

Hepatic Intra-arterial Cetuximab in Combination with 5-Fluorouracil and Cisplatin as Salvage Treatment for Sorafenib-refractory Hepatocellular Carcinoma

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Abstract. *Background:* Sorafenib is the only therapy approved for advanced hepatocellular carcinoma no longer eligible for transcatheter arterial chemoembolization. Hepatic intra-arterial chemotherapy has been shown to be an effective and safe therapy for advanced hepatocellular carcinoma. Cetuximab has been administered intravenously to patients with advanced hepatocellular carcinoma, showing encouraging results in terms of its safety and toxicity profile. *Aim:* Our purpose was to evaluate the safety and feasibility of hepatic arterial chemotherapy with cetuximab, cisplatin and 5-fluorouracil for patients with advanced hepatocellular carcinoma, not responsive or not eligible for sorafenib therapy. *Patients and Methods:* From January 2010 to January 2011, 12 patients received a 2-day course of chemotherapy consisting of repeated daily hepatic arterial administration of 20 mg of cisplatin as 2-h infusion, 5-fluorouracil at 500 mg/m² as 5-h infusion and cetuximab 500 mg/m² as 12-h infusion. Cycles were repeated every 14 days. *Results:* After a mean of four months of therapy, computed tomography revealed five partial responses, five cases of stable disease and two of progressive disease. The toxicity profile was favourable, with

no G4 gastrointestinal, hematologic or skin side-effects, or severe deterioration of liver function. *Conclusion:* Hepatic intra-arterial chemotherapy with cetuximab is a safe and feasible treatment for advanced hepatocellular carcinoma, with promising results in patients with initial poor prognosis.

Hepatocellular carcinoma (HCC) is the sixth most common tumor worldwide and, due to its poor prognosis, ranks as the third common cause of death from cancer (1). Unresectable HCC is an aggressive disease, with a median survival at diagnosis of 16 to 20 months for intermediate disease, and of only 6 months for untreated patients at advanced stages.

Sorafenib, a multikinase inhibitor that targets serine/threonine and receptor tyrosine kinases in order to reduce tumor growth and angiogenesis, was approved in 2007 for the treatment of patients with advanced HCC. Two randomized phase III double-blind placebo-controlled clinical trials conducted in patients with advanced HCC showed a modest but statistically significant improvement in survival and in time to radiologic progression in the patients treated with sorafenib compared to the group treated with placebo (2, 3).

No other treatment is currently approved or is known to be effective for patients with advanced HCC whose disease is in progression, or who are intolerant to sorafenib. Life expectancy for these patients, often presenting with portal vein invasion or extrahepatic disease, is about six months (4).

Hepatic intra-arterial chemotherapy (HAIC) with cisplatin and 5-fluorouracil (5-FU) was demonstrated to be safe and partially effective in severely advanced cases of HCC (5-7).

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Table I. Patient characteristics.

Patient	Age (years)	Gender	Cause of hepatic disease	ECOG PS	Histology	Grading	Child-pugh class
1	67	M	Alcohol	0	Trabecular	2	A5
2	50	F	HCV	0	Trabecular	3	A5
3	74	M	HBV	1	Pseudoglandular	3	B7
4	72	F	HCV, HBV	0	Trabecular	1	A5
5	69	M	HCV	2	n.a.	n.a.	A5
6	69	M	HBV, alcohol	0	HCC clear	2	A6
7	83	M	HCV	1	n.a.	n.a.	B7
8	76	M	Alcohol	0	Trabecular	2	A5
9	69	M	HBV	0	Trabecular	2	B7
10	66	M	HBV	0	n.a.	n.a.	A6
11	63	M	-	1	Trabecular	1	B7
12	70	M	HCV	0	n.a.	n.a.	A5

HCV: Hepatitis C virus, HBV: hepatitis B virus, ECOG: Eastern Cooperative Oncology Group, PS: performance status, n.a.: not applicable.

