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RECHALLENGE WITH ANTI-EGFR MONOCLONAL ANTIBODIES IN PRETREATED METASTATIC COLORECTAL CANCER PATIENTS: BEYOND THE LIMITS OF ACQUIRED RESISTANCE

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ABSTRACT

Background: Scientific data provide today the evidence that secondary K-RAS mutations do not occur during anti-EGFR therapy in CRC 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 re-treated 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 4 patients (10.2%). Median time to progression was 6.6 months. The correlation between skin toxicity during first cetuximab therapy and during cetuximab rechallenge was significant (p= .01). **Conclusions:** 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.

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 TGF-α and EGF. Several phase II and phase III trials 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, 3, 4, 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 [6,7,8]. 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 with or without 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 OS was significantly improved in patients with WT K-ras versus patients with mutated K-ras (P = .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 DCR than patients with K-ras mutations (P = .0003)[10].

Then, other mutations downstream of EGFR could affect its anti-EGFR effectiveness such as BRAF, Src[11, 12], and PI3KCA. BRAF is a serine-threonine kinase and the principal effector of K-ras. BRAF mutation (exons 15 and 21) in 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, 15, 16].

PIK3CA is also commonly mutated in colorectal cancer (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 pTEN protein, a negative regulator of PI3K, has also been associated with resistance to anti-EGFR therapy [20, 21 ,22].

K-ras mutation is an early pathogenic step in colorectal cancer 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 for at least 6 months or clinical response) 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 re-treated 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 wild type (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 ≤ 1 and a life expectance > 3 months were included. Bone marrow function requirements included an absolute neutrophil count ≥ 1500/mm³, a platelet count ≥ 100000/ mm³ and haemoglobin ≥ 10.0 g/100 ml. Preserved renal function (serum creatinine ≤ 1.5 mg/dL and normal creatinine clearance), hepatic function (total bilirubin ≤ 1.5 mg/dL, AST and ALT ≤ 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 m2 followed by weekly infusions of 250 mg m-2. Irinotecan was given at dose of 180 mg m2 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. Tumor response was evaluated every 8 weeks with the use of consistent imaging techniques (CT or MRI). 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]. Toxic effects were assessed according to the National Cancer Institute Common Toxicity Criteria, version 2 (National Cancer Institute Common Toxicity Criteria, 1998).

Toxicity and Dose Modifications

Toxic effects were assessed according to the National Cancer Institute Common Toxicity Criteria (National Cancer Institute Common Toxicity Criteria, 1998). Modifications of the dose of cetuximab were performed 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 haematologic or nonhematologic toxic effects. Cumulative toxicity was evaluated and recorded before each treatment cycle. Irinotecan administration was stopped for ≥ G2 hematological toxicity and was restarted in case of toxicity regression to G0-1. Reduction of 25% in Irinotecan dosing was applied for G3 non-hematological toxicity and G4 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 KPS), electrocardiogram (ECG), chest X-ray and tumor measurements, based on the appropriate imaging techniques (i.e. computed tomography 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 computed tomography scan were performed if clinically indicated. Patients could remain on treatment until disease progression (evaluated with the best instrumental exams applicable in case of metastatic lesions every one 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 performed to reject a 30% response rate in favour 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 ≤ 6 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 more than 16 responses were observed in these 39 patients further assessment could be suggested. Time to progression 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) was used for statistical analysis.

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/ 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%). Best responses after first cetuximab-based therapy: 6 complete responses, 29 partial responses and 4 stable diseases lasting at least 6 months. Median time to progression 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%); 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%). A total of 514 of weekly cetuximab-based cycles and a total of 94 bi-weekly cetuximab-based cycles have been administered.

Antitumor efficacy

All patients enrolled in the study were assessable for antitumor efficacy. In the first stage of the study 6 partial responses and 1 complete response were obtained. For this reason we proceed to the second stage of the study. Considering all the included patients, the overall response rate according to the IRC assessment was 53.8% (95% CI 39.1% to 63.7%) with 19 partial responses (48.7%) and 2 complete responses (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 4 patients (10.2%). The median time to progression was 6.6 months (95% C1: 4.1-9.1). 18 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 a partial to a complete response and from stable disease to partial response respectively (Table 3). Both, stable disease lasting at least 6 months and partial response during the first cetuximab-based therapy have been demonstrated to predict clinical benefit after cetuximab retreatment. The Kaplan-Meier curves for median time to progression are depicted in Figure 1.

