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ORIGINAL ARTICLE

Epidermal growth factor receptor serum (sEGFR) level may predict response in patients with EGFR positive advanced colorectal cancer treated with gefitinib?

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Abstract
Purpose Epidermal growth factor receptor-overexpression reported in colorectal cancer, justifies therapeutic use of EGFR-inhibitors. We have recently conducted a phase II study in 57 patients with EGFR-positive advanced colorectal cancer (ACC) who received gefitinib-FOLFOX6 followed by gefitinib-single agent as maintenance. Main biological objective was to assess sEGFR as surrogate marker of tyrosine kinase inhibition and as predictor of response.
Methods sEGFR, evaluated by quantitative ELISA, was investigated as predictive factor both taking into account the basal value only, and its whole pattern over time. sEGFR was collected at baseline and at every 2-months assessment in 42 cases. Thirty-three patients reported CR/PR as best objective response (BOR), while nine showed SD/PD.
Results Retrospectively, on average, the sEGFR values reported by both responders (CR/PR) and not responders (SD/PD) were already different at baseline (49.4 ± 6.2 and 42.4 ± 8.4 ng/ml respectively). This difference was statistically significant ($p = 0.042$). Although sEGFR trend over time confirmed the basal difference ($p = 0.032$), this result should be taken with caution, due to the small number of patients reporting EGFR values besides the basal one.
Conclusions Higher sEGFR at baseline was associated to BOR and may be considered a significant predictor of outcome in patients with ACC.

Keywords Serum EGFR · Gefitinib · Advanced colorectal cancer · Chemotherapy

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Introduction

The Epidermal growth factor receptor or Human Epidermal Growth Factor Receptor 1 (HER1) is a 170-kDa polypeptide tyrosine kinase growth factor receptor. Along with HER2, HER3 and HER4, it is a member of the HER receptor family and an important mediator of cell proliferation, differentiation, and survival. Binding of ligand leads to dimerization of the receptor with another HER molecule, followed by autophosphorylation of intracellular tyrosine residues. Signal transduction cascades via the Ras, ERK1/2, PI3K/Akt, and STAT pathways are activated promoting cellular proliferation, adhesion, differentiation, angiogenesis, and apoptosis [31].

EGFR overexpression has been demonstrated in up to 60 to 80% of colorectal cancer and has been associated to worse prognosis and decreased survival [22]. Nowadays therapeutic agents that target EGFR constitute an important progress in the treatment of metastatic disease and include

monoclonal antibodies, such as cetuximab and panitumumab, while the role of small-molecule tyrosine-kinase inhibitors has to be still determined [8, 24, 32].

Gefitinib (ZD1839, Iressa; AstraZeneca Pharmaceuticals, Wilmington, Del) is a potent orally-active small-molecule inhibitor of tyrosine kinase domain of EGFR. This agent has demonstrated ability to inhibit tumor growth and to control development of metastases in several human cancer cell lines expressing high levels of EGFR and human tumor xenografts including also colon cancer [5, 34].

In pre-clinical model the inhibition of EGFR signalling pathway can sensitize tumor cells to chemotherapy and combination of gefitinib with cytotoxic agents can enhance antitumor activity of chemotherapy in vivo and in vitro [5, 28].

In clinical setting, gefitinib monotherapy has shown antitumor activity in patients with tumors of epithelial origin such as head and neck cancers and in a subgroup of heavily pre-treated cases with recurrent or refractory non-small-cell lung cancer (NSCLC) [10]. Despite first encouraging results, two subsequent phase III trials that randomized previously untreated patients with advanced NSCLC to standard platinum-based chemotherapy (INTACT-1, cisplatin and gemcitabine; INTACT-2, carboplatin and paclitaxel), ± gefitinib failed to demonstrate a difference in response rate, time to progression, or 1-year or overall survival with combined treatment [11, 14].

