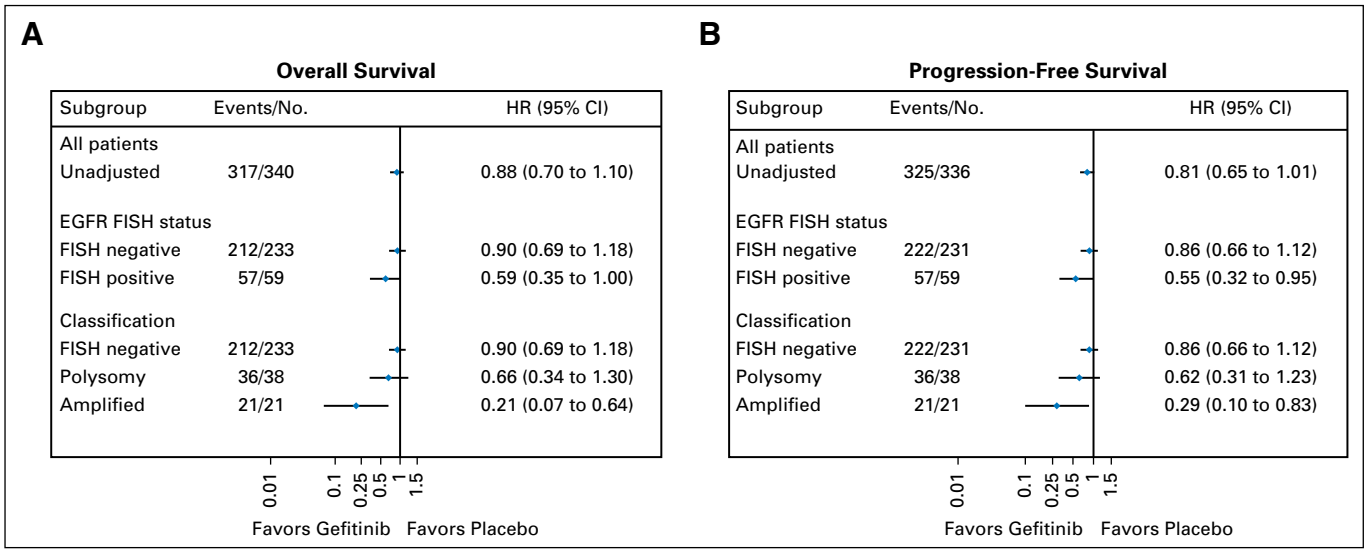

Fig 3. Forest plot for *EGFR* FISH-positive and -negative patients, and *EGFR* high polysomy and amplification. (A) OS. (B) PFS. CNG, copy number gain; EGFR, epidermal growth factor receptor; FISH, fluorescent in situ hybridization; HR, hazard ratio.

chromosomal instability leading to structural aneuploidy including CNGs of oncogenes such as *EGFR* is common.[17-20,23,36] In our analysis, we found no significant difference in the frequency of *EGFR* CNG between adenocarcinomas and squamous cell carcinomas.

There was insufficient tissue available to analyze EGFR protein expression. In esophageal cancer, *EGFR* FISH-positive tumors almost invariably overexpress EGFR by immunohistochemistry, but up to 50% of *EGFR* FISH-negative tumors also strongly