Since HCC is a highly vascular tumor with predominantly arterial blood supply, it is particularly suitable for intra-arterial chemotherapy (8).

Recently, some reports indicated that epidermal growth factor receptors (EGFR) are frequently expressed in human HCC. EGFR expression appears to play an important role in hepatocellular carcinogenesis, and most likely contributes to the aggressive pattern of growth of this type of tumor (9-11). EGFR is a promising target for innovative strategies in advanced HCC. Cetuximab is a chimeric monoclonal IgG1 antibody targeting EGFR that has already been approved in advanced colorectal cancer (*KRAS* wild-type) and in advanced head and neck cancer, binding specifically to EGFR resulting in an inhibition of the receptor function (12). Some studies have reported preliminary experience in the clinical use of cetuximab in patients affected by advanced HCC, with conflicting results. When cetuximab was used as monotherapy, although it was well tolerated, it showed no antitumor activity (13). Conversely, when it was combined with gemcitabine and oxaliplatin, it appeared to be active with manageable toxicity (14).

Our purpose was to evaluate the use of intrahepatic arterial infusion of cetuximab, in combination with cisplatin and 5-FU, in patients with advanced HCC, not eligible for sorafenib therapy, or with a disease that progressed after sorafenib treatment.

Patients and Methods

Patients. Between January 2010 and January 2011, 12 consecutive patients with advanced HCC not responsive to (n=7) or not eligible for sorafenib therapy (n=5) were enrolled in the study.

Patients were not eligible for sorafenib because of poor hepatic functional reserve (n=4), or a recent acute cerebrovascular event (n=1). Diagnosis of HCC had been previously confirmed by

contrastographic imaging techniques and tumor biopsy in eight cases and by imaging techniques alone in four cases, according to the recent diagnostic guidelines (15).

Eleven patients had previously undergone one or more treatments either surgical (n=1) or nonsurgical, such as percutaneous ethanol injection (n=5), percutaneous radiofrequency thermal ablation (n=6) and transcatheter arterial chemoembolization (n=10). Only one patient had not received any previous locoregional treatment for HCC. All enrolled patients had multinodular hepatic disease, without extrahepatic metastases and with portal tumor invasion in 8 out of 12 cases. Liver functional reserve, evaluated using the Child-Pugh classification (16), corresponded to grade A score 5 in six patients, grade A score 6 in two patients and grade B score 7 in four patients. Tumor stage was determined by Barcelona Clinic Liver Cancer (BCLC) and Cancer of the Liver Italian Program (CLIP) classifications (17, 18). A consent form, approved by the Institutional Ethics Review Committee, was obtained from each participant. Pre-treatment investigations included a complete medical history and physical examination, alpha-fetoprotein (α -FP) assay, electrocardiogram, hematologic and biochemical profiles, contrast-enhanced abdominal computed tomography (CT) and chest x-ray. Body weight, performance status (PS) and clinical examination were recorded before each cycle. A summary of patient and tumor characteristics is reported in Table I and Table II.

Hepatic artery infusion chemotherapy. Celiac angiography was performed following the Seldinger method and using the transfemoral arterial approach in four patients and the trans-subclavian arterial approach in eight patients. Arteriography of the celiac trunk and superior mesenteric artery was performed (using a 4F sheath, a radiopaque catheter and a 0.035-inch hydrophilic guidewire) to visualize the arterial vascularization of the liver and of the tumor lesions. The gastroduodenal artery was occluded with micro-coils to prevent gastroduodenal injury from a reflux of the anticancer agents. A dedicated catheter was placed in the left, right, or common hepatic artery depending on the topography of the tumor lesions. After confirming the correct location of the catheter tip, a reservoir connected to the catheter was implanted in a subcutaneous pocket in the left anterior chest wall or in the right groin region, depending on the access, subclavian or femoral, respectively.

