Lack of usefulness of epidermal growth factor receptor expression determination for cetuximab therapy in patients with colorectal cancer

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In patients with metastatic colorectal cancer, the use of cetuximab currently requires a documented tumoral epidermal growth factor receptor positivity. Responses to cetuximab, however, have been described in patients with epidermal growth factor receptor-negative tumors. We have used cetuximab in all eligible patients with metastatic colorectal cancer, whether their tumor expressed epidermal growth factor receptor or not. We assessed the cetuximab efficacy with regard to tumoral epidermal growth factor receptor expression. Twenty patients with metastatic colorectal cancer were treated off study with cetuximab and irinotecan after failure of oxaliplatin- and irinotecan-based regimens. Tumors were analyzed in all patients for epidermal growth factor receptor expression by immunohistochemistry. Tumors were positive for epidermal growth factor receptor in 12 cases and negative in eight cases. An objective response to cetuximab-based therapy was obtained in four patients (20%). Tumors of these four patients were negative for epidermal growth factor receptor expression. These results provide further evidence for the lack of usefulness of epidermal growth factor receptor detection by immunohistochemistry for cetuximab therapy in patients with metastatic colorectal cancer. *Anti-Cancer Drugs* 17:855–857 © 2006 Lippincott Williams & Wilkins.

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Introduction

Within the last decade, there have been important advances in the medical treatment of metastatic colorectal cancer (CRC). The combination of oxaliplatin and irinotecan has considerably improved the efficacy of 5-fluorouracil. More recently, cetuximab, a monoclonal antibody against the epidermal growth factor receptor (EGFR), has shown significant activity when combined with irinotecan, after the failure of an irinotecan-based regimen [1,2]. Clinical studies on cetuximab were performed in patients with EGFR-positive tumor cells. In clinical practice, the detection of EGFR expression is currently required before beginning a cetuximab-based therapy. Yet, objective responses to cetuximab have also been reported in patients with EGFR-negative tumors [3,4]. Here, we present the results of our clinical experience with cetuximab in patients with metastatic CRC with regard to tumoral EGFR expression.

Patients and methods

We analyzed all the patients treated between June 2004 and December 2005 for metastatic CRC with cetuximab and irinotecan in a nonstudy setting in the University Hospital of Lille, France. All patients provided informed consent. In all, there were 20 patients: 15 men/five women; median age 62 years (range 39–75). The primary tumor was colon in 15 cases and rectum in five cases. The primary tumor had been resected in 19 cases. Metastases were synchronous in 11 cases and metachronous in nine cases. Metastatic sites were liver in all cases, chest in eight cases and peritoneum in three cases. Surgical resection of liver or pulmonary metastases was performed in 10 cases. In all these 10 cases, a tumor relapse had been observed. As first-line therapy, patients received an oxaliplatin-based regimen in 14 cases (Folfox in nine cases, oxaliplatin and capecitabine in four cases, oxaliplatin and tegafur uracil in one case) and an irinotecan-based regimen in six cases (Folfiri). As second-line therapy, 14 patients received Folfiri and six received Folfox. Tumors became resistant to oxaliplatin in 17 cases and to irinotecan in all 20 cases. Oxaliplatin treatment had to be stopped for toxicity in three cases (two sensory neuropathies and one hypersensitivity).

EGFR expression was analyzed by immunohistochemistry (IHC) in all patients. Arbitrarily, when primary and metastatic tumor samples were available, the test was preferentially performed on metastases because therapy

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Table 1 Patient characteristics and efficacy of cetuximab-based therapy

