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ORIGINAL REPORT

Epidermal Growth Factor Receptor Gene Copy Number and Clinical Outcome of Metastatic Colorectal Cancer Treated With Panitumumab

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ABSTRACT

Purpose
In a previous cohort study, we proposed that responsiveness of metastatic colorectal cancer (mCRC) to anti–epidermal growth factor receptor (EGFR) monoclonal antibodies has a genetic basis, being associated with increased *EGFR* gene copy number (GCN) as measured by fluorescence in situ hybridization (FISH) in individual tumors. The present study was aimed at assessing the predictive role of *EGFR* GCN, in terms of clinical outcome, in patients treated with panitumumab.

Patients and Methods
Patients with mCRC refractory to standard therapies were a subset of patients from a phase III trial of panitumumab plus best supportive care (BSC; n = 58) versus BSC alone (n = 34) who were selected on the basis of availability of tumor samples adequate for FISH.

Results
In patients treated with panitumumab, a mean *EGFR* GCN of less than 2.5/nucleus or less than 40% of tumor cells displaying chromosome 7 polysomy within the tumor predicted for shorter progression-free survival (PFS; $P = .039$ and $P = .029$, respectively) and overall survival ($P = .015$ and $P = .014$, respectively). None of the treated patients with mean *EGFR* GCN of less than 2.47/nucleus or less than 43% of tumor cells displaying chromosome 7 polysomy obtained objective response compared with six of 20 and six of 19 patients with values greater than these cutoff limits, respectively ($P = .0009$ and $P = .0007$, respectively). Evaluation of BSC-treated patients showed no correlation between *EGFR* GCN or chromosome 7 polysomy status and PFS.

Conclusion
In a larger and more homogeneous series than in previous studies, present exploratory data suggest that mCRC patients with tumors distinguishable by FISH analysis of *EGFR* as homogenously disomic or with low chromosome 7 polysomy have a reduced likelihood of response to panitumumab.

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INTRODUCTION

The anti–epidermal growth factor receptor (EGFR) monoclonal antibody (moAb) panitumumab is effective in prolonging survival in patients with metastatic colorectal cancer (mCRC) after failure of conventional chemotherapy.[1,2] Clinical evidence shows that approximately 10% of patients achieve objective tumor response to anti-EGFR moAbs.[1-3] The identification of patients who are likely to benefit from EGFR-targeted moAbs is increasingly crucial for improving therapeutic strategies, as well as for reducing the financial burden of health care systems.[4]

We previously reported that, in mCRC patients, objective tumor response to the EGFR-targeted moAbs cetuximab and panitumumab occurs in a fraction of patients whose tumors have increased gene copy number (GCN) of the *EGFR*, as assessed by fluorescent in situ hybridization (FISH).[5] The predictive role of *EGFR* GCN in mCRC was then evaluated in subsequent studies, where an association with objective tumor response[6] and overall survival (OS)[7] was demonstrated after treatment with cetuximab. Nevertheless, because of partial discrepancies and difficult technical reproducibility,[7] as well as because of the heterogeneity and paucity of evaluated patients, at the present

time, no predictive assay has reached the clinical setting to select candidates to anti-EGFR moAb treatment.

The present study was performed to clarify the predictive role of *EGFR* GCN, in terms of objective response, progression-free survival (PFS), and OS, in patients treated with panitumumab monotherapy. In a larger and more homogeneous patient series than previously performed, we analyzed tumors from patients treated in a randomized phase III trial comparing panitumumab and best supportive care (BSC) with BSC only for treatment of mCRC after failure of regimens containing a fluoropyrimidine, oxaliplatin, and irinotecan.[1,2] The opportunity to also study patients treated with BSC only has allowed us to assess the role of *EGFR* GCN as a prognostic factor.

PATIENTS AND METHODS

Patients

We assessed tumor samples from 64 mCRC patients treated in nine institutions in Italy according to the panitumumab (Amgen, Thousand Oaks, CA) phase III, open-label, randomized 408 trial and the panitumumab open-label continuation 194 trial. Patients had mCRC progressing on or after standard treatments and expressed EGFR by immunohistochemistry, as reported elsewhere.[1,2]

The subset of patients for the present study was selected among the 92 patients recruited in Italy based on the availability of tumor tissue adequate for further FISH analysis. Tumor response or resistance to therapy was confirmed radiologically by investigators according to Response Evaluation Criteria in Solid Tumors. Patients gave written informed consent for *EGFR* analysis and for receiving the study treatment. This study was authorized by the institutional ethical committee (amendment 4).

