Complete Response in 5 Out of 38 Patients with Advanced Hepatocellular Carcinoma Treated with Stem Cell Differentiation Stage Factors: Case Reports from a Single Centre

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Abstract: Hepatocellular carcinoma (HCC) represents the third cause of cancer-related death. Because HCC is multicentric with time, excluding the few transplanted patients, sooner or later it becomes untreatable with loco-regional therapies and, until some years ago, it was not responsive to systemic therapies. In 2005 a randomized trial indicated the efficacy of a product containing stem cell differentiation stage factors (SCDSF) taken from zebra fish embryos during the stage in which the totipotent stem cells are differentiating into the pluripotent adult stem cells. In such a trial the patients, with "intermediate" and "advanced" HCC according to BCLC/AASLD guidelines, presented benefit in terms of performance status (PS) and objective tumoral response, with some cases (2.4%) of complete response (CR). The aim of this cohort study is to report the experience of a tertiary referral center on the evidence of cases of CR in patients with "advanced" stage HCC treated with SCDSF as supportive care. CR was regarded as sustained disappearance of the neoplastic areas or blood supply therein, accompanied by normalization of AFP levels. Out of 49 patients consecutively recruited and retrospectively evaluated, 38 had "advanced" stage and 11 "terminal" stage. In 5 patients with "advanced" stage a sustained CR was reported (13.1%). Improvement on PS was obtained in 17 patients (34.6%). No side effects occurred. SCDSF treatment confirmed its efficacy in patients with "advanced" HCC, in terms of PS and tumoral response.

Keywords: Hepatocellular carcinoma, advanced stage, systemic therapy, stem cells, biological response modifiers, sorafenib, targeted molecular therapy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common tumor whose incidence is increasing worldwide, and represents the third cause of cancer-related death [1]. HCC usually affects patients with cirrhosis, mainly of viral origin. According to the stage, one disease will prevail over the other. When the tumor is at "early" stage (stage A according to the BCLC/AASLD guidelines) [2], radical treatment such as liver transplantation or curative/palliative treatments such as surgical resection or percutaneous ablation techniques, can definitely cure the patient or prolong his survival, respectively [2]. However, most patients cannot benefit from these options because the neoplastic disease is too advanced at first detection, or becomes advanced during the follow-up after treatment, since it becomes multi-centric with time. These patients are usually candidates to receive only not radically curative regional intra-arterial treatments, the gold standard being conventional trans-arterial chemoembolization (TACE), or systemic treatment (ST), respectively. The former is performed in multi-nodular presentations without neoplastic portal thrombosis (stage B or "intermediate" according to BCLC/AASLD guidelines). A cumulative review considering previous meta-analyses and new contributions concluded that such treatment does improve 2-year overall survival (ranging from 27% to 49%) in comparison with the best supportive therapy available [3]. The latter, generally performed in patients not treatable with TACE because of their deteriorated general conditions or in patients with "advanced" or C stage disease (according to BCLC/AASLD guidelines) because of the presence of neoplastic portal thrombosis or extra-hepatic disease, until some years ago proved useless in terms of overall survival. Only a small randomized controlled trial (RCT), that was never validated, comparing treatment with megestrol versus no treatment demonstrated a favorable influence on the course of disease in selected patients [4,5]. A meta-analysis of STs used in RCTs published in 2005 concluded that none provided an overall survival advantage to patients with B and C stage [6]. Recent studies have demonstrated that mutations in growth factor receptor pathways have been found in HCC. EGFR mRNA is up-regulated in tissue samples from patients with HCC. Likewise, an increase in the amount of EGFR ligands that can activate these receptors, such as TGA-alfa, has been found in HCC cell lines. Constitutively, activated growth factor receptors are another type of mutation associated with hepatocarcinogenesis; thus, even in the absence of ligand, the pathway can be activated [7]. Following such an indication, RCTs using molecular targeted therapy were activated, demonstrating a modest but significant gain in survival versus placebo [8]. This benefit in survival was not correlated to the evidence of tumor shrinkage on imaging according to

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standard clinical trial criteria, but only to an induced stable disease. These favorable data for sorafenib were confirmed in a second study in Asia [9].