Recent experiences of gefitinib combined with standard regimen containing fluorouracil and oxaliplatin has further suggested the role of this small-molecule to enhance the antitumor efficacy of chemotherapy in patients with ACC pre-treated or not [17, 35].

While the degree of EGFR expression does not seem to influence outcome, unclear remains the role of EGFR overexpression in predicting response in patients treated with chemotherapy combined with gefitinib [4].

Serum EGFR (sEGFR) that is the extracellular binding domain of EGFR detected in blood samples either in healthy individuals either in cancer patients, is evaluated in some clinical trials on lung, breast, ovarian cancers, as surrogate marker of prognosis [2, 12, 29].

In the experience conducted on patients affected by lung cancer, sEGFR values were modified during treatment with gefitinib and were associated with response, progression-free survival, regardless of performance status, stage, and histology [12].

In another study conducted on patients with metastatic breast cancer, in a multivariate analysis a decreased sEGFR level remained a significant prognostic factor for decreased survival [29].

Within this scenario, the topic of our report is to evaluate in patients with ACC selected for positive tissue EGFR overexpression, the correlation between sEGFR, patients characteristics and outcome [35, 36].

Patients and methods

Patient selection

Patients with newly diagnosed or recurrent ACC not pre-treated for metastatic disease who have positive tumor cells stained for EGFR overexpression, were enrolled in a multicenter phase II trial.

Eligible patients had histologically confirmed adenocarcinoma arising in the colon or rectum and radiographic evidence of synchronous or metachronous metastatic disease. Histological EGFR assessment was performed in at least one metastatic site and all specimens were reviewed by a centralized laboratory. Other patient's characteristics were described in our previous report [35].

Methodology for EGFR determinations

EGFR expression in tissue was detected by DAKO-test, as previously reported [35].

Sample collection and serum EGFR determination

Serum levels for EGFR were determined at baseline and every tumor assessment. Samples were aliquoted and stored at −80°C until use. The EGFR extracellular binding-domain was evaluated by quantitative enzyme-linked immunoadsorbent (Oncogene Science Biomarker Group, part of Siemens Medical Solutions Diagnostics, Cambridge, MA, USA), a sandwich immunoassay with a mouse monoclonal capture antibody and an alkaline phosphatase-labeled mouse monoclonal antibody as detector. Both capture and detector reagents specifically recognize the extracellular domain of EGFR.

As a control group we tested 38 healthy subjects: mean serum EGFR values were 58.4 ng/ml (standard deviation [SD] 7.3; range 45.4–75.0).

Study design

All patients received gefitinib concomitantly to the first cycle of chemotherapy.

Combination therapy in details was: Gefitinib 250 mg orally once daily continuously plus FOLFOX-6 recycled every 14 days. Tumor assessment and biopsy for histological examination and EGFR were performed within 3 weeks before study entry.

Blood tests including sEGFR and tumor markers were performed within 1 week before study start were repeated every 2 months concomitantly to tumor re-assessment.

Patients with not-progressive disease after a maximum of 10 FOLFOX-cycles continued on gefitinib monotherapy.

Statistical methods

Baseline characteristics of study patients were summarized in terms of frequencies and percentages for categorical variables and by the mean ± standard deviation and median, minimum, and maximum values for continuous variables. Predictive value of baseline sEGFR for binary best overall response (CR + PR vs. SD + PD) was modelled by the multivariate logistic regression model [13, 15]; when using sEGFR as time-dependent covariate (i.e., when using the whole sEGFR profile instead of the baseline value only), its predictive value was estimated by the Cox's regression model [7]. Univariate analysis for progression-free survival (PFS) and overall survival (OS) were analysed by the Kaplan–Meier approach and the association with predictors evaluated by the log-rank test [23]. Multivariate analysis on PFS and OS were performed by the proportional hazard Cox's model. OS was measured from the start of the treatment to death from any cause and censored to the last follow up. PFS was measured from the start of the treatment to relapse or death from any cause and censored to the last follow up. To guarantee a minimum of stability of the estimates produced by the multivariate analysis, the choice of the number of possible predictors to include into each multivariable model was related to the number of reported events [13]. For this reason each multivariable model included centre, age, gender, and site of primary tumor as the only adjusting factors. Serum-EGFR and CEA at baseline were evaluated both as continuous and categorical classification. The pertinent cut-offs (46 ng/ml and 30 ng/ml respectively) were detected by the area under the ROC (Receiver Operating Characteristics) curve approach, and were identified as those maximizing sensitivity and specificity. Statistical analysis were performed using the SAS statistical software (version 8.02 for windows).

