Resumen

Objetivo: Evaluar la respuesta a cetuximab en términos de tiempo hasta progresión y supervivencia global en pacientes con cáncer colorrectal (CCR) con determinación del receptor del factor de crecimiento epidérmico (EGFR) indetectable.

Método: Se seleccionaron nueve pacientes con cetuximab EGFR negativo, confirmado mediante estudio inmunohistoquímico. Variables recogidas: datos demográficos, diagnóstico, tratamientos previos, tiempo desde la primera metástasis hasta inicio con cetuximab, reacciones adversas y marcadores tumorales. La respuesta se monitoreó mediante marcadores tumorales y progresión de la enfermedad. La evaluación de la calidad de vida mediante estado funcional de Karnofsky (KPS) o Eastern Cooperative Oncology Group (ECOG).

Resultados: 22% hombres (2/9) con una mediana de edad de 48 años (31-63). La mediana de tiempo desde el diagnóstico de enfermedad metastásica hasta inicio de tratamiento con cetuximab fue 19 meses (12-48). Todos los pacientes habían fracasado a un esquema que incluyó irinotecán, el 77,77% (7/9) también a uno con oxaliplatino. La mediana de ciclos con cetuximab fue de 14 (rango 6-32). El principal efecto adverso fue la aparición de una erupción cutánea acnéiforme presente en el 100% de los casos. La mediana de tiempo hasta progresión fue 7 (rango 3-16) meses y la supervivencia global 10,2 meses (rango 4-24). Los resultados en calidad de vida mostraron KPS entre 80-100% y ECOG < 2. Los resultados obtenidos en nuestro estudio en supervivencia global y tiempo hasta progresión son superiores a los del estudio pivotal, 10,2 vs. 8,6 meses y 7 vs. 4,1 meses respectivamente.

Conclusiones: Con los resultados obtenidos se puede cuestionar la utilidad de la determinación de la expresión del EGFR, al menos mediante la técnica de inmunohistoquímica, como predictor de respuesta al tratamiento con cetuximab. Esto sugiere que la selección de los pacientes mediante la determinación rutinaria de este receptor pudiera ser inapropiada, ya que excluye a pacientes que potencialmente pueden beneficiarse del tratamiento. No obstante, se requieren más ensayos clínicos en este ámbito que corroben estas conclusiones.

Palabras clave: Cetuximab. Cáncer colorrectal. EGFR negativo. Inmunohistoquímica.
**Conclusions:** According to the results obtained, the use of assessing the EGFR expression (by the immunohistochemistry technique at least), as a means of predicting response to treatment with cetuximab may be questioned. This suggests that selecting patients using the routine assessment of this receptor is inappropriate, since it excludes patients who may potentially benefit from the treatment. However, more clinical trials are required in this area in order to confirm these conclusions.

**Key words:** Cetuximab. Colorectal cancer. EGFR-negative. Immunohistochemistry.

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**INTRODUCTION**

19,000 new cases of colorectal cancer (CRC) are diagnosed in Spain every year and, if we include both sexes, it has the highest incidence rate, representing the second highest cause of death by cancer. Survival rates in Spain are slightly higher than the European average (the five-year survival rate is approximately 50%), and near or higher than the average in the most developed countries such as Switzerland, France or Germany. In general, surgery with or without adjuvant treatment, cures half of CRC patients, while there is a recurrence of the disease in the remaining half, and palliative treatment is therefore required. According to data from the National Cancer Institute, approximately 50% of CRC patients will be diagnosed with hepatic metastasis, whether this is at the time of diagnosis or as a result of a recurrence of the disease, and only a small proportion of these patients are suitable for surgical resection.

The median overall survival for patients with advanced CRC has increased from 12 months to 18-21 months in the last decade. This increase is mainly due to the introduction of new drugs such as irinotecan, oxaliplatin and oral fluoropyrimidines. However, even with the progress made in comparison with traditional chemotherapy, these treatments still have their limitations.

