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Cetuximab rechallenge in metastatic colorectal cancer patients: how to come away from acquired resistance?

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Background: Scientific data provide the evidence that secondary *K-RAS* mutations do not occur during anti-epidermal growth factor receptor therapy in colorectal cancer patients. This multicenter phase II prospective study aims to investigate the activity of a retreatment with a cetuximab-based therapy.
Patients and methods: We enrolled 39 irinotecan-refractory patients who had a clinical benefit after a line of cetuximab- plus irinotecan-based therapy and then a progression of disease for which underwent a new line chemotherapy and finally, after a clear new progression of disease, were retreated with the same cetuximab- plus irinotecan-based therapy.
Results: Median number of therapeutic lines before accrual was 4. Median interval time between last cycle of first cetuximab-based therapy and first cycle of the retreatment was 6 months. Overall response rate was 53.8% with 19 partial responses (48.7%) and 2 complete responses (5.1%). Disease stabilization was obtained in 35.9% of patients and progression in four patients (10.2%). Median progression-free survival was 6.6 months. The correlation between skin toxicity during first cetuximab therapy and during cetuximab rechallenge was significant ($P = 0.01$).
Conclusion: Rechallenge with the same cetuximab-based therapy may achieve a new important clinical benefit further delaying the progression of disease and improving the therapeutic options.
Key words: cetuximab, colorectal neoplasms, clinical trial, phase II, retreatment

introduction

Cetuximab (ERBITUX®) is a chimeric IgG1 monoclonal antibody that bind extracellular domain of epidermal growth factor receptor (EGFR) [1] preventing its linkage with endogenous ligands such as transforming growth factor-α and epidermal growth factor. Several phase II and phase III trials

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supported cetuximab combination in first-line treatment of metastatic colorectal cancer (mCRC) reporting a clinical benefit, progression-free survival (PFS), and overall response rate (ORR) increase and higher rates of liver resections [2–5]. Other studies supported the use of cetuximab as a single agent or in combination with irinotecan for patients who had progressed on a previous chemotherapy [6–8]. The EGFR activation leads to the activation of intracellular effectors involved in intracellular signaling pathways, such as the G protein K-ras.

Moreover, oncogene *K-RAS* mutations affect the clinical response to anti-EGFR therapy. In fact, a large retrospective analysis evaluated *K-RAS* mutation status in 113 patients affected by irinotecan-refractory mCRC treated with cetuximab or in combination with irinotecan in clinical trials. An ORR of 41% was observed in 27 of 66 patients with wild-type (WT) *K-RAS* versus 0 of 42 in *K-RAS*-mutated patients. The median overall survival was significantly improved in patients with WT *K-RAS* versus patients with mutated *K-RAS* ($P = 0.02$). Decrease in tumor sizes was significantly larger in WT *K-RAS* patients [9]. Another prospective trial observed that patients whose tumors do not have *K-RAS* mutations have a significantly higher disease control rate than patients with *K-RAS* mutations ($P = 0.0003$) [10].

Then, other mutations downstream of EGFR could affect its anti-EGFR effectiveness such as *BRAF*, Src [11, 12], and *PI3KCA*. B-raf is a serine-threonine kinase and the principal effector of K-ras. *BRAF* mutation (exons 15 and 21) in colorectal cancer (CRC) occurs in a low percentage of cases (5%–12% of cases) [13], but several studies have suggested that it is associated with a decreased response to anti-EGFR therapy [14–16].

PIK3CA is also commonly mutated in CRC (20% of cases) [17]. It encodes the p110a subunit of PI3K regulating its function. It has been associated with cetuximab resistance in preclinical studies [18, 19]. Moreover, *PI3KCA* mutations have been associated with panitumumab and cetuximab resistance in retrospective analyses including patients affected by mCRC [15]. Loss of phosphatase and tensin homolog protein, a negative regulator of PI3K, has also been associated with resistance to anti-EGFR therapy [20–22].