Previously, following a different mode of approaching carcinogenesis mutations, an open RCT was published in 2005 [10]. The trial assessed the efficacy of a product containing stem cell differentiation stage factors (SCDSF), taken from zebra fish embryos during the stage in which the totipotent stem cells are differentiating into the pluripotent adult stem cells, versus conservative treatment. The rationale for using such treatment was based on *in vitro* and *in vivo* experimental studies which demonstrated that tumor development in an embryo is reduced or suppressed when processes of cell differentiation are in progress. In 167 patients (76.5% with B stage not treatable with TACE and 23.5% with C stage), 33 presented (19.8%) an objective response, of whom 2.6% cases with complete response (CR) and 17.2% with partial response. Evaluation of survival showed significant difference between responders versus non responders, with negligible side effects. Additionally the majority of patients improved their initial Performance Status (PS).

Primary aim of this study was to validate the efficacy of SCDSF treatment for patients with "advanced" and "terminal" HCC, reporting the experience of a tertiary referral center.

MATERIAL & METHOD

This study is a retrospective trial based on the experience of a tertiary referral center, reporting the results of a cohort of patients with "advanced" (stage C) and "terminal" (stage D) HCC treated with SCDSF as supportive care (the product is patented only for supportive care and therefore can be officially administrated exclusively in this setting). The main end-points of the study was to evaluate the impact on PS and, if any, the rate of CR. Inclusion criteria were: 1) HCC diagnosed on a histopathological basis or following the non invasive criteria proposed by BCLC/AASLD guidelines; 2) no eligibility for potentially local curative therapies (liver transplantation, hepatic resection, percutaneous ablation therapies) or intra-arterial therapies; 3) patients with recurrent disease after previous therapies; 4) no concomitant ST during SCDSF treatment. Before therapy, each patient was evaluated with computed tomography (CT) to assess tumor stage and with dosage of serum alpha-fetoprotein (AFP). After the first 3-4 months of treatment, and every 3-4 months until discontinuation of the therapy, the patients were evaluated for the same parameters. Treatment was discontinued whenever the patient reported side effects had developed, or when disease progression without improvement in PS occurred. Treatment was continued in patients with neoplastic disease progression but showing improvement in PS. CR of disease was regarded as disappearance of the neoplastic areas or blood supply inside them (i.e. no enhancement during arterial and portal phase after contrast media), accompanied by normalization of AFP levels (<200 ng/ml, when > 200 ng/ml at baseline). Patients received the product (conceived and prepared by Biava P.M. and now currently present on the pharmaceutical market) containing stem cell differentiation stage factors taken at different precise moments of zebra fish embryo development. The product was administered in an oral dose of 1-1.5 ml/day (sublingual dose of 30 drops for patients < 60 kg in weight and 45 drops for patients > 60 kg, three times daily). In case of patients presenting CR, every case was individually presented.

Overall, from September 2005 to September 2009, 49 patients who fulfilled the inclusion criteria were censored and consecutively treated in the departments of hepatology and surgery, and followed-up until March 2010.

RESULTS

Out of 49 patients, 37 were men and 12 women, with a median age of 70 years (range: 46-83). Of these,70% were positive for serum HCV antibodies, 15% for serum hepatitis B virus surface antigen, and 12% for alcohol consumption over 40 g/day. The rate of recurrent disease after previous therapies was 87%, while the rate of SCDSF as first treatment was 12%. At the baseline, 38 patients (77.5%) presented an "advanced" stage and 11 (22.5%) presented a "terminal" stage, according to BCLC/AASLD staging system. At the end of the study no patient presented side effects or discontinued treatment.