The recent discovery that growth factors are significantly involved in regulating cell proliferation and differentiation has led to the development of new therapeutic agents for treating cancer. The epidermal growth factor receptor (EGFR) was the first to be identified from the family of receptors known as tyrosine kinase type I or ErbB or HER-1 receptors. When the epidermal growth factor is bound to its receptor it causes phosphorylation of the tyrosine kinase, which in turn produces a signal transduction cascade, which affects DNA synthesis. The EGFR signal pathways are involved in controlling cell survival, cell cycle progression and angiogenesis, as well as cell migration and invasion or cellular metastasis.

The EGFR is overexpressed in 25-77% of the cells of CRC patients, and in 72-82% of the cells of patients with irinotecan-refractory CRC. This overexpression has been associated to a worse prognosis and it has been studied as a possible target of other antitumour agents.

EGFR expression in the tumour tissue may be quantified using different techniques such as Western blot, ELISA, polymerase chain reaction and genome amplification among others. However, an immunohistochemistry study is the currently preferred method due to its relative simplicity and low cost.

Recent advances in genetic engineering have led to the development of monoclonal, human or chimeric antibodies, which are able to block these signalling cascades which lead to cell cycle progression.

Cetuximab is an IgG1 chimeric monoclonal antibody targeting the EGFR. Its affinity is approximately 5 to 10 times greater than that of the endogenous ligands, thus inhibiting the receptor function. It also induces EGFR internalisation, which therefore reduces the number of receptors available on the cell surface. This drug has been approved in Spain, to be used in combination with irinotecan, for treating patients with metastatic CRC who express the EGFR, following the failure of cytotoxic treatment which may have included irinotecan.

The linear correlation between the degree of EGFR expression and response to cetuximab is currently being questioned, since several authors have described a response in EGFR-negative patients.

The aim of this study is to evaluate the efficacy of cetuximab in CRC patients in which the EGFR is undetectable (negative) in an immunohistochemistry study.

**METHOD**

Patients with metastatic CRC (EGFR-negative) who were being treated with cetuximab from August 2004 to October 2006 were selected from the pharmacy department’s centralised cytostatic unit. The analysis data were obtained from reviews of medical records.

The following variables were collected:

- Demographic data: age at diagnosis and sex.
- Diagnosis: histological classification and staging at diagnosis, according to the TNM classification.
- Treatment regimes followed.
- Time between locating the first metastasis and start of treatment with cetuximab.
- Location of the metastases when treatment with cetuximab was begun.
- Adverse events experienced during treatment with cetuximab, assessing their clinical severity in accordance with the Common Terminology Criteria for Adverse Events (CTCAE).
- Tumour markers during treatment with cetuximab: Carcinoembryonic antigen (CEA) and CA 19.9.

The following response variables were established in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST): time until disease progression (defined as the period between the date on which treatment with cetuximab started, until disease progression was first observed), and overall survival (the period...
between the date on which treatment with cetuximab started until the death of the patient due to any cause or the last monitoring date).

The response was monitored based on the evolution of tumour markers and disease progression, confirmed using nuclear magnetic resonance and computed axial tomography.

High serum levels of CEA before treatment have a negative significance on prognosis. CA 19.9 is useful in monitoring resected tumours so that tumour recurrence may be detected early.

Well-being was evaluated using the Karnofsky performance status (KPS), or the Eastern Cooperative Oncology Group (ECOG) scale. This was assigned by the doctor based on observations of the patients’ ability to perform ordinary tasks.

The statistical analysis of the data was performed using the SPSS program version 14.0.

**RESULTS**

A total of nine patients were selected.

In all cases the samples for assessing the HER-1 were obtained from the primary tumour, and in two of these, metastasis was located using a fine needle aspiration biopsy (FNAB). An immunohistochemistry study was used to assess the EGFR expression, and negative results were found in all samples.

Of all the patients, 22% were men (2/9) and the remaining 78% were women (7/9), with a median age of 48 (31-63). All tumours were histologically classified as mucinous adenocarcinoma and the most frequent location was in the sigmoid (55.5%), followed by the rectum and caecum. At the time of diagnosis, 6 patients already presented with hepatic metastases, which are considered irresectable (Table I).