Results

Between January 2003 and December 2004, 57 patients enrolled into the study: 42 patients were considered evaluable for biological endpoint. Data were missing in fifteen due to collecting failure or uncorrected collection timing. Baseline characteristics of 42 patients are listed in Table 1. Thirty-one patients (73.8%) had synchronous metastatic disease. The most common distant localization was in liver and in 29 cases (69%) only one disease site was documented. The median number of courses was 8 (range 1–12): one patient, who achieved PR after 8 courses of chemotherapy, received 12 total cycles. Median duration of treatment was 29 weeks (range 1–141).

Seventy-three percent of patients received gefitinib maintenance-treatment as single-agent for a median period of 16.1 weeks (range 3.7–46.4).

Best response obtained was CR in 3 patients (7.2%), PR in 30 (71.4%), SD in 8 (19.0%) and PD in 1 case (2.4%). Best overall response, was obtained in 33 patients (78.6% 95% CI 66.1–99.0%) (Table 2).

Table 1 Baseline characteristics (42 patients valid for efficacy analysis)

Variables	*N* (%)
Center	
Milan—EIO	24 (57.1%)
Ancona	13 (31.0%)
Siena	5 (11.9%)
Age (years)	
Mean ± SD	57.8 ± 9.5
Median (range)	57 (38–76)
≤60	26 (61.9%)
>60	16 (38.1%)
Gender	
Male	21 (50.0%)
Female	21 (50.0%)
Site of primary tumor	
Rectum	8 (19.0%)
Colon-Sigma	34 (81.0%)
Performance status (ECOG)	
0	28 (66.7%)
1	11 (26.2%)
2	3 (7.1%)
Metastatic sites (lung, liver, peritoneal effusion)	
1	29 (69.0%)
≥2	13 (31.0%)
Stage at diagnosis	
Synchronous	31 (73.8%)
Metachronous	11 (26.2%)
Previous Chemotherapy	
No	35 (83.3%)
Yes	7 (16.7%)
Serum EGFR at baseline (ng/ml)	
>46	25 (59.5%)
≤46	17 (40.5%)
Mean ± SD	47.9 ± 7.3
Median (range)	47.9 (23.3–6.3)
CEA at baseline (ng/ml)	
≤30	14 (33.3%)
>30	16 (38.1%)
Not collected	12 (28.6%)
Median (range)	55.0 (0.4–2863.0)