10 out of 11 patients with "terminal" stage (20.4%) died before the first evaluation, 20 (40.8%) showed progression disease without benefit on PS, 12 (24.4%) with progression disease, stable disease or partial response presented an improvement of PS of 1 or 2 points in ECOG score.

Five out of 38 patients with "advanced" stage (13.1%) presented CR. One of these patients (case 2) during follow-up presented the appearance of a small new nodule < 2 cm in size, successfully treated with selective TACE. All these patients are alive and free of disease at the end of the study, after 53, 29, 27, 26 and 26 months of follow-up, respectively. Clinical details and CT scans for all these patients are reported. Overall improvement on PS (17 patients) was obtained in 34.6% of cases.

CASE 1

In October 2005, a 49-year-old-male with HVC-related cirrhosis in Child-Pugh's class A visited our surgery outpatient department under suspicion of HCC. CT scans showed a diffuse not homogeneous neoplastic lesion in the right lobe and a massive bi-lobar portal thrombosis reaching the main branch (Fig. (**1a**)), untreatable by resection. AFP level was 7883 ng/ml. The patient was considered by the oncologists of our center for enrolment into a phase III trial (SHARP trial, sorafenib vs placebo) but, because of the high level of transaminases, the enrolment was postponed.

Meanwhile, in October 2005, the patient visited another center where he was given standard SCDSF treatment, without the knowledge of the oncologists. In January 2006, after the normalization of transaminase levels, the patient was enrolled into the SHARP trial. CT scans performed from March 2006 to March 2007 showed a gradual decrease in size of the neoplastic lesions and of AFP levels. In May 2007 CT scans showed CR (Fig. (**1b**)). AFP level was 4 ng/ml. In July 2007, the opening of the blinded trial revealed that the patient was enrolled in the placebo group. In March 2010 the patient is alive and free of disease.

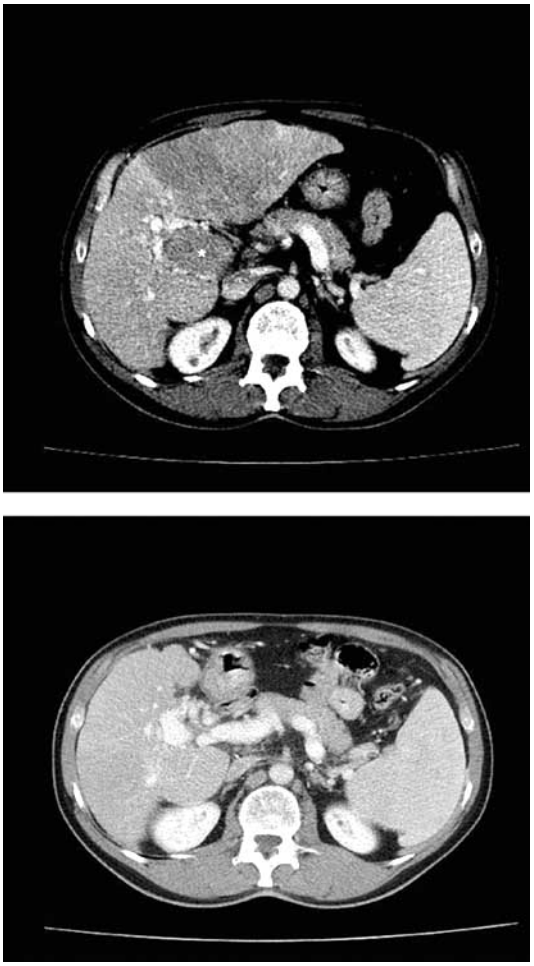

Fig. (1). a. CT scan during the portal phase performed prior to treatment shows a vascularized neoplastic thrombus (asterisk) occupying and enlarging the right portal branch and reaching the main trunk. **b.** CT scan during the portal phase performed after therapy shows the disappearance of the portal thrombus and the patency of the portal flow. The same pattern was confirmed along the follow-up.