K-RAS mutation is an early pathogenic step in CRC development, and it seems to remain the same during tumor progression [23]. In fact, the same *K-RAS* mutations can be detected in most adenoma and in more than a half of the tumor adjacent mucosa [24]. One study analyzed *K-RAS* status of CRC primary tumor and its metastasis sites in 21 patients. It was observed that anti-EGFR therapy do not change *K-RAS* status concordance between CRCs and corresponding metastasis in 20 of 21 cases [25]. These data provided first evidence that secondary *K-RAS* mutations do not occur during anti-EGFR therapy in CRC patients.

Basing on the hypothesis that *K-RAS* status remain the same during the history of the disease, despite the treatments received, we designed a phase II prospective study with the aim of demonstrating that patients who responded and then progressed during a cetuximab-based therapy can receive, after a new line of therapy, a further line containing the same cetuximab-based therapy gaining a clinical benefit.

materials and methods

eligibility criteria

This is a multicentric phase II trial that examines irinotecan-refractory patients who had a clinical benefit [confirmed stable disease (SD) for at least 6 months or clinical response] after a line of cetuximab- plus irinotecan-based therapy and then a progression of disease, during cetuximab-based therapy, for which underwent a new line chemotherapy and finally, after a clear new progression of disease, were retreated with the same or another Cetuximab- plus Irinotecan-based therapy. Eligible patients had histologic or cytologic confirmation of CRC, with measurable metastatic disease in at least one site identified by instrumental examinations. All patients were required to be *K-RAS* WT (codons 12 and 13) with RT-PCR and *K-RAS* status was centrally assessed. Patients aged between 40 and 80 years with an Eastern Cooperative Oncology Group (ECOG) performance status one or less and a life expectance >3 months were included. Bone marrow function requirements included an absolute neutrophil count $\geq 1500/mm^3$, a platelet count $\geq 100\,000/mm^3$ and hemoglobin ≥ 10.0 g/100 ml. Preserved renal function (serum creatinine $\leq$ 1.5 mg/dl and normal creatinine clearance), hepatic function (total bilirubin ≤ 1.5 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤ 2.5 times normal). The study has been conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki and all patients signed a written consent form prior to the enrollment. Patients were excluded if adequate follow-up was not possible (environmental or geographic difficulties, no compliance to undergo necessary clinical-instrumental investigations, etc.).

treatment plan

Cetuximab was given at a loading dose of 400 mg/m^2 followed by weekly infusions of 250 mg/m^2. Irinotecan was given at dose of 180 mg/m^2 as a 90-min infusion day. A histamine-receptor antagonist and atropine (0.25 mg) were given as premedication before every infusion. Moreover, dexamethasone was given at the dose of 20 mg before the induction course and at the dose of 8 mg in the further courses. A standard antiemetic drug was always given in the premedication and in the following days according to the physician's opinion. All the patients were to be treated until disease progression or unacceptable toxic effects; no treatment holiday was permitted. Tumor response was evaluated every 8 weeks with the use of consistent imaging techniques [computed tomography (CT) or magnetic resonance imaging]. The response to the treatment, both during cetuximab treatment and rechallenge, prior or further treatments, was evaluated centrally by two different radiologists and confirmed by the investigators, according to Response Evaluation Criteria in Solid Tumors (RECIST) [26].

toxicity and dose modifications

Toxic effects were assessed according to the National Cancer Institute—Common Toxicity Criteria, 1998. Modifications of the dose of cetuximab were carried out only in case of toxic effects to the skin not restoring after 2 weeks of rest, and modifications in the dose of irinotecan were made in case of hematologic or non-hematologic toxic effects. Cumulative toxicity was evaluated and recorded before each treatment cycle. Irinotecan administration was stopped for more than or equal to G_2 hematological toxicity and was restarted in case of toxicity regression to G_0–G_1. Reduction of 25% in Irinotecan dosing was applied for G_3 non-hematological toxicity and G_4 hematological toxicity in the previous cycle. The use of hemopoietic growth factors for white and red cell lines was allowed when necessary.