The median time between diagnosis of the metastatic disease and the start of treatment with cetuximab was 19 months; however, this figure varies considerably if patients are evaluated individually (12-48) (Table II).

Before beginning therapy with cetuximab, all patients had failed an irinotecan-based chemotherapy regime. 77.77% (7/9) of the patients had also failed a regime which included oxaliplatin. Cetuximab was essentially the last treatment option possible for these patients and it was administered as a fourth line treatment to 44.4% (4/9) of the patients, and as a third line treatment to 22.2% (2/9). The patients were monitored until the death of 8 patients and a change in the line of treatment due to disease progression in the remaining one. This patient was monitored until the study end date (October 2006) (Table III).

In all cases, there was correlation between disease progression and an increase in specific tumour markers.

Taking into account the fact that a cycle refers to two weeks of treatment, the median number of cetuximab treatment cycles received was 14 (6-32). Cetuximab was combined with irinotecan in all cases (initial dose of 400 mg/m², subsequent doses of 250 mg/m² every 7 days and modified FOLFIRI), except for one in which irinotecan had to be suspended due to the appearance of intestinal pseudobstruction following the first cycle. In other cases the cetuximab dose was reduced by 25% due to toxicity (onychopathy grade III).

**Table I. Characteristics of the patients at the beginning of the study**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Primary diagnosis</th>
<th>TNM Classification (stage)</th>
<th>Location of metastasis at start of treatment with cetuximab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>Female</td>
<td>Adenocarcinoma of the sigmoid</td>
<td>T4NxM1 (IV)</td>
<td>Liver</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>Male</td>
<td>Adenocarcinoma of the sigmoid</td>
<td>pT3N0M1 (IV)</td>
<td>Liver</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>Female</td>
<td>Adenocarcinoma of the caecum</td>
<td>pT3N1M1 (IV)</td>
<td>Liver</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>Male</td>
<td>Adenocarcinoma of the sigmoid</td>
<td>pT4N2M0 (IIIC)</td>
<td>Liver</td>
</tr>
<tr>
<td>5</td>
<td>48</td>
<td>Female</td>
<td>Adenocarcinoma of the sigmoid</td>
<td>T3NxM1 (IV)</td>
<td>Liver</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>Female</td>
<td>Adenocarcinoma of the rectum</td>
<td>pT3Pn2M0 (IIIC)</td>
<td>Lungs</td>
</tr>
<tr>
<td>7</td>
<td>58</td>
<td>Female</td>
<td>Adenocarcinoma of the rectum-sigmoid</td>
<td>pT3N0M0 (IIIC)</td>
<td>Lungs</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>Female</td>
<td>Adenocarcinoma of the sigmoid</td>
<td>pT4N2M2 (IV)</td>
<td>Liver</td>
</tr>
<tr>
<td>9</td>
<td>63</td>
<td>Female</td>
<td>Adenocarcinoma of the rectum</td>
<td>T4aN1M1 (IV)</td>
<td>Liver</td>
</tr>
</tbody>
</table>

**Table II. Characteristics of the treatments received**

<table>
<thead>
<tr>
<th>Previous treatments</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU/LV (Mayo Clinic Regime)</td>
<td>4</td>
</tr>
<tr>
<td>425 mg/m² 5-Fluorouracil (5-FU) i.v. bolus + 20 mg calcium folinate x 5 days every 28 days</td>
<td></td>
</tr>
<tr>
<td>Modified FOLFIRI</td>
<td>9</td>
</tr>
<tr>
<td>400 mg/m² (5-FU) i.v. bolus/leucovorin-5-FU</td>
<td></td>
</tr>
<tr>
<td>2,400 mg/m² continuous infusion 46 h - irinotecan</td>
<td></td>
</tr>
<tr>
<td>180 mg/m² every 14 days</td>
<td></td>
</tr>
<tr>
<td>Modified FOLFOX 4</td>
<td>7</td>
</tr>
<tr>
<td>5-FU 400 mg/m² (5-FU) i.v. bolus/leucovorin-5-FU</td>
<td></td>
</tr>
<tr>
<td>2,400 mg/m² continuous infusion 46 h - oxaliplatin</td>
<td></td>
</tr>
<tr>
<td>85 mg/m² every 14 days</td>
<td></td>
</tr>
<tr>
<td>Capecitabine</td>
<td>5</td>
</tr>
<tr>
<td>Oral capecitabine 1,250 mg/m² x 14 days every 21 days</td>
<td></td>
</tr>
</tbody>
</table>