The association between *EGFR* GCN and objective tumor response, PFS, and OS was evaluated in 58 patients (33 men and 25 women; median age, 61 years; range, 44 to 78 years) who received panitumumab (408 trial or 194 continuation trial) and in 34 patients (21 men and 13 women; median age, 61 years; range, 44 to 76 years) who were initially randomly assigned to the BSC arm of the 408 trial. The latter group was evaluated for PFS only. Among the 58 patients who received panitumumab, 30 patients received panitumumab from the beginning, and 28 patients started in the BSC arm of the study and then, on progression, received panitumumab in the 194 trial. Among the 34 patients who were analyzed during BSC, six did not cross over to panitumumab. Thus, data from the 28 patients who crossed over to panitumumab treatment were analyzed twice regarding association between GCN and clinical outcome (ie, with and without panitumumab treatment; Fig 1). Total time at risk for calculating OS was 439 patient-months, and median follow-up time was 7.1 months (range, 0.8 to 17.7 months); cumulative time at risk for PFS was 183.5 months, with a median follow-up time of 1.8 months (range, 0.8 to 11.2 months).

Among patients treated with panitumumab (n = 58), six (10.4%) achieved objective tumor response (one complete response and five partial responses), 14 (24.1%) had stable disease, and 38 (65.5%) had progressive disease. Among patients treated with BSC only (n = 34), two (5.9%) demonstrated stable disease after 8 weeks, and 32 (94.1%) had progressive disease. Overall, this outcome is representative of the expected clinical benefit exerted by panitumumab both in the pivotal phase III 408 trial (overall response rate, 10%; PFS, 8 weeks; OS, 6.4 months) and in the 194 continuation trial (overall response rate, 11.6%; PFS, 9 weeks; OS, 6.3 months).[1,2]

FISH Analysis of EGFR *GCN*

Thin tissue sections (2 μm) were placed in a pretreatment solution for 10 minutes at 96°C, allowing them to cool in the same solution for 30 minutes at room temperature. After a wash in buffer solution, the slides were put into pepsin solution for 30 minutes at 37°C. Tissue sections were then stained with a propidium iodide solution (0.6 μg/mL; QBiogene, Irvine, CA) to verify whether nuclei of tumor cells were adequately digested for a subsequent optimal permeabilization of probes. If enzymatic digestion was considered incomplete, further steps of tissue digestion were performed at intervals of 15 minutes. Dual-color, dual-target FISH assays were performed as previously described.[4] Analysis was performed with the Imager Z1 fluorescence microscope (Zeiss, Gottingen, Germany) and the ISIS FISH Imaging System (MetaSystems, Altlussheim, Germany) for acquisition and elaboration of images. *EGFR* gene, which is located on the short arm of chromosome 7, was visualized as a red signal with a tetramethylrhodamine isothiocyanate filter, whereas the α-centromeric probe of chromosome 7 (CEP7) was visualized as a green signal with a fluorescein isothiocyanate filter, and nuclei were visualized as a blue signal with 4,6-diamidine-2-phenylindole filter. Representative images of samples were acquired by the CV-M4 digital double-speed megapixel progressive scan camera (Jai Europe, Copenhagen, Denmark) as monochromatic layers that were subsequently merged by the ISIS software. At least 200 nonoverlapping interphasic nuclei were scored for the number of *EGFR* and CEP7 copies at $\times$400 magnification, after initial overlook at $\times$200 magnification, to detect the pattern of fluorescence. *EGFR* gene status was scored as the average number of *EGFR* red signals per nucleus and as the ratio between *EGFR* red signals and CEP7 green signals. Normal controls consisted of a cultured retinal pigment epithelial cell line and healthy-appearing colorectal mucosa contiguous to the malignant component for each patient, whereas the positive control for amplified *EGFR* was the A431 cell line. Clinical outcome relative to 10 patients reported in a previous study[4] using a different FISH methodology was known by pathologists before present analysis. Polysomy of *EGFR* gene consisted of an increase of *EGFR* red signals ($\geq$ three signals per nucleus) paralleled by the same increase of chromosomes 7 (on which the *EGFR* gene is located) as measured by the number of CEP7 green signals per nucleus.

Statistical Analyses

The present study was promoted by the coordinating center of the 408 and 194 panitumumab trials in Italy. Participation was offered to 10 recruiting centers, and nine of these centers provided 64 samples of 92 total enrolled patients in Italy. Sample size of the study was determined by the highest