Table 2 Predictors for best overall response

	Best overall response		Multivariate analysis	
	CR + PR (N = 33)	SD + PD (N = 9)	OR (95% CI)	p (Wald)
Gender				
Males	17 (80.9%)	4 (19.1%)		
Females	16 (76.2%)	5 (23.8%)		0.71[a]
Age (years)				
≤60	21 (80.8%)	5 (19.2%)		
>60	12 (75.0%)	4 (25.0%)		0.67[a]
Site of primary tumor				
Rectum	7 (87.5%)	1 (12.5%)		
Colon-Sigma	26 (76.5%)	8 (23.5%)		0.48[a]
Performance status (ECOG)				
0	24 (85.7%)	4 (14.3%)		
1–2	9 (64.3%)	5 (35.7%)		0.17[b]
Metastatic sites				
1	23 (79.3%)	6 (20.7%)		
≥2	10 (76.9%)	3 (23.1%)		0.59[b]
Stage at diagnosis				
Synchronous	23 (74.2%)	8 (25.8%)		
Metachronous	10 (90.9%)	1 (9.1%)		0.25[b]
Previous Chemotherapy				
No	26 (74.3%)	9 (25.7%)		
Yes	7 (100%)	0 (–)		0.13[b]
CEA at baseline (ng/ml)				
≤30	13 (92.9%)	1 (7.1%)		–
>30	11 (68.7%)	5 (31.3%)		0.15[b]
Not collected	9 (75.0%)	3 (25.2%)		–
Serum EGFR at baseline (ng/ml)				
Categorical classification				
>46	23 (92.0%)	2 (8.0%)	1	–
≤46	10 (58.8%)	7 (41.2%)	10.2 (1.4–74.0)	0.022[b]
Linear trend (continuous)				
Mean ± SD	49.4 ± 6.3	42.4 ± 8.4	0.84 (0.71–0.99)	0.042[b]

[a] Adjusted by centre

[b] Adjusted by age, gender, site of primary tumor and centre

Samples for sEGFR at baseline, 2 and 4 months post-treatment start, were obtained from 42, 28 and 24 patients respectively. During mono-therapy phase (6 and 8-months after the beginning of treatment) the patients with serum samples decreased to 16 and 2 respectively (Fig. 1).

The patients were separated into two groups: those who, at any time during treatment, reached an objective response (CR/PR) and those who didn't (SD/PD).

No significant correlations between major patient characteristics in our study and response to therapy were reported (data not shown).

Retrospectively, on average, the sEGFR values reported by both responders (CR/PR according to RECIST criteria) and not responders (SD/PD according to RECIST criteria) were already different at baseline (49.4 ± 6.2 and 42.4 ± 8.4 ng/ml respectively). This difference was statistically significant. As a matter of fact, the multivariate model revealed sEGFR as the single significant predictor for BOR (p = 0.042, OR = 0.84: 95% CI 0.71–0.99, Table 3). The meaning of this result was that the greater the serum EGFR at baseline, the lower the risk of no clinical response (SD or PD). In other terms, for each EGFR increase of a unit, corresponded a 16% decrease of the risk of no response to treatment. This difference, despite the small number of patients providing sEGFR values from the 4th month of treatment onwards, was confirmed by using sEGFR as time-dependent covariate (p = 0.032, Fig. 1).

No further predictor of positive response to treatment but sEGFR at baseline was found (Table 2).

No correlation was detected between EGFR overexpression in the tumor tissue (DAKO-test: 1+ in 23, 2+ in 4 and 3+ 15 cases) and the sEGFR at baseline (p = 0.97 if sEGFR

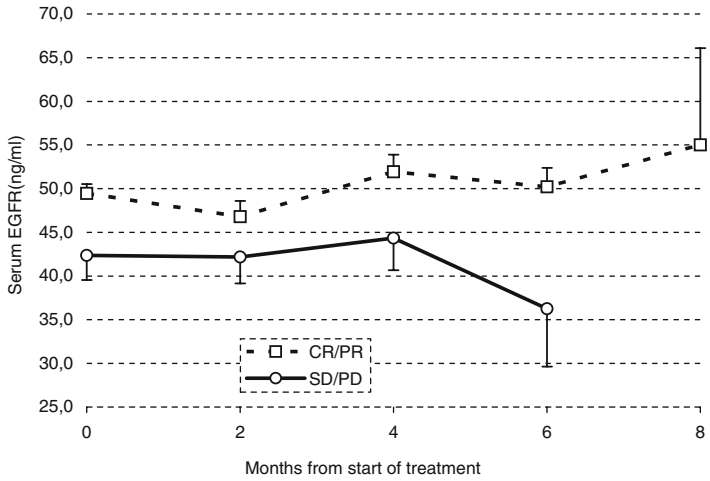


Fig. 1 Serum Epidermal Growth Factor Receptor (ng/ml) at baseline (time 0) and during treatment (month 2, 4, 6, 8) according to tumor response