Table II. *Tumor characteristics.*

Patient	Histology grading	BCLC stage	CLIP stage	EGFR	KRAS	BRAF	Macrovascular invasion	Previous locoregional treatment	Previous sorafenib
1	Trabecular G2	C	3	1	Wild-type	Wild-type	Yes	No	Yes
2	Trabecular G3	C	2	2	Wild-type	Wild-type	Yes	No	Yes
3	Pseudoglandular G3	C	3	1	Wild-type	Wild-type	Yes	TACE, RFTA	No
4	Trabecular G1	B	1	2	Wild-type	Wild-type	No	TACE	Yes
5	n.a. n.a.	C	1	n.a.	n.a.	n.a.	No	TACE, RFTA, PEI	Yes
6	HCC clear G2	C	1	2	Wild-type	Wild-type	Yes	TACE	Yes
7	n.a. n.a.	B	1	n.a.	n.a.	n.a.	No	TACE, RFTA, PEI	No
8	Trabecular G2	C	2	1	Wild-type	Wild-type	Yes	Surgery, TACE, RFTA, PEI	Yes
9	Trabecular G2	C	2	1	Wild-type	Wild-type	Yes	TACE, RFTA, PEI	No
10	n.a. n.a.	C	3	n.a.	n.a.	n.a.	Yes	TACE	Yes
11	Trabecular G1	C	3	2	Wild-type	Wild-type	No	TACE	No
12	n.a. n.a.	C	2	n.a.	n.a.	n.a.	Yes	TACE, PEI, RFTA	No

G: Grading, n.a.: not applicable, HCC: hepatocellular carcinoma, BCLC: Barcelona Clinic Liver Cancer, CLIP: Cancer of the Liver Italian Program, EGFR: epidermal growth factor receptor, TACE: transhepatic arterial chemoembolization, RFTA: radiofrequency thermal ablation, PEI: Percutaneous ethanol injection.

A 2-day course of chemotherapy consisted of daily intra-arterial administration of cisplatin at 20 mg as a 2-hour infusion, 5-fluorouracil at 500 mg/m² as a 5-hour infusion and cetuximab at 500 mg/m² as a 12-hour infusion. Treatment courses were repeated every 2 weeks until evidence of progressive disease or unacceptable toxicity. Prophylactic antiemetic drugs, administered intravenously before each course of chemotherapy, included: 8 mg of ondansetron, 8 mg of dexamethasone and 50 mg of chlorphenamine. Topical or oral antibiotics, or both, were also given in cases of skin toxicity.

Evaluation of therapeutic response and adverse events. Objective responses, defined as the sum of complete and partial responses, were evaluated by Response Evaluation Criteria in Solid Tumours (RECIST) criteria (19). Tumor responses were assessed by contrast-enhanced CT every 2 months (after 4 cycles of chemotherapy), or earlier in patients with suspected disease progression. Complete responses (CR) were defined as the complete disappearance of all assessable disease. Partial responses (PR) were defined as a decrease of >30% in the sum of the largest dimensions of target lesions. Stable disease (SD) was defined as a decrease of <30% or an increase of <20% in measurable lesions. Progressive disease (PD) was defined as an increase of at least 20% in measurable lesions or the appearance of new malignant lesions. AFP dosing was carried out at each cycle of chemotherapy. Adverse events (AEs) were evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 3.0) at every cycle. Liver

function was also reassessed before every cycle of chemotherapy, using Child Pugh score, in order to detect a deterioration of liver function. An abdominal plain x-ray was performed before the administration of each cycle to ensure the correct positioning of the catheter tip.

Immunohistochemical and molecular analysis. Formalin-fixed paraffin-embedded (FF-PE) sections derived from core needle biopsy of the tumor lesions were prepared for the assessment of EGFR status by immunohistochemical analysis (IHC) and a molecular analysis in 8 out of 12 patients enrolled in the study. EGFR expression was classified as negative (0), weakly positive (1+), positive (2+), or strongly positive (3+) (20).