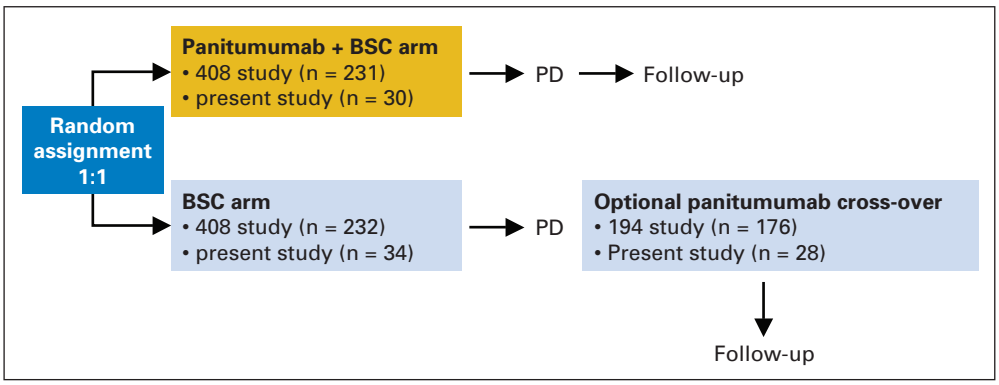

Fig 1. Patient disposition. Patients with metastatic colorectal cancer refractory to standard therapies evaluated in the present study were a subset of patients enrolled onto the 408 phase III trial of panitumumab plus best supportive care (BSC) versus BSC alone.[1] Twenty-eight of 34 patients who were randomly assigned to the BSC arm of the study were allowed, at progression of disease (PD), to receive panitumumab in the 194 continuation study trial.[2]

Table 1. Objective Tumor Response and *EGFR* Gene Copy Number Evaluated by FISH in Tumors of Patients With Metastatic Colorectal Cancer Treated With Panitumumab

	FISH Analysis			
	EGFR Gene Copy Number Ratio			
Patient No.	CEP7	Nucleus	Chromosome 7 Polysomy (%)	Best Objective Response
1	1.13	2.47	50	PR
2	1.03	3.29	75	PR
3	1.01	4.04	85	SD
4	1.10	3.84	70	PR
5	1.03	3.67	65	PR
6	1.04	1.88	0	SD
7	1.06	2.16	0	SD
8	1.00	3.48	85	PD
9	1.00	2.76	55	SD
10	0.91	1.70	0	PD
11	1.02	2.00	0	PD
12	1.03	2.00	0	PD
13	1.18	2.10	0	PD
14	0.92	1.90	0	PD
15	1.00	1.88	0	PD
16	0.99	1.90	0	PD
17	0.97	1.68	0	PD
18	0.94	1.60	0	PD
19	0.91	1.78	5	PD
20	0.97	1.73	0	PD
21	1.08	1.98	0	PD
22	1.01	3.51	85	PD
23	1.04	2.37	40	PD
24	0.90	1.84	0	SD
25	0.90	1.84	0	SD
26	1.00	1.67	0	PD
27	0.81	1.50	0	PD
28	1.05	1.65	0	PD
29	1.05	2.10	5*	SD
30	0.97	1.20	0	PD
31	1.03	2.50	43	PR
32	0.96	1.34	0	PD
33	1.02	3.23	65	CR
34	0.98	2.23	10	PD
35	1.01	3.38	75	PD
36	1.21	2.72	50	PD
37	1.05	2.70	50	PD
38	1.01	3.68	90	SD
39	1.01	2.85	65	SD
40	1.09	2.00	0	PD
41	1.01	2.01	10	PD
42	1.03	1.80	10	SD
43	1.00	1.79	0	PD
44	1.03	3.26	80	PD
45	1.03	2.00	15	SD
46	1.03	3.03	65	PD
47	1.05	3.32	75	SD
48	1.10	1.97	0	PD
49	1.04	2.02	20	SD
50	1.07	3.64	65	PD
51	1.02	2.40	38	PD
52	0.97	1.88	0	PD
53	0.97	1.70	0	PD
54	1.09	1.62	0	SD
55	1.01	2.29	40	PD
56	1.03	2.20	5*	PD
57	1.07	2.57	40	PD
58	1.11	2.12	15	PD

NOTE. *EGFR* gene status was evaluated as the mean value of *EGFR* gene/nucleus, as the mean value of *EGFR* gene/CEP7 (see Patients and Methods), and as the percentage of chromosome 7 polysomy (≥ 3 signals per nucleus) scoring 200 tumor cells. Tumor response was evaluated by Response Evaluation Criteria in Solid Tumors.

Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; CEP7, α-centromeric probe for chromosome 7; PR, partial response; SD, stable disease; PD, progressive disease; CR, complete response.

*Focal area of amplification.

number of patients collected based on availability of tumor specimens suitable for FISH analysis. All data were first analyzed and described by mean and standard deviation or by median and range, according to their distribution. Binomial end points have been analyzed by means of univariate logistic regression; models as a whole were evaluated by likelihood ratio test and by their pseudo-R^2 measure, whereas the significance of the single independent variables was evaluated by means of the Wald test. For binomial independent variables, a further cross-tabulation followed by the Fisher's exact test was also carried out. All statistical tests were two sided. Time-to-event analysis was performed using the Kaplan-Meier product-limit method; the equality of the survivor function was then evaluated using the log-rank test. For patients who received panitumumab, OS was defined as the time from random assignment onto the panitumumab arm of protocol 408 or, for those who crossed over, from the optional assignment onto protocol 194 until death from any cause, censoring patients who had not died at the date last known alive; PFS was defined as the time from assignment onto protocols until tumor progression. For patients treated with BSC only, PFS was defined as the time from random assignment onto the BSC arm of the 408 trial to progression.