BEST OVERALL RESPONSE	Mean±SD (n)	Mean±SD (n)	Mean±SD (n)	Mean±SD (n)	Mean±SD (n)
CR/PR	49.4 ± 6.2 (33)	46.8 ± 8.2 (21)	51.9 ± 8.7 (20)	50.2 ± 7.8 (13)	55.0 ± 15.7 (2)
SD/PD	42.4 ± 8.4 (9)	42.2 ± 8.0 (7)	44.3 ± 7.3 (4)	36.3 ± 11.5 (3)	- (0)
p-value (baseline value only)	0.042				
p-value (whole sEGFR profile)			0.032		

as categorical variable; $p = 0.82$ if sEGFR as continuous variable)

Concerning sEGFR at baseline, besides considering their original values, the patients were classified into the two over-mentioned categories. The cut-off value separating the two categories (46 ng/ml) was detected by the area under the ROC curve techniques; that was the value that provided the maximum sensitivity (70%) and specificity (78%) and created two different clusters with significantly different probability of clinical response to treatment. The positive and negative predictive values (PPV and NPV) of such response-to-treatment predictor were 92% (23/25) and 41% (7/17) respectively. In a multivariate model the probability of no clinical response to treatment for patients exhibiting a sEGFR at baseline lower than or equal to 46 ng/ml, was tenfold greater than those with sEGFR greater than 46 ng/ml (95% CI 1.4–74.0, $p = 0.022$, Table 2).

We reported a median time to progression of 7 months (range 2–33 months) in our patients treated with gefitinib plus FOLFOX-6.

On average the median probability of disease progression or death was reached after 7 months from start of treatment (95% CI 6–9 months). Neither sEGFR, nor any other potential predictor, but CEA at baseline, resulted a significant predictor for the 2-year disease progression. Concerning CEA, the patients with a moderate baseline value ($\leq$30 ng/ml) took 9 months (7–10) to reach the median probability of disease progression, while those with a higher value (>30 ng/ml) reached it 3 months early (6 months; 95% CI 5–7, $p = 0.01$). Sensibility and specificity of CEA above or below 30 ng/ml, as predictor of 2-year disease progression, were 56% and 67% respectively. PPV and NPV were 94% (15/16) and 14%(2/14) respectively In the multivariate model the hazard of 2-year disease progression for patients with CEA > 30 ng/ml resulted 2.7 times greater than that exhibited by those with CEA $\leq$ 30 ng/ml ($p = 0.03$, Table 3).

At median follow up of 18 months (range 4–33 months), 25 (59.5%) patients were alive. At the time of present analysis overall survival was about 50%: 80.7% were alive at 1 year and 50.1% at 2 years.

The estimated probability of 2-year overall survival was 51%. For the patients with sEGFR below/above 46 ng/ml, the estimates were 40.6 and 55.9% respectively ($p = 0.10$, Fig. 2). The loss of information caused by the dichotomisation is the main reason for the not statistically significant result. As the matter of fact, the association of the original variable (which considers the original values before dichotomisation) with 2-year OS was widely statistically significant ($p = 0.02$). This result was totally confirmed even after adjustment for the above-mentioned fixed covariates ($p = 0.011$); the greater the serum EGFR at baseline, the lower the risk of death. Even by using the dichotomous classification (cut-off: 46 ng/ml) instead of the continuous one, the multivariate result on sEGFR was almost confirmed ($p = 0.068$), Table 4. Further baseline variable significantly associated to 2-year OS was the performance status ($p = 0.04$, multivariate model).