CASE 2

In January 2008, a 74-year-old-male with HCV-related cirrhosis in Child-Pugh's A class visited our hepatology outpatient department because of advanced HCC. Since 1999, in another hospital, he had undergone several RFA and TACE to treat bi-lobar multinodularity. We subsequently performed total body CT scan, which showed multiple hepatic nodules, thrombosis of the left portal vein, thrombosis of the middle hepatic vein reaching the vena cava, and bilateral lung metastases (Fig. (**2a**,**b**)). AFP level was 72625 ng/ml.

In January 2008 he was given standard SCDSF treatment. A CT scan 4 months after treatment showed the decrease in volume of the hepatic nodules and thrombosis, and disappearance of lung metastases. AFP level decreased to 458 ng/ml. In August 2009 appearance of a new small nodule (< 2 cm) successfully treated with selective TAE. In January 2010, CT scan showed a CR because of the additional decrease in volume of hepatic nodules without enhancement during arterial and portal phase, and the disappearance of hepatic vein and vena cava thrombosis (Fig. (**2c**,**d**)). AFP level was 143 ng/ml.

CASE 3

In December 2008, a 75-year-old-male with HCV-related cirrhosis in Child-Pugh's class A visited our hepatology outpatient department because of advanced HCC. Since 2002, in another hospital, he had undergone resection, RFA and PEI to treat three HCC nodules. Because of progression of disease, the patient received sorafenib 400 mg twice every day from July to November 2008, which was discontinued for side effects. We subsequently performed CT examination, which showed several bi-lobar nodules and thrombosis of the left portal vein reaching the main portal trunk (Fig. (**3a**)). AFP level was 25136 ng/ml.

In December 2008 he was given standard SCDSF treatment. CT scans 4 months after treatment showed decrease in volume of the hepatic nodules, disappearance of thrombosis in the main portal trunk and in the left portal vein substituted by cavernoma. AFP level decreased to 5 ng/ml. In October 2010, CT scan showed CR. In March 2010, CT scans confirmed the previous pattern (Fig. (**3b**)). AFP level was 2 ng/ml.

CASE 4

In February 2008 a 73-year-old man with HCV-related cirrhosis in Child-Pugh's class A was admitted to our hepatology department because of HCC, 9 cm in size at segment 4 and two small nodules in other segments. AFP level was 5 ng/ml. He was given TACE. In April 2008 a CT scan showed partial Lipiodol uptake in the main nodule and persistence of enhanced tissue in remnant areas. In July 2008 he was given oral sorafenib 400 mg twice every day, which was discontinued in December 2008 because of progression of disease (appearance of portal thrombosis in the left portal vein reaching the main trunk) (Fig. (**4a**)).

In January 2009 he was given standard SCDSF treatment. In April 2009, CT scan showed a decrease in volume of hepatic nodules accompanied by a reduction of contrast enhancement, with persistence of portal thrombosis. In February 2010, CT scan showed CR, because of the disappearance of smaller nodules or additional decrease in volume without contrast enhancement in arterial and portal phases of the main lesion, and disappearance of portal thrombosis (Fig. (**4b**)). AFP level was 3 ng/ml. In March 2010 the patient was resected for the main lesion and the tributary portal vein, the aim being to ablate the neoplastic area before a possible future recurrence. Histopathology showed complete necrosis of the neoplastic tissue.

CASE 5

In September 2008, a 64-year-old-female with HVC-related cirrhosis in Child-Pugh's class B visited our surgery outpatient department. CT scan showed an infiltrating HCC in the left lobe with neoplastic thrombosis into the left hepatic vein reaching the vena cava (Fig. (**5a**)). AFP level was 21 ng/ml. The patient was submitted to TACE, followed by partial response.