Receiver operating characteristic (ROC) curve analysis was carried out to determine a possible cutoff point for the *EGFR* GCN continuous variable; for each value, sensitivity, specificity, and total accuracy were obtained as percentages. Statistical significance was set at $P < .05$ for each analysis; all analyses were carried out using STATA SE 9.2 software (STATA Corp, College Station, TX) running on a Windows XP machine (Microsoft, Redmond, WA).

RESULTS

Results of *EGFR* gene analysis by FISH in 58 patients with mCRC treated with panitumumab are listed in Table 1; mean values of *EGFR* GCN and the percentage of cells displaying chromosome 7 polysomy (*EGFR* gene/nucleus ≥ 3) and/or *EGFR* gene amplification (*EGFR* gene/CEP7 ≥ 2) are reported. None of the tumors was found to show homogeneous amplification of the *EGFR* gene, whereas two patients showed focal areas of amplification in $\leq 5\%$ of tumor area. Figure 2 shows representative patterns of *EGFR* gene signals evaluated by FISH. The hypothesis of an association between *EGFR* GCN and objective response to panitumumab was first evaluated with logistic regression, showing a statistically significant positive correlation between increase in mean GCN and probability of response (odds ratio = 5.62; 95% CI, 1.506 to 20.974). This model showed a 98.1% specificity (95% CI, 89.7% to 99.9%), and therefore, it seems particularly suited to identify nonresponders (89.5% negative predictive value; 95% CI, 78.5% to 96.0%).

A ROC analysis was then set up to define a cutoff value of mean *EGFR* GCN (Fig 3). The value of ≥ 2.47 *EGFR* copies/nucleus emerged as the best cutoff value to discriminate responders versus nonresponders to panitumumab, with an overall accuracy of 75.9% (95% CI, 62.8% to 86.1%). None of the patients had tumor response when the *EGFR* GCN was less than this value, thus accounting for a sensitivity of 100% (95% CI, 54.1% to 100%), whereas six of 20 patients with *EGFR* GCN ≥ 2.47/nucleus achieved objective response ($P = .0009$; Table 2). Given these findings, we pragmatically elected a GCN ≥ 2.5/nucleus as the cutoff value for subsequent survival analysis. Log-rank test showed a significant difference favoring, for both PFS and OS, those patients with tumors with the increased GCN values ($P = .039$ and $P = .015$, respectively; Fig 4).

Because of the nonhomogeneous pattern of *EGFR* GCN in individual mCRC tumors, frequently showing variable ratios of disomy versus polysomy of chromosome 7 and/or *EGFR* gene amplification, we elected to also evaluate GCN as the percentage of cells displaying