Discussion

Although the addition of novel cytotoxic drugs as oxaliplatin and irinotecan to the classical gold standard fluorouracil

Table 3 Predictors for progression-free survival (PFS)

	Events (%)	Univariate analysis		Multivariate analysis	
		Time to median PFS months (95% CI)	p (log-rank)	HR (95% CI)	*p* (Wald)
Overall	39 (92.8)	7 (6–9)			
Gender					
Male	20 (95.2)	7 (6–8)			
Female	19 (90.5)	7 (6–9)	0.61		0.61[a]
Age					
≤60	25 (96.2)	7 (6–9)			
>60	14 (87.5)	6.5 (6–9)	0.93		0.93[a]
Site of primary tumor					
Rectum	7 (87.5)	8.5 (6–11)			
Colon-Sigma	32 (94.1)	6.5 (6–8)	0.28		0.32[a]
Performance status (ECOG)					
0	26 (92.9)	7.5 (6–9)			
1–2	13 (92.9)	6 (5–8)	0.19		0.31[b]
Metastatic sites					
1	26 (89.7)	7 (6–9)			
≥2	13 (90.0)	7 (6–8)	0.28		0.07[b]
Stage at diagnosis					
Synchronous	28 (90.3)	7 (6–9)			
Metachronous	11 (100.0)	7 (5–9)	0.68		0.79[b]
Previous chemotherapy					
No	32 (91.4)	6 (6–8)			
Yes	7 (100.0)	9 (7–11)	0.45		0.43[b]
Serum EGFR (ng/ml) at baseline					
Categorical classification					
>46	15 (88.2%)	7 (6–10)		1	
≤46	24 (96.0%)	7 (6–8)	0.74	1.13 (0.53–2.37)	0.75[b]
CEA at baseline (ng/ml)					
≤30	12 (85.7%)	9 (7–10)		1	–
>30	15 (93.7%)	6 (5–7)	0.01	2.70 (1.10–6.64)	0.03[b]
Not collected	12 (100.0%)	6 (6–9)		2.50 (0.78–8.04)	0.12[b]

[a] Adjusted by centre

[b] Adjusted by age, gender, site of primary tumor and centre

have improved response rate and prolonged overall survival, advanced unresectable colorectal cancer remains an incurable disease.

EGFR signalling inhibition represents a recent area of interest for the application of molecular target therapies in colorectal cancer. From research in cancer cell biology, a series of EGFR inhibitors as monoclonal antibodies and tyrosine kinase inhibitors have been tested demonstrating the potential therapeutic role of the EGFR.

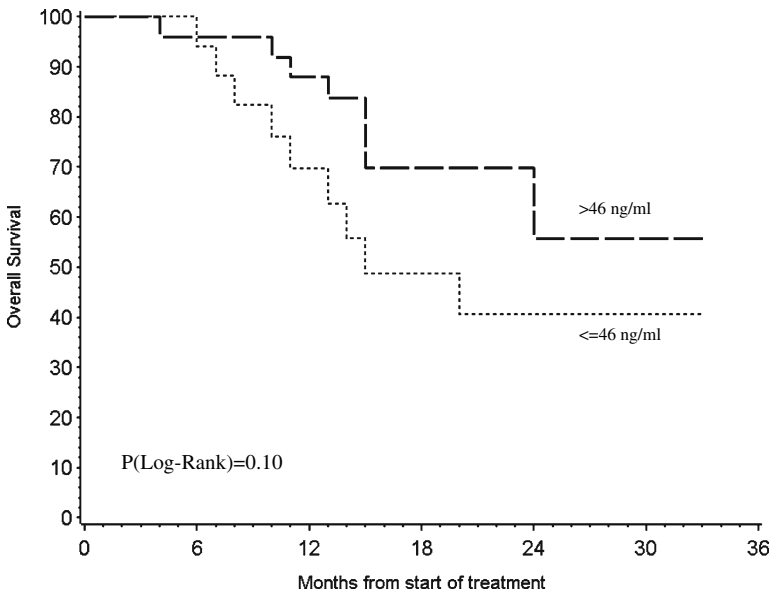
Preclinical data have shown a synergism between EGFR inhibitors and cytotoxic agents by increasing (or restoring) susceptibility of tumor cells to therapeutic apoptosis induction that has justified clinical trials.