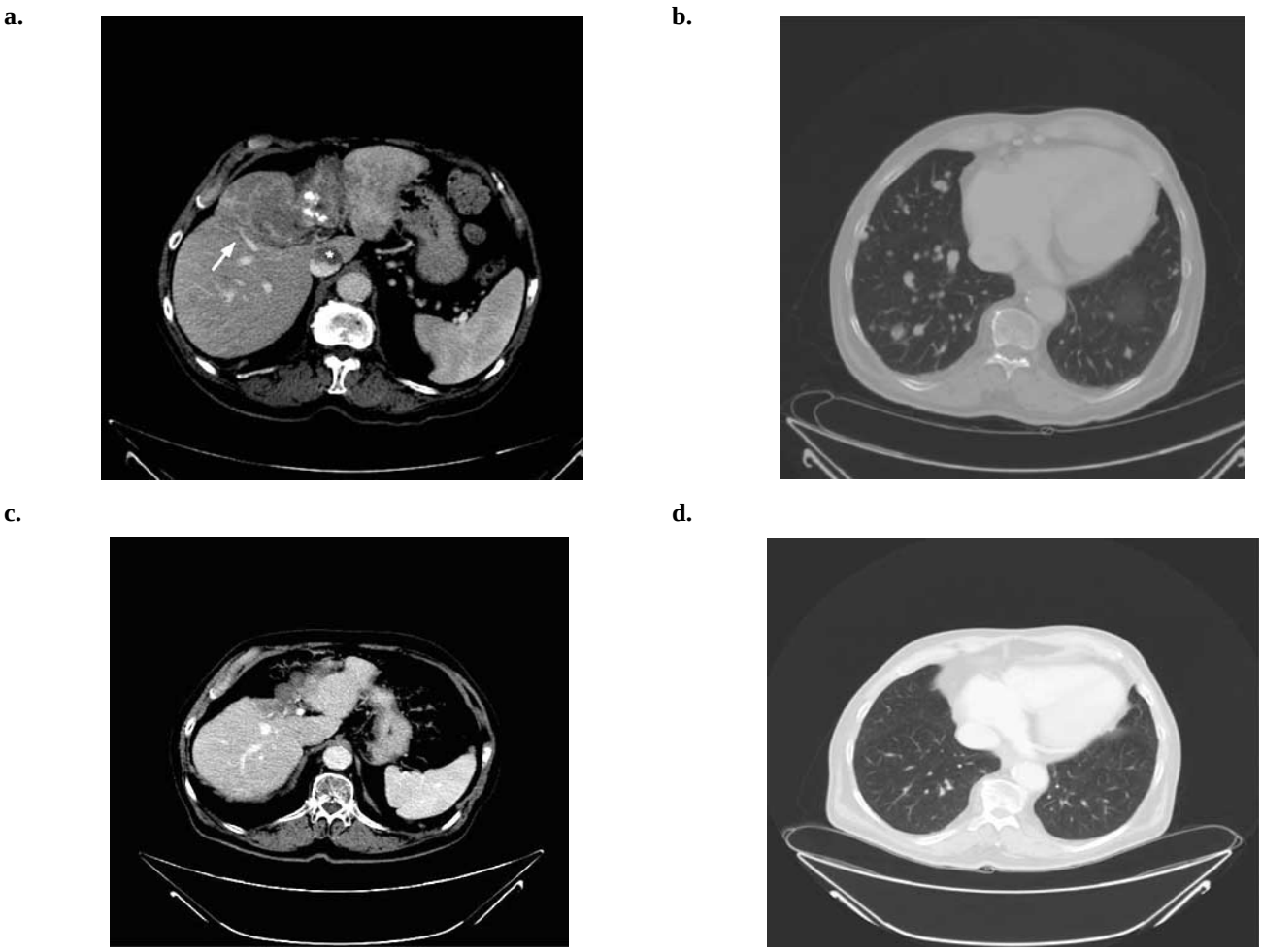

Fig. (2). a. CT scan during the portal phase performed prior to treatment shows one of the HCC lesions (arrow) reaching the vena cava (asterisk) through the hepatic veins. **b.** CT scan performed prior to treatment shows several bi-lobar lung metastatic nodules. **c.** CT scan performed during the portal phase after therapy shows the shrinkage of the HCC nodule without enhancement inside, and the disappearance of the neoplastic thrombus inside the vena cava. The same pattern was confirmed along the follow-up. **d.** CT scan performed after therapy shows the disappearance of the metastatic nodules. The same pattern was confirmed along the follow-up.