Genomic DNA extraction was performed using a commercial kit (Nucleospin Tissue; Macherey-Nagel, Düren, Germany), according to the manufacturer's recommendations. KRAS exon 2 and BRAF exon 15 were individually PCR-amplified, and the PCR products were directly sequenced bidirectionally by dye-terminator sequencing after PCR purification with AMPure Magnetic Beads (Agencourt, Beverly, MA, USA). Sequencing products were purified using CleanSEQ Magnetic Beads (Agencourt) and separated by capillary electrophoresis on a CEQ 8800 DNA Analyzer (Beckman-Coulter, Milan, Italy). Primers for PCR and sequence are summarized in Table III. Sequence data were analyzed using BioEdit software (21), and manually reviewed.

Table III. *BRAF* and *KRAS* exons and corresponding primers used for mutation analysis for the study.

<i>BRAF</i> ex 15-F	TGCTTGCTCTGATAGGAAAATG
<i>BRAF</i> ex 15-R	AGCATCTCAGGGCCAAAAAT
<i>BRAF</i> ex 15-S	TGTTTCCTTTACTTACTACACCTCA
<i>KRAS</i> ex 2-F	GGTGGAGTATTTGATAGTGTATTAACC
<i>KRAS</i> ex 2-R	AGAATGGTCCTGCACCAGTAA
<i>KRAS</i> ex 2-S	TCATTATTTTATTATAAGGCCTGCTG

Results

Clinical efficacy. A total of twelve patients were enrolled in this study. All patients with histological samples available for immunohistochemical and molecular analysis (n=8) were EGFR positive and *BRAF* and *KRAS* wild type. At the time of data analysis, the patient population had received a total of 111 cycles of the intra-arterial chemotherapy regimen containing cetuximab. The median number of cycles administered per patient was 9 (range 5-19). Seven out of twelve patients were still continuing the treatment at the time of data analysis. Reasons for early discontinuation of study treatment included extrahepatic disease progression (n=4) and a sudden non cancer-related death in a patient with a history of chronic ischemic heart disease.

According to the RECIST criteria, at the first tumor assessment (after 4 cycles of chemotherapy), a PR was observed in five patients (42%), and no patient had a CR. SD was recorded in five patients (42%) and PD was detected in two patients (16%). We observed a long maintenance of the tumor response, ranging from 9 to 15 months (mean 12 months) in the group of four patients treated with more than 12 cycles of chemotherapy.

Toxicity. Table IV summarizes all the reported toxicities. Overall, we recorded a small number of AEs ranging from grade 1 to 3. No grade 4 toxicities were observed. The most common toxicities for all grades were skin rashes, observed in 9 out of 12 patients. Grade 3 skin rash was observed in two patients, requiring oral administration of doxycycline, topical application of vitamin K1 analog cream and a one-week delay in the administration of chemotherapy. Other major symptoms were skin xerosis and asthenia. One patient with previously known mild renal insufficiency experienced a worsening of renal function after two cycles of chemotherapy, requiring a dose reduction of cisplatin. Hematological toxicities were not frequent. Many patients in the study had a moderate or severe grade of pancytopenia at baseline related to liver disease. However, there were no delays in administering the cycles of chemotherapy caused by the worsening of pancytopenia. The most common catheter-related problems were the displacement of the catheter tip from the hepatic artery and the formation of

Table IV. Treatment-related toxicities.

Toxicity	Grade 1-2		Grade 3	
	n	%	n	%
Skin rash	9	75	2	16
Skin xerosis	6	50	-	-
Nail and periungual lesion	1	8	1	8
Asthenia	5	41	-	-
Nausea	4	33	1	8
Stomatitis	1	8	1	8
Constipation	2	16	-	-
Diarrhea	-	-	-	-
Infusion reaction	-	-	-	-
Ocular toxicity	3	25	-	-
Hypomagnesemia	2	16	2	16
Hypoalbuminemia	6	50	-	-

hematoma in the subcutaneous pocket of the reservoir. Catheter displacement occurred in three cases, shown by abdominal plain x-ray. The tip of the catheter was correctly placed again into the hepatic artery in all cases, but the procedure caused a delay of two weeks in the administration of chemotherapy.