Patient Gender/age/PS[a]	Tissue tested for EGFR	Interval between tissue fixation and EGFR testing (months)	Result of EGFR testing	Best response to cetuximab-based therapy	Progression-free survival (months)	Overall survival (months)
M/61/0	liver metastasis	30	negative	PR	11+	11+
F/75/2	primary colon	7	negative	PR	5+	5+
M/39/2	primary rectum	35	negative	PR	9	10
F/59/1	liver metastasis	53	negative	PR	4	6
M/62/0	liver metastasis	25	negative	SD	5	7
F/53/1	pulmonary metastasis	10	negative	SD	4+	4+
M/71/2	primary colon	21	negative	PD	–	7
M/68/2	liver metastasis	28	negative	PD	–	3
M/67/1	liver metastasis	23	positive	SD	4	6
F/40/0	pulmonary metastasis	6	positive	SD	6	7
M/73/2	liver metastasis	26	positive	PD	–	4
M/72/2	liver metastasis	25	positive	PD	–	5
F/70/1	liver metastasis	18	positive	SD	6	8+
M/69/1	primary colon	31	positive	SD	5	8
M/51/1	primary colon	19	positive	SD	7	8
M/66/2	primary colon	8	positive	SD	8+	8+
M/46/3	primary rectum	24	positive	PD	–	2
M/54/0	primary rectum	23	positive	SD	4	6
M/70/2	primary colon	20	positive	PD	–	5+
M/72/1	primary rectum	11	positive	SD	6+	6+

EGFR, epidermal growth factors receptor; PR, partial response; SD, stable disease; PD, progressive disease (according to RECIST criteria)[5].
[a]Performance status according to the WHO classification.

was directed against metastases (in all but one patient, the primary tumor had been removed). Expression analysis was performed on metastases in 10 cases and primary tumors in 10 cases (Table 1). The immunohistochemical study was performed on 4-μm-thick, deparaffinized sections using a streptavidin–biotin complex and a hematoxylin counterstain. A mouse monoclonal EGFR antibody clone 31G7 (Zymed, San Francisco, California, USA) was used as previously described [3]. Antigen retrieval and immunostain were performed on the Ventana Benchmark automated immunostainer (Ventana Medical System, Strasbourg, France). The antibody Zymed kit was used because it was compatible with the automated immunostainer. In each case, positive and negative controls were tested. A tumor was considered positive for EGFR when at least 1% of cells presented membranous staining.

All patients received cetuximab on a weekly basis (400 mg/m^2 in week 1 and 250 mg/m^2 in the subsequent weeks). Irinotecan was administered every 2 weeks (180 mg/m^2). Tumor evaluation by computed tomography scan was performed every 8 weeks and the tumoral responses were graded according to the RECIST criteria [5].

Results

Tumors were considered positive for EGFR in 12 cases and negative in eight cases (Table 1). The positivity rate was 7/10 in primary tumors and 5/10 in metastases. The mean duration between tumor fixation and EGFR testing was 19.5 months (range 6–31) for positive tumors and 26.3 months (range 7–53) for negative tumors.

A median number of eight treatment courses were delivered (range 5–28). Therapy did not have to be stopped in any of the patients as a result of limiting toxicity. A partial response was obtained in four cases (20%), stable disease in 10 cases (50%) and tumor progression in six cases (30%). In all four patients with an objective response, EGFR expression was considered negative.

The median progression-free survival and overall survival were 7.3 and 9.5 months, respectively.

Discussion

A correlation between the EGFR expression level and cetuximab efficacy has been suggested by experimental studies [6]. Yet, this correlation seems to be speculative in the clinical setting. No relationship has been found between the intensity of the IHC staining for EGFR and the response rate in clinical trials [1,7]. Consequently, we have treated all patients with cetuximab, whether their tumors expressed EGFR or not.

In the present series, all the patients had metastatic CRC, and most experienced failure with prior irinotecan- and oxaliplatin-based regimens. An objective response under irinotecan and cetuximab combination was obtained in 4/20 patients (20%). This rate is consistent with those obtained in patients pretreated with irinotecan and oxaliplatin [4]. Yet, all the objective responses were observed in patients in whom no tumoral EGFR expression had been detected.

Another recent report mentioned significant cetuximab activity in EGFR-negative patients [3]. Four objective

responses were obtained in 16 EGFR-negative patients, which is similar to results observed in EGFR-positive patients. In the study by Lenz *et al.* [4], an objective response was obtained by using single-agent cetuximab in 2/9 EGFR-negative patients (22%).