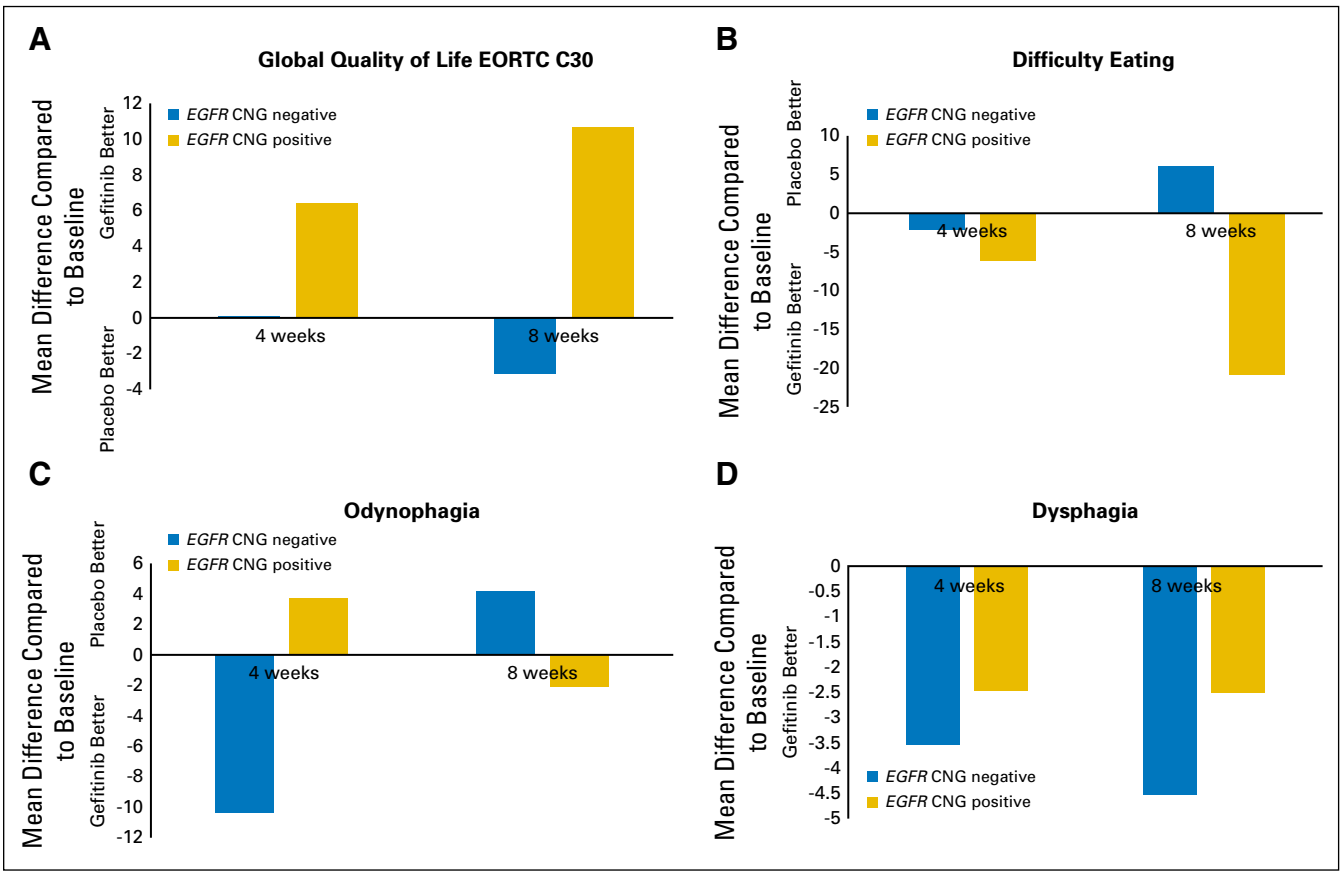

Fig 4. Patient-reported outcomes. (A) Global quality of life. (B) Difficulty eating. (C) Odynophagia. (D) Dysphagia. CNG, copy number gain; *EGFR*, epidermal growth factor receptor; FISH, fluorescent in situ hybridization.

overexpress EGFR protein.[39,40] Together with our demonstration of lack of benefit from gefitinib in *EGFR* FISH-negative patients, this suggests that *EGFR* FISH may be more a reliable predictive biomarker than EGFR immunohistochemistry, although this needs to be confirmed by additional investigation.

This study was retrospective and, therefore, subject to limitations. The results of *EGFR* CNG and the mutation analysis may not be representative of the intention-to-treat population from the original randomization. However, the cohort of patients tested did not show significant differences in clinical features compared with the intention-to-treat population. Furthermore, molecular testing and analysis were hypothesis driven, performed to diagnostic standard in a reference laboratory with clinical pathology accreditation, blind to patient treatment and outcomes, had a prospectively determined statistical analysis plan formulated before molecular results were available, and used data from a large, randomized controlled trial. Therefore, this study robustly evaluated *EGFR* CNG determined by FISH as a predictive biomarker.

In conclusion, *EGFR* FISH appears to predict a benefit from gefitinib in patients with esophageal cancer whose disease has progressed after previous chemotherapy. The role of gefitinib and other anti-EGFR therapies should be explored in prospective clinical trials in different settings in *EGFR* FISH-positive esophageal cancer, particularly in EGFR-amplified tumors, in which the impact of these agents is likely to be greatest.

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Disclosures provided by the authors are available with this article at jco.org.

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