study schedule and evaluations

Screening assessments including medical history, physical examination (including vital signs, height, weight, and Karnofsky Performance Scale (KPS)), electrocardiogram (ECG), chest X-ray, and tumor measurements, based on the appropriate imaging techniques (i.e. CT scan) or physical examination, were conducted within 14 days before treatment initiation. Laboratory data including complete blood count, blood chemistry, and urinalysis were also obtained. During treatment, weekly assessments included vital signs, physical measurements, KPS, complete blood counts, and blood chemistry. For patients continuing treatment beyond 18 weeks, these assessments were carried out at three-weekly intervals. Urinalysis, chest X-ray, ECG, and brain CT scan were carried out if clinically indicated. Patients could remain on treatment until disease progression (evaluated with the best instrumental exams applicable in case of metastatic lesions every 1 month of therapy) or the development of unacceptable toxicity or patient's refusal. All tumor measurements were reviewed and confirmed by an independent panel of radiologists and oncologists.

sample size and statistical considerations

The efficacy analysis was based on the intent-to-treat population. The primary end point was overall confirmed response rate. The Simon minimax two-stage design was used with early termination of the trial if a predetermined minimum level of activity was not observed after the first stage of accrual. The sample size calculation was carried out to reject a 30% response rate in favor of a target response rate of 50%, with a significance level of 0.05 and a statistical power of 80%. The preliminary activity of cetuximab-based rechallenge was assessed through the accrual of 19 patients. If there were less than or equal to six responses, accrual needed to be terminated. Otherwise 20 additional patients needed to be entered in the second stage to achieve a target sample size of 39 evaluable patients for tumor response. If >16 responses were observed in these 39 patients, further assessment could be suggested. PFS was calculated from inclusion date to progression documented or death date. Treated patients would have been followed until disease progression. Safety was analyzed in all patients who received at least one dose of study medication. SPSS software (version 11.05, SPSS, Chicago, IL) was used for statistical analysis.

Table 1 Patient characteristics

Characteristics	No. of patients (%)
Total number	39 (100)
Male/female	28/11 (71/29)
Age (years)	
Median	59
Range	44–82
Performance status	
Median	0
Range	0–1
Primary tumor site	
Colon	19 (49)
Rectum	9 (25)
Rectosigmoid	11 (29)
Tumor differentiation	
Well differentiated	4 (10)
Moderately differentiated	15 (39)
Poorly differentiated or undifferentiated	20 (51)
Median number of metastatic sites (range)	2 (1–6)
1	11 (28)
2–3	18 (46)
>3	10 (26)
Sites of metastases	
Liver	26 (67)
Lung	14 (36)
Nodes	17 (44)
Local	4 (10)
Other	8 (21)

results

patient characteristics

Demographic and other baseline characteristics of patient population are summarized in Table 1. From February 2007 to January 2010, a total of 39 patients were enrolled into the study.

All patients, 11 females and 28 males, were assessable for treatment efficacy and safety. The median age of study population was 59 years (range: 44–82 years), all patients had an ECOG performance status of 0 or 1, and 71.2% had two or more metastatic sites (28 patients) involving the liver (26 patients; 66.6%), the lung (14 patients; 35.9%), and the nodes (17 patients; 43.6%). Primary tumor site was colon in the 48.7% of patients, rectosigmoid junction in the 28.2%, and rectum in the 25.3% of patients. Median number of therapeutic lines before study accrual, including original cetuximab containing regimen, was 4 (3–7). All patients were irinotecan refractory at the moment of the first cetuximab-based therapy. Chemotherapy protocols associated during first cetuximab-based therapy were the following: irinotecan monotherapy (53.9%), FOLFIRI (46.1%). All the patients received a weekly cetuximab schedule. Best responses after first cetuximab-based therapy: 6 complete responses (CRs), 29 partial responses (PRs), and 4 SDs lasting at least 6 months. Median PFS with first treatment with cetuximab: 10 months (3–30) (Table 2). The median interval time between last cycle of first cetuximab-based therapy and first cycle of the following cetuximab retreatment was 6 months (2–12). All patients have been considered in progression at the moment of the study entry. Chemotherapy protocols administrated after the first cetuximab-based therapy were the following: 5-fluorouracil-based therapy (17.9%), oxaliplatin-based therapy (51.3%);