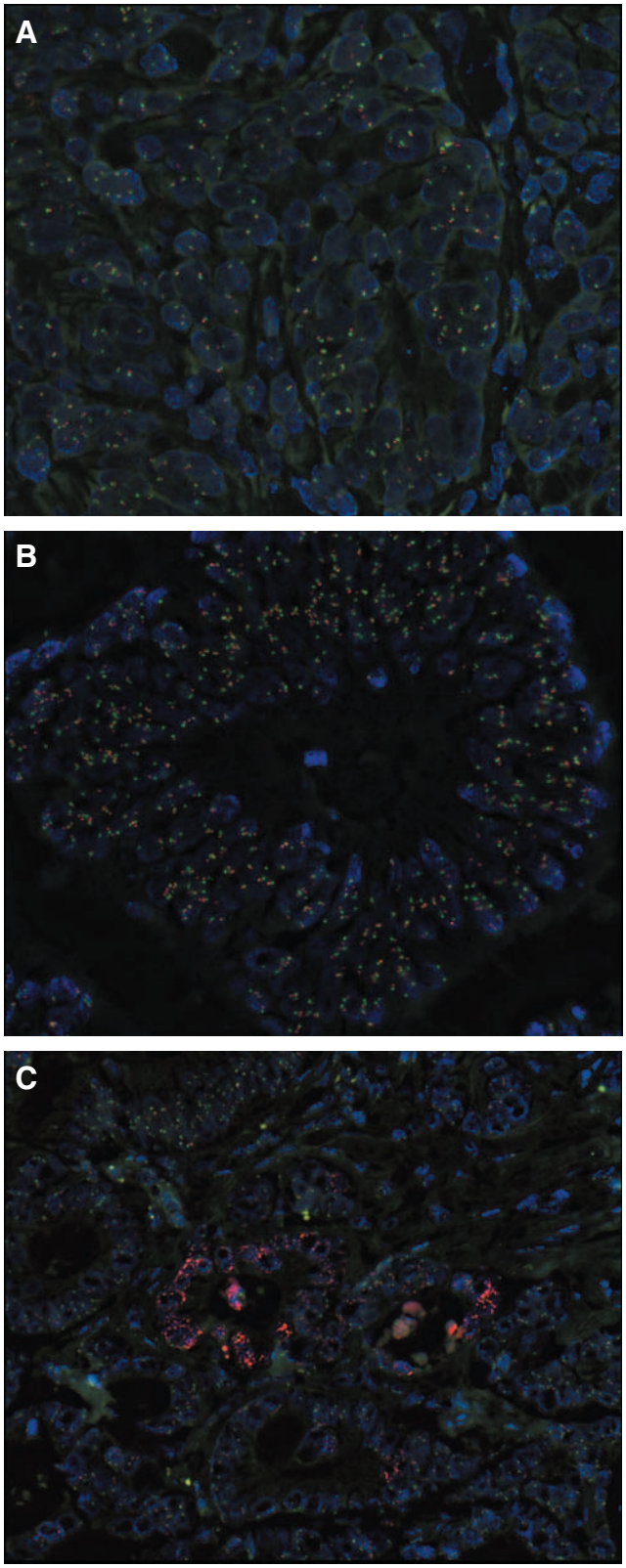

Fig 2. Representative fluorescence in situ hybridization (FISH) analyses showing the following microscopic patterns (magnification ×200): (A) no gain in *EGFR* gene copy number, with a homogeneous disomic pattern (patient 48); (B) increase in *EGFR* gene copy number by polysomy of chromosome 7 (patient 4); and (C) *EGFR* gene focal amplification in less than 5% of tumor cells (patient 29).

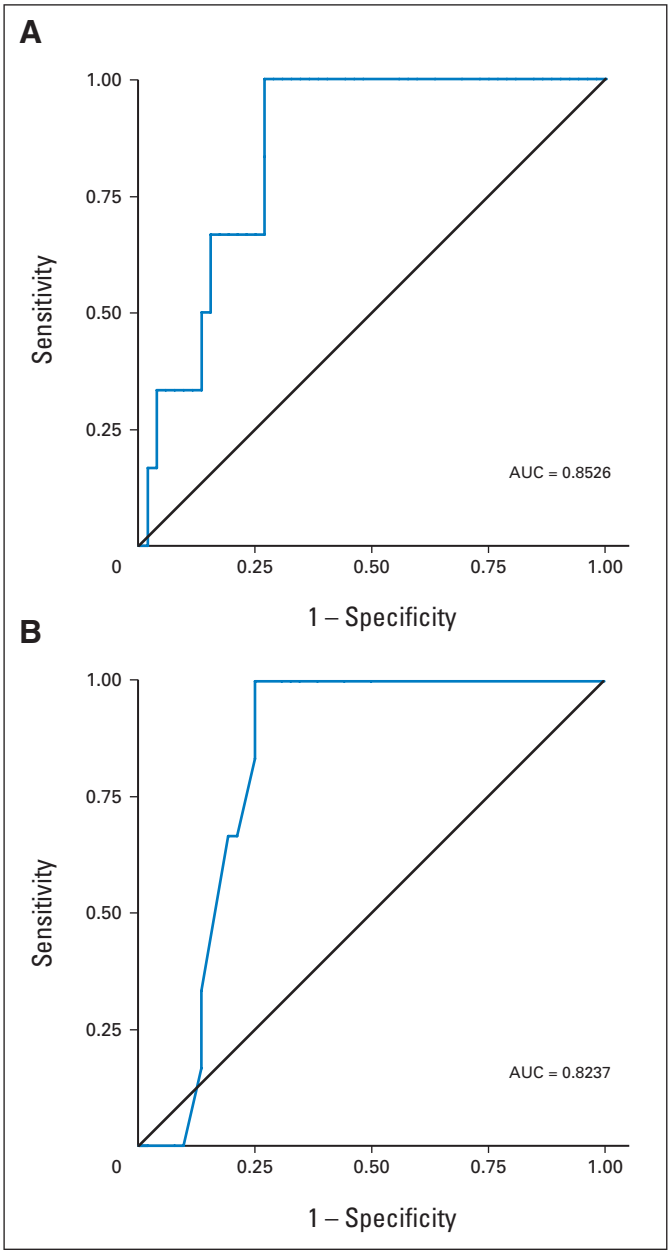

Fig 3. Receiver operating characteristic analysis relative to logistic model based on mean (A) *EGFR* gene copy number or (B) fraction of cells displaying chromosome 7 polysomy, with objective tumor response to panitumumab as the end point. Both models achieve specificity of approximately 75% and maintain 100% sensitivity. AUC, area under the curve.