This apparent discrepancy may be explained by the fact that EGFR expression is generally assessed in primary tumors. Scartozzi *et al.* [8] showed that EGFR status in primary colorectal tumors was not correlated with EGFR expression in related metastatic sites. Especially, EGFR expression was found positive in seven metastases from 46 EGFR-negative primary tumors (15%). In our series, EGFR expression was assessed in the metastatic sites in 10/20 cases. Among these 10 cases, five were considered negative for EGFR. Yet, an objective response was obtained in two of these five cases.

The inaccuracy of IHC may also explain the discrepancy. The interpretation of this method is somewhat subjective. The positivity level (1% of stained tumor cells) seems to be very small and is probably inappropriate to predict the efficacy of cetuximab-based therapy. A recent study suggests that the gene copy number of EGFR could more accurately predict the efficacy of cetuximab [9]. In another study performed in patients receiving single-agent cetuximab, a prognostic value was found for the mRNA amount of EGFR [10].

An additional explanation for the lack of correlation between membranous EGFR expression and antitumor activity may be the potential for cetuximab to induce antibody-dependent cell-mediated cytotoxicity, which leads to indirect antitumor activity by the recruitment of cytotoxic host effector cells [11].

The interval between tumor sample fixation and IHC may interfere with the detection of EGFR expression. In a prospective study performed on 40 patients having colorectal or noncolorectal tumors, each tumor sample was evaluated for EGFR expression at six time points extending to 24 months [12]. A dramatic decline in EGFR immunostaining intensity with increasing storage time of the tumor sample, leading to many more EGFR-negative results in older specimens, was observed. This could contribute to explain false-negative cases in the present study because the mean storage time was higher for EGFR-negative tumors than for the positive tumors (26.3 v 19.5 months, respectively). Yet, both mean intervals are important and, in the study by Atkins *et al.* [12], the EGFR test was shown to lose its sensitivity within only 9 months. This should be confirmed in a larger series.

Altogether, these results provide further evidence that tumoral EGFR status, as assessed by available immunohistochemical methods, is not appropriate enough to select patients suitable for cetuximab therapy. Especially, the discrepancies between EGFR expression in primary and secondary lesions could represent an important limitation.

References

1 Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, *et al.* Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**:337–345.

2 Starling N, Cunningham D. Cetuximab in previously treated colorectal cancer. *Clin Colorectal Cancer* 2005; **5**:S28–S33.

3 Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A, *et al.* Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. *J Clin Oncol* 2005; **23**:1803–1830.

4 Lenz HJ, Mayer RJ, Gold PJ, Mirstching B, Stella PJ, Cohn L, *et al.* Activity of cetuximab in patients with colorectal cancer refractory to both irinotecan and oxaliplatin. *Proc Am Soc Clin Oncol* 2004; **22**:247S.

5 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**:205–216.

6 Castillo L, Etienne-Grimaldi MC, Fischel JL, Formento P, Magne N, Milano G. Pharmacological background of EGFR targeting. *Ann Oncol* 2004; **15**:1007–1012.

7 Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004; **22**:1201–1208.

8 Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Fabris G, Cascinu S. Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. *J Clin Oncol* 2004; **22**:4772–4778.

9 Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, DiNicolantonio F, *et al.* Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol* 2005; **6**:279–286.

10 Vallbohmer D, Zhang W, Gordon M, Yang DY, Yun J, Press OA, *et al.* Molecular determinants of cetuximab efficacy. *J Clin Oncol* 2005; **23**: 3536–3544.

11 Milano G. Pharmacological skills for targeting EGFR and VEGF. *Bull Cancer* 2005; **92**:S17–S20.

12 Atkins D, Reiffen KA, Tegtmeier CL, Winther H, Bonato MS, Storkel S. Immunohistochemical detection of EGFR in paraffin-embedded tumor tissues: variation in staining intensity due to choice of fixative and storage time of tissue sections. *J Histochem Cytochem* 2004; **52**:893–901.