Discussion

Since two randomized phase III, double-blind, placebo-controlled clinical trials demonstrated a significant, although clinically modest, improvement in overall survival (2, 3), sorafenib has been considered the standard treatment for good performance status patients with advanced hepatocellular carcinoma. To date, no other treatment is currently approved for patients with advanced HCC whose disease is in progression, or who are intolerant to sorafenib. Life expectancy for these patients, often presenting with portal vein invasion or extrahepatic disease, is very poor, not exceeding 6 months (4).

Epidermal growth factor receptor (EGFR)-dependent pathway plays an important role in the development and progression of human epithelial cancer, including HCC. Approximately 70% of human HCCs express this receptor (9-11) and its activation is involved in hepatic carcinogenesis, tumor growth and dissemination. Cetuximab is a chimeric monoclonal IgG1 antibody targeting EGFR that binds specifically to EGFR with an affinity that is approximately 5 to 10-fold higher than endogenous EGFR ligands, resulting in an inhibition of the receptor function (12). *In vitro* studies of HCC in human cell lines showed that cetuximab inhibits growth of p53 wild-type HepG2 HCC cells in a time and dose-dependent manner; moreover, cetuximab treatment results in arresting the cell cycle in the G₁/G₀ phase by the modulation of cell cycle regulator expression, such as cyclin D1, and in inducing apoptosis

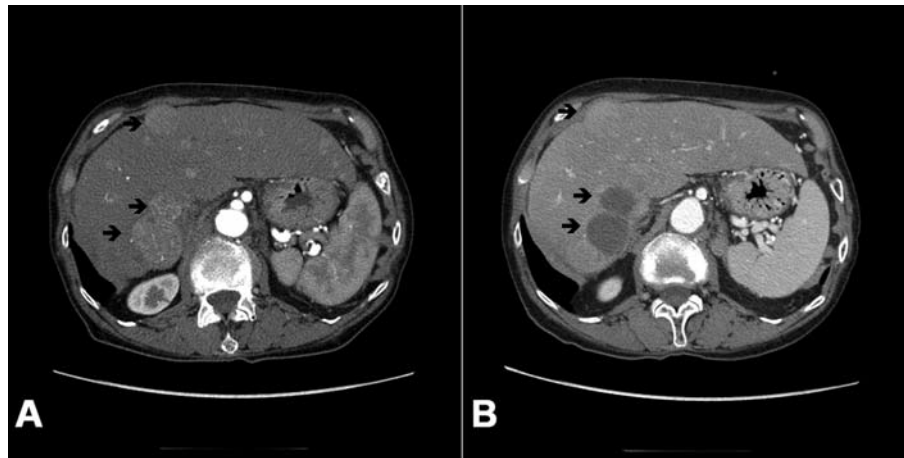


Figure 1. Arterial contrast-enhanced CT scan before (A) and after (B) 4 cycles of HAIC (arrows).

(22). Huether *et al.* have also evaluated the possible synergistic antineoplastic effects of cetuximab plus cytostatic drugs in HCC cell lines, particularly testing doxorubicin and cisplatin. Their results show that combining doxorubicin with cetuximab has a remarkable synergistic effect, which could otherwise be obtained only with high concentration of cisplatin (22).

On the basis of these findings, in the past three years, initial clinical experience with cetuximab in patients affected by advanced HCC has been gained. In the literature there are two phase II studies testing cetuximab in patients with advanced HCC. Zhu *et al.* (13) administered cetuximab intravenously (at an initial dose of 400 mg/m² with subsequent weekly doses of 250 mg/m²) to 30 patients affected by unresectable or measurable metastatic HCC, reporting no responses in terms of efficacy and survival improvement, but showing a tolerable toxicity profile; they also analyzed the pharmacokinetics profile of the monoclonal antibody in relation to hepatic metabolism, showing that no dosing modification of cetuximab was required in patients with mild to moderate hepatic impairment. Conversely, Asnacios *et al.* (14) first reported the promising results of cetuximab in combination with gemcitabine and oxaliplatin in the treatment of 45 untreated patients with advanced-stage HCC. They obtained disease stabilization in 40% of patients, and a median progression-free survival time and overall survival time of 4.7 months and 9.5 months, respectively. The toxicity profile was acceptable, and no treatment-related death was recorded (14).