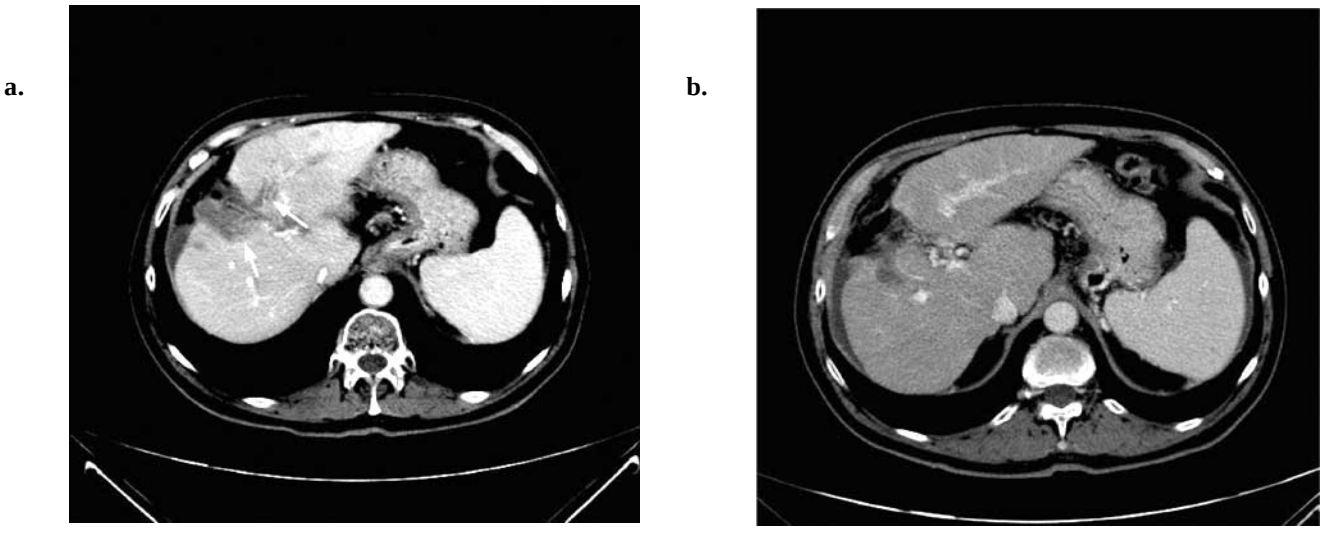

Fig. (3). a. CT scan during the portal phase performed prior to treatment shows one of the HCC lesions (arrow) partially occupying the left portal vein (arrow). **b.** CT scan performed during the portal phase after therapy shows the shrinkage of the HCC nodule without enhancement inside, and the disappearance of the neoplastic thrombus inside the portal vein. The same pattern was confirmed along the follow-up.