**Table III. Characteristics of the treatments received**

<table>
<thead>
<tr>
<th>Number of previous treatments</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU/LV (Mayo Clinic Regime)</td>
<td>3</td>
<td>(1-5)</td>
</tr>
<tr>
<td>Modified FOLFIRI</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Modified FOLFOX 4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Capecitabine</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5-FU/LV (Mayo Clinic Regime)</td>
<td>3</td>
<td>(1-5)</td>
</tr>
</tbody>
</table>
For all nine patients, the results obtained for median time until disease progression was 7 months (3-16), and 10.2 months (4-24) for overall survival.

In terms of well-being, the results obtained showed that the majority of patients were able to carry out ordinary tasks and work without requiring special care during treatment with cetuximab. In the 6 patients assessed using the KPS, the result varied between 80 and 100%. Only one case with a KPS of between 60 and 70% required a care plan. In all 3 of the patients in which the ECOG scale was used, the result was < 2.

The main adverse event associated to cetuximab was the appearance of an acneiform rash which presented in 100% of cases, as grade I in 5 patients, grade II/III in 3 and grade IV in 1.

### DISCUSSION

In Spain the officially approved indication for the use of cetuximab in patients with metastatic CRC is currently limited to those who express the EGFR (normally assessed using an immunohistochemistry study), and have failed an irinotecan-based chemotherapy regime.

The use of cetuximab in metastatic CRC patients who do not express the EGFR is currently very topical, given that, surprisingly, responses have been noted in EGFR-negative patients treated with monoclonal antibodies which target this receptor. These findings seem to show that overexpression of the EGFR is not a predictive factor of the response to cetuximab.

The immunohistochemistry technique, used to detect the EGFR, uses a specific antibody that has been previously marked with a substance that becomes visible, without affecting the ability of the antibody to form a complex with the antigen. It is possible to locate and identify the antigen-antibody complex within the sample being studied, using some specific techniques such as peroxidases and fluoresceins. This technique is semi-quantitative and is limited by the sensitivity of the monoclonal antibody as well as tissue handling and processing. There are published cases in which the receptor expression level was drastically reduced as a result of the primary tumour sample having been stored for a long time. Variation also occurred depending on the binding technique used. New techniques are currently being investigated at the DNA level in order to assess the EGFR by genome amplification (FISH, Southern blot, PCR). The FISH technique (fluorescence in situ hybridization) is very sensitive and has the advantage of not being affected by the sample storage time. However this technique is not yet widely available and has not been standardised for detecting the EGFR gene amplification. The use of the Southern blotting technique is limited since it uses radioactivity. Two factors must be borne in mind for all methods used to measure the genome amplification at DNA level: that the protein expression (EGFR) is visible in the absence of genome amplification and that amplification is uncommon in most human tumours.

Some preclinical data suggest that there is a linear correlation between the EGFR expression in tumour cells and response to therapy targeting the EGFR. However, it would appear that this concept has become a preconceived assumption, and many studies use an adequate level of EGFR expression to predict sensitivity to therapy, without taking into account, for example, the percentage of carcinogenic cells stained or the intensity of the stain.

It would seem that tumour response to the EGFR inhibition not only depends on the receptor expression level, but the subtype and/or location of this. The immunohistochemistry technique does not detect these differences in receptors.

In the pivotal study (BOND study), a considerable proportion of CRC patients with a high level of EGFR expression were refractory to the treatment, while patients with tumours with lower levels of expression responded to the therapy. This is exactly what Vallböhmer et al. confirmed, thus showing that authors of previous studies could not be certain when describing the significant correlation between the EGFR expression, based on immunohistochemistry, and response to treatment with cetuximab and irinotecan, as well as cetuximab alone as monotherapy. Curiously, in phase II of this trial,
one of the patients with an undetectable EGFR responded to the treatment.