In the period preceding the introduction of biological drugs in clinical practice, the treatment of advanced or metastatic HCC with conventional chemotherapy presented conflicting results. The most active *in vitro* and *in vivo* agents were doxorubicin, 5-FU and cisplatin. Systemic doxorubicin had

been evaluated in more than 1000 patients within clinical trials and provided partial responses in around 10% of cases, without any evidence of survival advantages (23). Combination chemotherapy containing cisplatin, doxorubicin, interferon alpha2b and fluorouracil (PIAF) was compared to doxorubicin in a large randomized clinical trial: response rates were of 20.9% for the PIAF regime and 10.5% for doxorubicin, and median survival rates for the respective groups were 8.67 and 6.83 months without significant differences between the groups (24). Hepatic arterial infusion of chemotherapy is an alternative approach to systemic chemotherapy. The main reason why HAIC may be particularly well-suited for this tumor is that HCC receives most of its blood supply from the hepatic artery, whereas normal hepatic tissue receives most of its blood from the portal vein (25, 26). This provides a theoretical basis for the administration of chemotherapeutic agents via the hepatic artery, with a relative sparing of normal hepatic tissues and with the possibility of prolonged drug exposure of the neoplastic tissue. Some studies have indicated the beneficial effects of intra-arterial infusion of 5-FU combined with cisplatin for HCC, obtaining response rates from 9-30% (27).

The purpose of our study was to evaluate the tolerability and safety of the intra-arterial hepatic infusion of cetuximab, in combination with cisplatin and 5-FU, in patients with advanced HCC, not eligible for sorafenib therapy, or with a disease that progressed after sorafenib therapy. To our knowledge this is the first time that cetuximab has been administered by this technique. Doses of cetuximab were calculated on the basis of the results obtained by Tabernero *et al.* (28). Even though the patient population is small, the results are very promising. On the first tumor assessment, according to RECIST criteria, we obtained PR in five patients, SD in five patients and PD in two patients;

furthermore, in the group of four patients treated with more than 12 cycles of chemotherapy, we observed a long maintenance of the tumor response, ranging from 9 to 15 months (mean 12 months). Response to treatment was always assessed by contrast-enhanced CT. We noticed two different X-ray patterns of response: a dimensional reduction or disappearance of some lesions, as normally observed after conventional chemotherapy, and the complete loss of the arterial contrast enhancement in other lesions without any change in their size (Figure 1). This is the first time that this typical radiological pattern of response to antiangiogenic drugs has been described for patients treated with cetuximab. This observation confirms the postulated antiangiogenic effect of cetuximab (29).

The toxicity profile of chemotherapy, and in particular of intra-arterial cetuximab, was tolerable, with no grade 4 AEs recorded. The most common AEs were skin rash and xerosis, and asthenia; moreover, the frequency of these events was lower than that observed after systemic administration of cetuximab. Symptomatic treatment was always able to successfully reduce the severity of grade 3 skin reactions to grade 2 or less. No toxic deaths occurred.

The implantation of the catheter was well tolerated by the patients; in three cases we recorded a displacement of the tip of the catheter, one probably due to the mechanical stress caused by the large hepatic tumor mass.

Seven out of twelve patients were still continuing the treatment at the time of data analysis, and we are still enrolling patients in order to increase the patient population.

Despite the small sample of patients, our results are promising: intra-arterial chemotherapy of cisplatin, 5-FU and cetuximab is well-tolerated treatment that appears to achieve survival benefits in a setting of patients very poor prognosis, candidates for supportive care alone. A phase I study to determine the correct dose of drugs in this setting of patients is ongoing.

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