Table 2 First cetuximab-based therapy: characteristics

Characteristics	No. of patients (%)
Total number	39 (100)
Irinotecan refractory	39 (100)
Protocol of association	
Irinotecan monotherapy	21 (54)
FOLFIRI	18 (46)
Best response	
Stable disease (>6 months)	4 (10)
Partial response	29 (74)
Complete response	6 (15)
Median time to progression—months (range)	10 (3–30)
Median number of lines before cetuximab retreatment (range)	4 (3–7)

irinotecan-based therapy (7.7%), oxaliplatin-based therapy with bevacizumab (12.8%), and irinotecan-based therapy with bevacizumab (2.6%), 5-fluorouracil-based therapy with bevacizumab (7.7%). Chemotherapy protocols associated during cetuximab rechallenge-based therapy were the same used during the first cetuximab-based therapy: irinotecan in 21 patients (53.9%) and FOLFIRI in the remaining 18 patients (46.1%).

antitumor efficacy

All patients enrolled in the study were assessable for antitumor efficacy. In the first stage of the study, six PRs and one CR were obtained. For this reason, we proceed to the second stage of the study. Considering all the included patients, the ORR according to the International Rescue Committee assessment was 53.8% [95% confidence interval (CI) 39.1% to 63.7%] with 19 PRs (48.7%) and 2 CRs (5.1%). Disease stabilization was obtained in 35.9% of patients (95% CI 24.7% to 51.6%) for a clinical control rate of disease of 89.8%. Progression occurred in only four patients (10.2%). The median PFS was 6.6 months (95% CI 4.1% to 9.1%). Eighteen patients (46.1%) showed the same type of response (SD, PR or CR) during cetuximab retreatment when compared with the response obtained during the first cetuximab-based therapy, 2 patients (5.1%) has increased the quality of clinical result, transiting from PR to CR and from SD to PR, respectively (Table 3). Both, SD lasting at least 6 months and PR during the first cetuximab-based therapy have been demonstrated to predict clinical benefit after cetuximab retreatment. The Kaplan–Meier curves for median PFS are depicted in Figure 1.

Table 3 Clinical response after first cetuximab-based therapy and second cetuximab-based therapy in 39 patients

Best response 1st cetuximab	Best response rechallenge	No. of patients	Total no. (%)
PR	CR	1	2 (5)
CR		1	
SD	PR	1	19 (49)
PR		14	
CR		4	
SD	SD	3	14 (36)
PR		10	
CR		1	
PR	PD	4	4 (10)

safety results

All patients enrolled in the study were assessable for safety. Most frequent grades 3–4 adverse events were skin rash and diarrhea. Skin rash occurred in almost all patients (37 patients; 94.9%) and, as expected, it was generally moderate to severe in intensity (grade 2: 41%; grade 3: 38.5%; no grade 4). It has been demonstrated a significant correlation between skin toxicity during first cetuximab therapy and cetuximab rechallenge ($P = 0.01$). Diarrhea occurred in 22 (56.4%) patients and was grades 3–4 in only 3 (7.7%) and grades 1–2 in 19 (48.7) patients. Seven (18%) patients developed grades 3–4 neutropenia and no one developed febrile neutropenia. Dose delays were necessary in 17 patients (43.6%), mainly due to skin toxicity. Cetuximab dose adjustment was made in 5