Table 2. Objective Tumor Response of Patients With Metastatic Colorectal Cancer Treated With Panitumumab According to the Proposed Cutoff Values Estimated by Receiver Operating Characteristic Analysis

Cutoff	Best Tumor Response (No. of patients)		Total No. of Patients
	PD + SD	CR + PR	
EGFR gene copy number*			
< 2.47	38	0	38
≥ 2.47	14	6	20
Total	52	6	58
Chromosome 7 polysomy or amplification†			
< 43%	39	0	39
≥ 43%	13	6	19
Total	52	6	58

Abbreviations: PD, progressive disease; SD, stable disease; CR, complete response; PR, partial response; EGFR, epidermal growth factor receptor.
*Fisher's exact test, $P = .0009$.
†Fisher's exact test, $P = .0007$.

chromosome 7 polysomy and/or *EGFR* gene amplification (Table 1). Also applying this criteria, an increase in the percentage of chromosome 7 polysomy was significantly associated with probability of response (odds ratio = 1.04; 95% CI, 1.007 to 1.074). In other terms, this is equivalent to say that 1 unitary increase in percent polysomy corresponds with a 4% increase in odds of response. Analogously to mean *EGFR* GCN, this model shows a 100% specificity (95% CI, 93.2% to 100%), with a high negative predictive value (89.7%; 95% CI, 78.8% to 96.1%). A suitable cutoff value of 43% of chromosome 7 polysomy was determined by ROC analysis, with an overall accuracy of 77.6% (95% CI, 64.7% to 87.5%) and a sensitivity of 100% (95% CI, 54.1% to 100%). According to this cutoff, six of 19 patients with chromosome 7 polysomy ≥ 43% achieved objective response compared with none of 39 patients with chromosome 7 polysomy less than 43% ($P = .0007$; Table 2). Assuming ≥ 40% as the cutoff, Kaplan-Meier curves showed better PFS and OS for patients with ≥ 40% of chromosome 7 polysomy ($P = .029$ and $P = .014$, respectively; Fig 4). Homogeneous chromosome 7 disomy was observed in 26 of 58 patients and represented the most frequent pattern among tumors with nonincreased *EGFR* GCN. Survival analysis of 34 patients treated with BSC without panitumumab (control arm of the phase III panitumumab 408 trial) showed no benefit in PFS for patients with either a mean *EGFR* GCN ≥ 2.5/nucleus or a percentage of chromosome 7 polysomy ≥ 40% (Fig 5).

DISCUSSION

We previously showed that, in mCRC, objective tumor response to the EGFR-targeted moAbs cetuximab and panitumumab is associated with increased GCN of *EGFR* as assessed by FISH in individual tumor samples.[5] Subsequently, in 30 patients with mCRC, Liévre et al[6] confirmed this finding by chromogenic in situ hybridization. Both studies were not conclusive because they evaluated limited patient series that were nonhomogeneously treated (ie, receiving cetuximab or panitumumab, single-agent moAb, or the latter in combination with chemotherapy, or moAb therapy as first-line or subsequent treatment). In a study by Lenz et al,[7] evaluation of *EGFR* GCN was performed on 34 patients by polymerase chain reaction and showed a lack of association of increased GCN with objective responses and PFS but a significant positive correlation with OS. The same authors concluded that such discrepancies with the previous findings of others[5,6] could be a result of different techniques (ie, FISH *v* polymerase chain reaction). As proposed in pathology studies, uncertain reproducibility may be explained by dilution of tumor lysates by nonmalignant tissue or sampling limitations.[8,9] Moreover, Lenz et al[7] speculate that the association of increased *EGFR* GCN with OS may reflect its role as an independent prognostic variable.