Safety results

All patients enrolled in the study were assessable for safety. Most frequent grade3–4 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%; grade3: 38.5%; no grade 4). It has been demonstrated a significant correlation between skin toxicity during first cetuximab therapy and cetuximab rechallenge (p= .01). Diarrhea occurred in 22 (56.4%) patients and was grade 3–4 in only 3 (7.7%) and grade 1–2 in 19 (48.7) patients. Seven (18%) patients developed grade 3–4 neutropenia and no one febrile neutropenia. Dose delays were necessary in 17 patients (43.6%), mainly due to skin toxicity. Cetuximab dose adjustment was made in 5 (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 toxicities and no toxic death or cardiac and thromboembolic event occurred. Only 2 (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 estabilish the best therapeutic option for metastatic colorectal cancer patients. The presence of K-Ras mutations in aberrant crypt foci[29] and in preneoplastic lesions[30] suggests that these event 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[31,32]. 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 metastatic colorectal cancer: in fact, the evaluation of K-Ras/BRAF status before and after anti-EGFR antibody treatment performed by Gattenlohner et al. is resulted highly concordant (95% for K-Ras, 100% for BRAF)[25]. However, approximately 5% to 10% of metastatic colorectal cancer show K-Ras molecular heterogeneity between primary, lymph node and distant metastases [33]. 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[34]. According to the evidence of intratumoral heterogeneity, the occurrence of a disease progression after the initial response in a wild-type 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. 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 wild-type metastatic colorectal cancer 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 wild-type metastatic colorectal cancer patients, even without modifing K-Ras gene status, could lead to the destruction of wild-type 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 wild-type 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. Results of this prospective study can be justified by this hypothesis. Moreover, the tumor cell entrance to epithelial to mesenchimal transition (EMT) or the reverse mesenchimal-to-epithelial transition (MET) 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 mesenchimal 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)[35] 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 downregulate 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.

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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)

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)

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

Best response 1st Cetuximab	Best response rechallenge	# patients	Total # (%)
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)

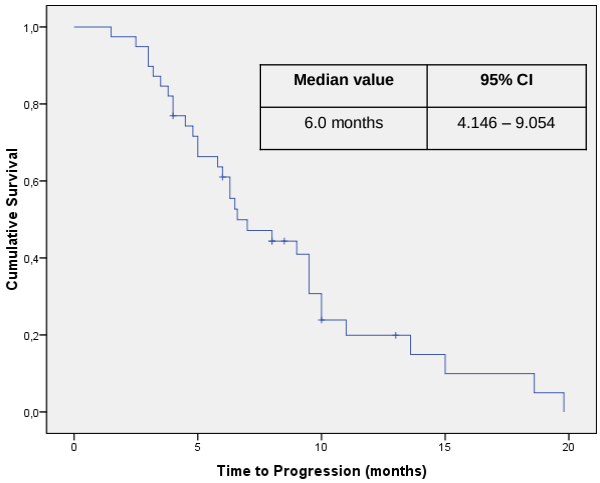

Figure 1. Kaplan-Meyer curve for Time-to-progression.

APPENDIX

LAST THREE YEARS CURRICULUM VITAE

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Oncologist
Date of Birth, 23rd January 1979
Place of Birth, Palermo
Nationality, Italian

Fellowship:

28.06.10 – 13.08.10: Clinical Training at Department of Nuclear Medicine, Clinica Rotger, Palma de Mallorca.

09.05.11 – 28.06.11: Clinical Training at Clinic of Gastrointestinal Tumors, Oncology Department, Hospital Son Llatzer, Palma de Mallorca.

Professional Career:

January 2009 – December 2011: Internship as Attending Physician at Department of Oncology, Policlinico "Paolo Giaccone", Palermo.

February 2010 – December 2011: Breast Cancer Screening Program at LILT, Palermo

Professional Societies Membership:

AIOM (Associazione Italiana di Oncologia Medica)

Scientific Activities:

Co-investigator and Data manager in Multicenter Clinical Trials, managed by different Clinical Research Cooperative Groups, according to Good Clinical Practice (ITMO, SICOG, GOIM).

Clinical trials:

- CISAPREP
- ERACLE
- CAPRI
- TAILOR
- TRIGGER
- ShortHER

Co-author of various papers in international journals, abstracts in international conferences, monographies, as listed below.

LIST OF THE SCIENTIFIC PUBLICATIONS

PAPERS

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