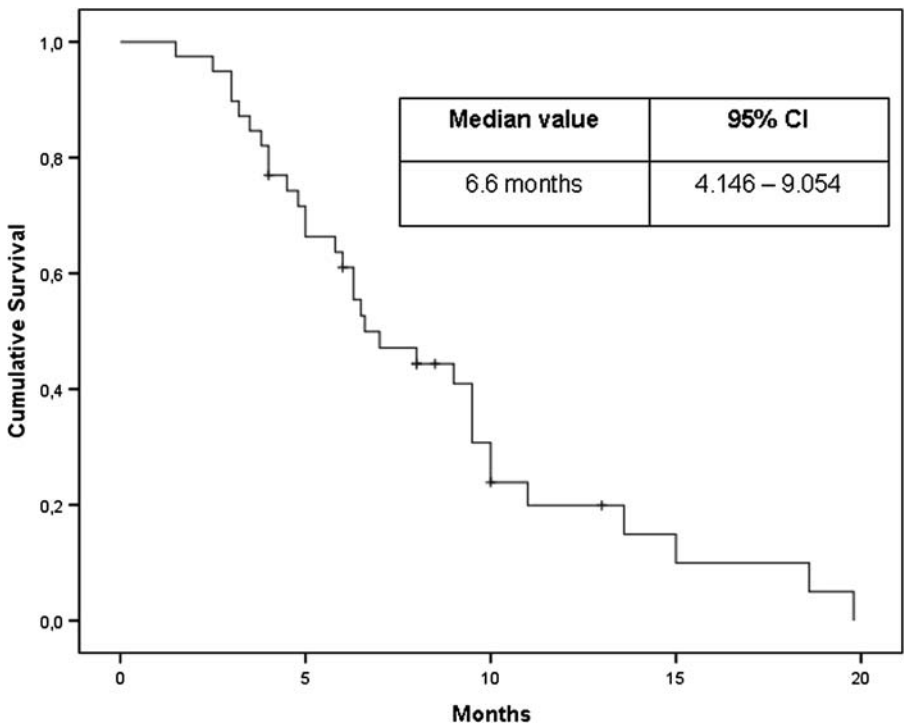

Figure 1. Kaplan–Meyer curve for progression-free survival. The median progression-free survival was 6.6 months [95% confidence interval (CI) 4.1% to 9.1%].

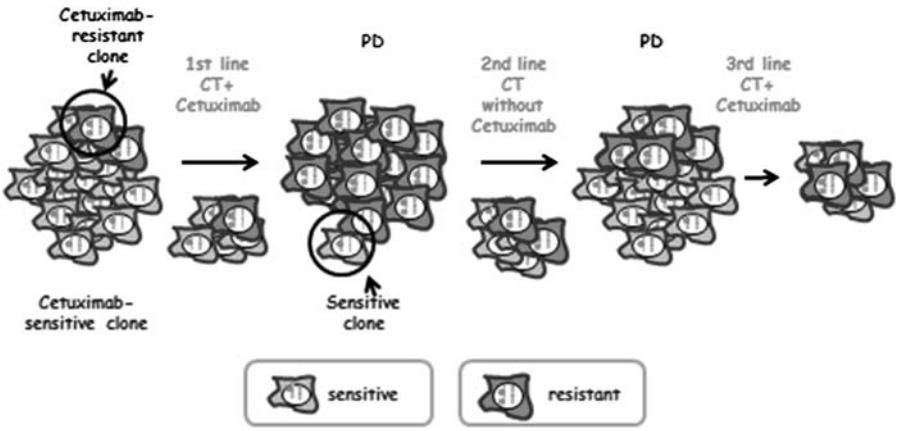

Figure 2. Hypothesis for the overcoming of acquired resistance to cetuximab. The tumor burden usually shows heterogeneity. So it is possible that before treatment both cetuximab-sensitive and -resistant are present. After the initial response, disease progression occurs in a wild-type *KRAS* tumor. This occurrence could be explained by the progressive prevalence of a mutated clone rather than by the late acquisition of a new mutation. This mechanism could be defined 'cetuximab-driven mutated genotype acquisition', which occurs during therapy.