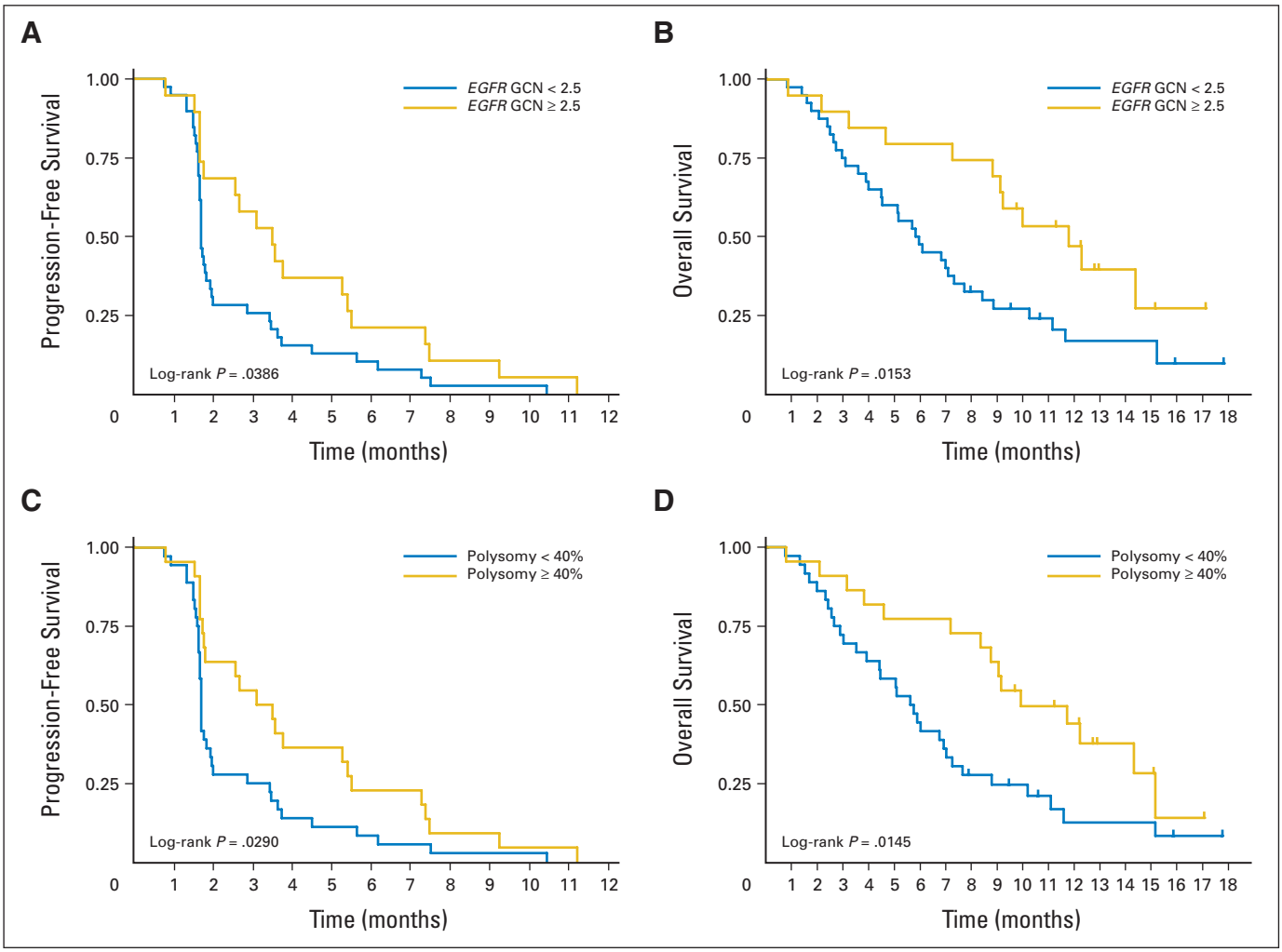

Fig 4. Progression-free survival and overall survival in metastatic colorectal cancer patients treated with panitumumab according to the proposed cutoff values of (A and B) *EGFR* gene copy number (GCN)/nucleus < 2.5 (n = 39) versus ≥ 2.5 (n = 19) and (C and D) < 40% fraction of chromosome 7 polysomy (n = 36) versus ≥ 40% (n = 22) as evaluated by fluorescence in situ hybridization in individual tumor specimens.

The present study was carried out in a homogeneous series of patients receiving panitumumab and BSC or BSC only within a single clinical trial setting of mCRC after failure of regimens including irinotecan and oxaliplatin. Our data produced mean values of ≤ 2.5 *EGFR* GCN/nucleus and ≤ 40% chromosome 7 polysomy as suitable cutoff values to identify patients who are less likely to respond to panitumumab, thus generating the hypothesis that these tumors are probably not driven by (not addicted to) the EGFR pathway and thus less susceptible to respond to panitumumab. In our series, the objective response rate in patients with tumors with mean ≥ 2.5 *EGFR* GCN/nucleus or ≥ 40% chromosome 7 polysomy was three times higher than in the unselected population (ie, approximately 30% *v* 10%, respectively). None of the responsive patients had tumors with *EGFR* GCN less than these cutoff values. Consistent with response rates, analyses of PFS and OS confirmed better clinical outcome for patients harboring increased GCN as defined by these two parameters. Because of the limited patient number evaluated in the present study, these cutoff values should be considered as exploratory and possibly useful for larger studies. In particular, their validation could take place by a new ROC analysis on a wider sample, paralleled by the application of present cutoffs values to evaluate prospectively if their sensitivity and specificity are confirmed.