Although the lack of correlation between the EGFR level and the efficacy of this therapy may be explained by certain mechanisms, it is possible that detection of the EGFR expression (normally in the primary tumour), is not correlated to the EGFR level in the metastases, and also makes these cells resistant to treatment. This was confirmed by Scartozzi et al., who stated that the expression of this receptor may vary between the primary tumour and the place where metastases are found. They came to this conclusion after finding that seven of the patients who did not express the EGFR in the primary tumour did indeed express this receptor in the metastases. Metastatic cells are often biologically different to the primary tumour, perhaps due to additional mutations, which allow them to grow and perform clonal breeding, as a result of previous exposure to multiple chemotherapeutic agents. Only a prospective clinical trial, which includes an assessment of the EGFR in the metastases, can definitively establish whether this practice is appropriate for selecting patients for treatment with cetuximab. In the present study, the receptor in the primary tumour and the hepatic metastases was only assessed in 2 patients, and the results were similar for both sites.

Recently Chung et al. carried out a retrospective study in which they included 16 metastatic CRC patients who had an undetectable EGFR (assessed using an immunohistochemistry study). In 25% of the patients, an objective response was obtained for a combination of cetuximab and irinotecan. This has been confirmed in subsequent studies, in which 22-25% response rates were obtained in CRC patients (EGFR-negative) who were refractory to irinotecan and oxaliplatin. These results are similar to those documented in trials carried out in which only those patients who did express the EGFR were included (22-25 vs. 22.9-25.2%). The main variable considered in the pivotal study as well as those mentioned above was objective response confirmed by radiology.

However, in the case of the metastatic disease it is worth monitoring ultimate objectives such as overall survival and well-being, rather than settling for significant results from intermediary variables. It is for this reason that overall response has not been used as the main variable in this study and ultimate variables have been used instead. It is believed that such variables have a greater impact in oncology.

Taking into account the limitations of such a small population, the results obtained in the present study in terms of overall survival do not differ significantly from the findings of the pivotal study, with patients who did express the EGFR (9.8 compared to 8.6 respectively). However, if the patient who is currently undergoing palliative treatment is included, there is a notable increase in overall survival, which is greater than 10.2 months. The same occurs with the variable for time until disease progression, which is higher than that found in the pivotal study (7 compared to 4.1 months). However, the differences between the populations compared must be taken into account, and the line of treatment which included cetuximab, as well as the chemotherapy regime followed, must be highlighted (irinotecan in the pivotal study and FOLFIRI in the present study).

The results obtained for the ultimate variables analysed were not at the expense of the patients’ well-being (KPS = 80-100%, ECOG < 2). This treatment allows patients to carry on with a relatively normal life, although it is not possible to state which drug contributed more to this, cetuximab or the combination of drugs in the FOLFIRI regime.

Furthermore, different authors have tried to establish a correlation between the presence of dermal toxicity, response and overall survival; however none of the studies obtained statistical significance. This is probably due to the small number of patients who responded, although those that did showed a high level of dermal toxicity. Although all of our patients presented with an acneiform rash (some to a greater extent than others), no relationship between toxicity severity and the efficacy parameters evaluated was found.

The statistical analysis of this study is limited due to the low number of patients. However, given the current level of interest in the use of cetuximab in CRC patients, independent of the EGFR expression, and the lack of published studies on this group of patients, the authors of the present work believe that it is important to make the efficacy results available and compare them with those obtained in clinical trials with patients who do express the EGFR.

In light of the results obtained, the use of assessing the EGFR expression (at least when using the immunohistochemistry technique) to predict response to treatment with cetuximab in metastatic CRC patients may be questioned. This suggests that selecting patients by a routine assessment of this receptor is inappropriate, since it excludes patients who may potentially benefit from this treatment. The same view is recommended in certain clinical practice guides such as the National Comprehensive Cancer Network, where the inclusion/exclusion of patients for therapy with cetuximab on the basis of results obtained using the current techniques is questioned.

However, more clinical trials in this area are required to confirm these conclusions.

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