(12.8%) patients because of skin toxicity. Six (15.4%) patients required reduction of irinotecan dose, mainly because of grade ≥ 2 diarrhea. No patient was hospitalized due to toxic effects and no toxic death or cardiac and thromboembolic events occurred. Only two (5%) patients discontinued treatment due to toxicity.

discussion

Data emerging from literature clearly pointed out that activating mutations of *K-RAS*, *BRAF*, and *PI3K* predict lack of response to cetuximab or panitumumab therapy [9, 27, 28]. Among these molecules, up to date, only *K-RAS* has been validated for diagnostic applications, and the search for *K-RAS* mutations in codons 12 and 13 of exon 2, described in 25% to 45% of patients, is today mandatory in order to establish the best therapeutic option for mCRC patients. The presence of *K-RAS* mutations in aberrant crypt foci [29] and in preneoplastic lesions [30] suggests that these events occur at a very early stage in human colorectal carcinogenesis. Moreover, despite the different lines of therapy administered, the *K-RAS* gene status seems to remain the same also in the advance phase of the disease, as shown by the high concordance of *K-RAS* testing results on the primary tumor and metastasis [23, 31]. The acquisition of secondary mutations, which is a frequent phenomenon in many other cancer types, do not seem to play a major role in therapy-related resistance to anti-EGFR antibody treatment in mCRC: in fact, the evaluation of *K-RAS*/*BRAF* status before and after anti-EGFR antibody treatment carried out by Gattenlöhner et al. [25] is resulted highly concordant (95% for *K-RAS*, 100% for *BRAF*). However, ~5% to 10% of mCRC show K-Ras molecular heterogeneity between primary, lymph node, and distant metastases [32]. Moreover, a recent study from Baldus et al. evaluated *K-RAS*, *BRAF*, and *PI3K* gene status into the primary tumor, comparing the tumor center and the invasion fronts. The intratumoral heterogeneity of *K-RAS*, *BRAF*, and *PIK3CA* mutations was observed in 8%, 1%, and 5% of primary tumors, respectively [33]. According to the evidence of intratumoral heterogeneity, the occurrence of a disease progression after the initial response in a WT *K-RAS* primary tumor could not be due to a late acquisition of the mutation rather to the progressive prevalence of a mutated clone, caused by a sort of 'cetuximab-driven mutated genotype acquisition' occurring during therapy (Figure 2). On the basis of these results and to our knowledge, we conducted the first phase II prospective trial evaluating the efficacy of a cetuximab rescue in *K-RAS* WT mCRC patients who experienced a clinical benefit followed by a progression with a previous cetuximab-based therapy. After the failure of an irinotecan-based first-line therapy, the recourse to a cetuximab-based therapy in *K-RAS* WT mCRC patients, even without modifing *K-RAS* gene status, could lead to the destruction of WT cells and to the prevalence of mutated clones, which lead to a first progression of disease. A further line of therapy without cetuximab could restore *K-RAS* WT clones, which could constitute the major part of the tumor mass at the time of a following progression of disease. At this point, a rescue through a cetuximab-based new line therapy may determine a further shrinkage of the disease. Moreover, the tumor cell entrance to epithelial-to-mesenchymal transition (EMT) or the reverse mesenchymal-to-epithelial transition may justify response or refractoriness, respectively, in patients retreated with Cetuximab. EMT is characterized by the combined loss of epithelial cell junction proteins such as E-cadherin and the gain of mesenchymal markers such as vimentin. Therefore, it is likely that the epithelial cells are more susceptible to EGFR-targeted therapies due to their activation of AKT primarily through EGFR-ErbB3. Mesenchymal cells activate AKT through alternative pathways like integrin-linked kinase (ILK) [34] and are largely resistant to EGFR inhibitors. Cetuximab-based therapy could lead during the time, after a first response, to activation of this alternative pathway, ILK-dependent, which allow the EMT. A further line without anti-EGFR therapy could down-regulate this process restoring cetuximab sensitiveness. In fact, we observed that rechallenge with the same cetuximab-based

therapy can achieve a new important clinical benefit delaying the progression of disease and improving the therapeutic options. The present phase II trial is the first demonstration in literature of a potential clinical benefit deriving from a rechallenge with cetuximab-based therapy in *K-RAS* WT colorectal patients previously treated with the same anti-EGFR-based protocol.

disclosure

The authors declare no conflict of interest.

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