Interestingly, in patients treated with BSC only, analysis of PFS did not show differences between increased and not increased *EGFR* GCN, thus indicating a predictive, rather than prognostic, value of this biologic characteristic. Analysis of OS among patients treated with BSC only was not performed because the cross-over design of the study permitted subsequent treatment with panitumumab on progression.

Different studies have described the nonhomogenous *EGFR* GCN pattern by FISH in mCRC specimens, potentially hampering the reproducibility of results of this analysis.[8,9] FISH analysis of *EGFR* in mCRC turned out to be different from *HER-2* evaluation in breast cancer, where specimens with increased copy number are mainly homogeneous and show clustered signals of *HER-2* gene amplification.[9] In our series, mCRC specimens with increased *EGFR* GCN frequently presented a nonhomogenous pattern, with variable ratios of chromosome 7 disomy versus polysomy and a low percentage of *EGFR* gene amplification that mainly occurred in focal areas (Fig 2). Given these features, in the attempt to improve technical quality and

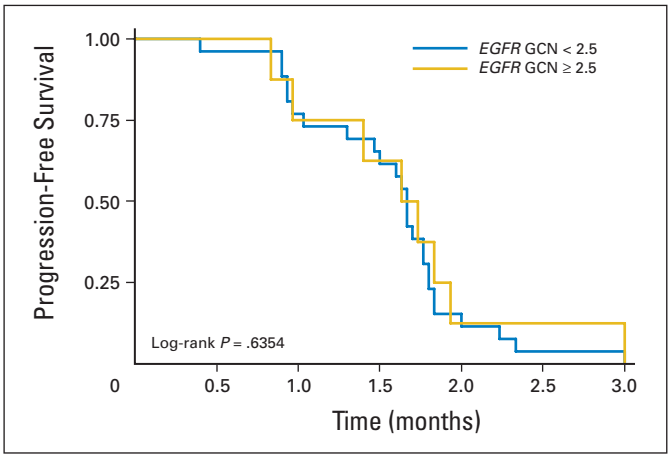

Fig 5. Progression-free survival in patients treated with best supportive care without panitumumab according to the proposed cutoff value of < 2.5 *EGFR* gene copy number (GCN)/nucleus (n = 26) versus ≥ 2.5 (n = 8). The same patients harboring increased *EGFR* GCN (≥ 2.5 copies/nucleus) also displayed a fraction of chromosome 7 polysomy ≥ 40%, thus demonstrating no difference in progression-free survival according to both cutoff values (P = .6354).

reproducibility of FISH results, we evaluated a high number of cells (at least 200 for each specimen) in thin sections of 2 μm to avoid overlapping of nuclei. Furthermore, we elected to evaluate specimens not only as mean *EGFR* GCN/nucleus but also in terms of fraction of chromosome 7 polysomy within the whole tumor specimen. Most of the tumors with nonincreased *EGFR* GCN displayed homogeneous disomy (26 of 58 patients had 100% chromosome 7 disomy), and according to our opinion, this pattern is more easily identifiable and assessable from a morphologic point of view. Because analysis of data revealed that the overall model is especially powerful to select patients less likely to respond to panitumumab by disomy, the clinical application of *EGFR* FISH analysis as predictive factor could be more effective than expected, mainly consisting of the detection of chromosome 7 disomy.

In our previous cohort study,[5] we described the association of increased GCN with tumor response. The data presented in the present article confirm that nonincreased *EGFR* GCN is associated with failure of response to anti-EGFR moAb therapy. In contrast, new evidence is presented indicating that only a fraction of tumors with increased *EGFR* GCN achieves objective response. The discrepancy with our previous findings is likely a result of the clinical enrichment strategy that was conducted to evaluate responsive patients who consistently were found to have tumors with increased *EGFR* GCN. Furthermore, in our previous study, we reported higher prevalence of *EGFR* amplification than in this study. This is because the aforementioned nonhomogeneous pattern of focal amplification was, in some cases, previously scored as amplified. In the present study, the use of a more sensitive apparatus allowed pathologists to discriminate signals even at low magnification, thus ensuring the evaluation of the whole tissue section.

It was recently demonstrated that *KRAS* and/or *BRAF* mutations in mCRC are predictors of resistance to the anti-EGFR moAbs cetuximab and panitumumab and are associated with a worse prognosis.[5,6,10] These genes are indeed cellular effectors that act downstream of epidermal growth factor signaling, and their malignant activation caused by mutations can independently impair the inhibitory effect of anti-EGFR moAbs, as elucidated in a cellular model where transfection of the G12V *KRAS* mutation reverted sensitivity to cetuximab.[10] In the future, the detection of *KRAS* and/or *BRAF* mutations in mCRC together with *EGFR* GCN could provide a better understanding of the molecular pathways that can be clinically exploited in individual mCRC patients for optimization of anti-EGFR moAb therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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