Gefitinib, an oral selective EGFR-targeted agent, has shown efficacy (response rate of 9–18%) in relapsed NSCLC patients and was responsible of interesting disease control rate (response rate 33%) when combined with chemotherapy as second line treatment in colorectal tumors [10, 17].

In our published experience conducted on 56 patients with EGFR-positive advanced colorectal tumor treated with gefitinib plus FOLFOX regimen as first line therapy, 71% objective responses were reported. Major investigational goal in our trial was directed to understand a difference in biological profile between responders and non-responders, with the aim to characterize a subpopulation who could take an advantage from target treatment.

The impact of EGFR expression levels on drug sensitivity to EGFR blockers is still an issue since preclinical data and clinical trial didn't describe correlation with response [6, 27].

Performance status, female gender, adenocarcinoma histology, and skin rash in NSCLC represent predictive factors



GROUP	OS (n)	OS (n)	OS (n)	OS (n)	OS (n)	OS (n)	OS (n)
SEGFR>46 ng/ml	100 (25)	96.0 (24)	88.0 (22)	69.8 (15)	55.9 (4)	55.9 (1)	-
sEGFR≤46 ng/ml	100 (17)	94.1 (16)	69.7 (11)	48.8 (6)	40.6 (3)	40.6 (1)	-

Fig. 2 Overall Survival (OS) according to sEGFR (ng/ml) value at baseline

in gefitinib or erlotinib treatment [10], while skin rash in head and neck tumors was correlated with outcome but not-specifically associated to EGFR-targeted treatments [30].

Several molecular pathways have been investigated that may be involved in the sensitivity of cells to gefitinib, including phosphorylation of EGFR and downstream receptor-depended molecules such as mitogen-activated protein kinases (MAPT), Akt, or p27, but their significance as biomarker predicting sensitivity towards EGFR-blocking agents has not yet been proven [1, 21].

Recently specific mutations in the EGFR gene were correlated with clinical responsiveness to gefitinib in a subgroup of patients with NSCLC, but have not yet been described for colorectal cancer [20].

Proteome-based technologies was also proposed in the attempt to understand the complex network of EGFR inhibition and in order to define proteins involved in potential resistance mechanism in experimental models but required further confirmations [19].

Interestingly results have been reported with high gene expression levels of EGFR, epiregulin and amphiregulin and with K-ras mutations, as potential predictive factors to antibody anti-EGFR (cetuximab or panitumumab) treatment in colorectal cancer [3, 16, 18, 25].

Recently was attributed a crucial role to nuclear factor-kB expression, a transcription factor that is also activated by the EGFR downstream signalling pathways, in predicting outcome in irinotecan-refractory colorectal cancer treated with cetuximab combination [26].

EGFR antibody-induced skin rash has been studied as clinical surrogate marker of efficacy. In patients treated with cetuximab skin rash is strongly correlated with response and survival [8] and recent data from EVEREST trial demonstrated that in patients who had no or mild skin reactions dose escalation may improve tumor response [33].

Among biomolecular markers we decided to investigate the role of sEGFR as predictive factor of response to gefitinib-containing treatment. Biological hypothesis was founded on the concept that extracellular binding domain of EGFR is proteolytically cleavated by an active tyrosine kinase and so detected in serum [9].

In our study all patients received gefitinib combined to chemotherapy, so we don't exclude potential effects of chemotherapy on sEGFR profile.

We evaluated the association between sEGFR levels over time and tumor response on 42 patients.

We chose to divide patients into the two categories previously described (CR/PR vs. SD/PD according to RECIST criteria) based on prognostic factors. By considering the patients who reported an objective response (complete or partial) and those who didn't (stable or progressive disease), we observed that the two groups were already different at baseline (49.4 ± 6.2 ng/ml vs. 42.4 ± 8.4 ng/ml; $p = 0.042$); this difference was maintained also over time ($p = 0.032$); responders showed higher sEGFR values than the not responders. The statistical analysis failed to validate other predictors of positive response, except for baseline sEGFR.

These results seemed to demonstrate that patients with higher pre-treatment EGFR serum levels were more suitable for response, suggesting that sEGFR may reflect the

Table 4 Predictors for overall survival (OS)

	Events (%)	Univariate		Multivariate	
		2-year OS (%)	p (log-rank)	HR (95% CI)	*p* (Wald)
Overall	17 (40.5)	51.0			
Gender					
Male	10 (47.6)	48.7			
Female	7 (33.3)	51.0	0.42		0.53[b]
Age					
≤60	10 (38.5)	50.7			
>60	7 (43.8)	46.4	0.69		0.69[b]
Site of primary tumor					
Rectum	2 (25.0)	65.6			
Colon-Sigma	15 (44.1)	50.1	0.19		0.20[b]
Performance status (ECOG)					
0	8 (28.6)	64.2		1	
1–2	9 (64.3)	26.5	0.02	3.07 (1.03–9.14)	0.04[c]
Metastatic sites					
1	10 (34.5)	49.1			
≥2	7 (53.8)	46.1	0.44		0.077[c]
Stage at diagnosis					
Synchronous	12 (38.7)	54.2			
Metachronous	5 (45.5)	50.5	0.25		0.57[c]
Previous Chemotherapy					
No	16 (45.7)	47.9			
Yes	1 (14.3)	83.3	0.28		0.21[c]
Serum EGFR (ng/ml) at baseline					
Categorical classification					
>46	8 (32.0%)	55.9		1	
≤46	9 (52.9%)	40.6	0.10	2.85 (0.92–8.78)	0.068[c]
Linear trend (continuous)	–	–	0.02[a]	0.91 (0.85–0.98)	0.011[c]
CEA at baseline (ng/ml)					
≤30	3 (21.4%)	77.9		1	–
>30	9 (56.3%)	21.4	0.15	3.53 (0.86–14.5)	0.08[c]
Not collected	5 (41.7%)	51.9		4.18 (0.56–31.1)	0.16[c]

[a] Wald's test

[b] Adjusted by centre

[c] Adjusted by age, gender, site of primary tumor and centre

need of functional activation of EGFR pathways in responding patient. This observation was previously reported in clinical investigation conducted on 46 patients with lung cancer [12] but a clinically relevant cut-off had not been established.

To discriminate patients at risk of poor treatment response, we identified a cut-off value (≤46 ng/ml). We are aware of the limits of this approach; firstly the cut-off value is highly data-dependent and, secondly, this estimate and, above all, the wideness of the confidence interval were deeply affected by the small sample size. However sensitivity and specificity, as well as the PPV, were great enough to make this results believable. We warrant that it should be validate in further clinical trials. It remains, however the analysis conducted on the original sEGFR values, where sEGFR at baseline was detected to be the single significant predictor of BOR ($p = 0.042$, OR = 0.84: 95% CI 0.71–0.99). The greater the serum EGFR at baseline, the lower the risk of no clinical response (SD or PD) or, alternatively, for each EGFR increase of a unit, corresponded a 16% decrease of the risk of no response to treatment. Differences beyond 4 months were never significant because of the small number of determinations available.

Although we had insufficient data to assess the prognostic utility of sEGFR titers, our results suggest that high baseline levels of sEGFR may represent a more sensible predictor of response to tyrosine kinase inhibitor. The small available sample size does not allow to perform valid clinical conclusions about predictive value of sEGFR trend over time.

Further evaluations on patients treated with EGFR-targeted antibodies most extensively used in this population

are needed to validate our hypothesis and to contribute to the successful use of these agents.

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