a. **b.**

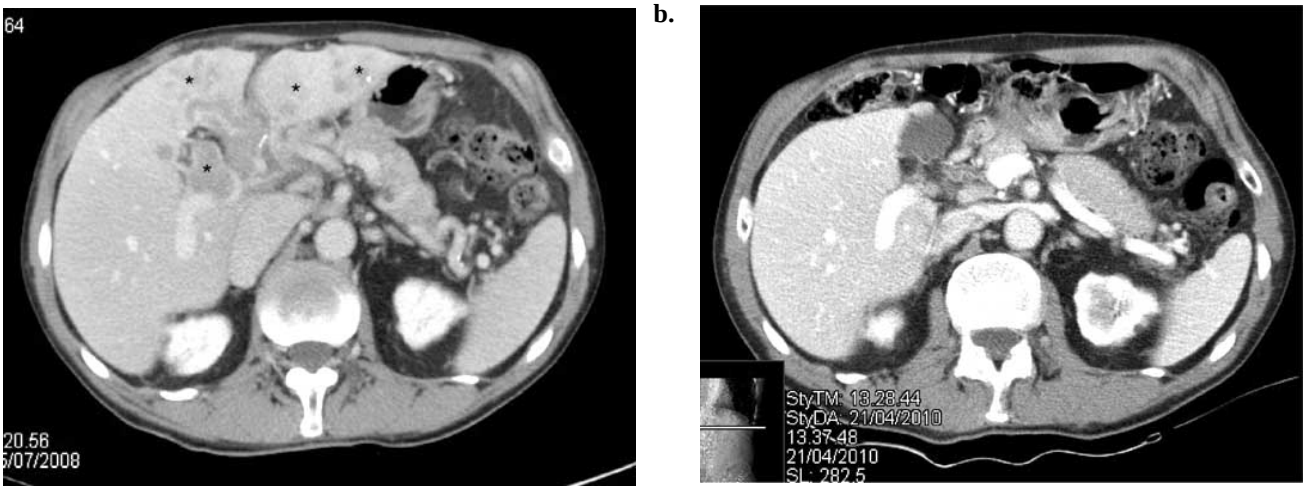

Fig. (4). a. CT scan during the portal phase performed prior to treatment shows a diffuse neoplastic thrombosis (asterisks) occupying all the portal system. **b.** CT scan performed during the portal phase after therapy shows the disappearance of neoplastic thrombosis and the patency of the portal system. The same pattern was confirmed along the follow-up.

In January 2009 she was given standard SCDSF treatment. Subsequent CT scans showed gradual decrease in volume and enhancement of the hepatic nodule, and disappearance of the thrombosis. In March 2010 the last CT scan confirmed CR (Fig. (**5b**)). AFP level was 16 ng/ml.

DISCUSSION

Despite the fact that surveillance programs have been widely implemented, curative therapies can only be applied to less than 30% of patients with HCC. Excluding the few transplanted patients, the others are likely to present "intermediate" and, at the end, "advanced" disease. A study on resected patients demonstrated that multi-centricity is already present in 50% of early stages and that 93% of patients with single, minute HCC presented other nodules within five years [11]. This means that sooner or later all the patients, treated or untreated, become untreatable with loco-regional therapies.

The rationale for using SCDSF derived from observational data on tumor suppression during embryo differentiation and experimental data on the effect of these factors on tumor growth both *in vivo* and *in vitro*. A model demonstrated that tumor cells are considered undifferentiated mutated cells, blocked in a step of multiplication comprised between two stages of cell differentiation. These cells have oncofetal antigens, which are maintained during phylogeny, and receptors for embryonic regulators of cell differentiation on their surface. It was demonstrated in prior studies that these regulators are able to stop or delay the growth of different human tumors *in vitro* through the control of important genes and proteins of the cell cycle, such as p53 and pRb [12-14].

The previous trial using SCDSF treatment demonstrated that it may be effective in terms of objective responses and stable disease in 38% of cases, and in terms of survival rate for responders [10]. In fact, the trial demonstrated a statistical difference (p=0.037) between treated and untreated patients before the stop of randomization (at the second interim analysis) comparing SCDSF treatment versus the best supportive care. In the patients presenting an objective response, excepting the cases where the tumor completely disappeared at first control, the pattern shown by CT scans was unusual. The typical neoplastic hypervascularization during the arterial phase decreased month by month (the so called "vanishing effect") due to neoangiogenesis reduction, whereas the tumor substantially maintained its baseline size until completely disappearing in cases of CR. The objective response ranged from 3 to 21 months (mean, 10.2), with some patients showing no disease progression at the end of the study. In such patients presenting longer responses some new, small (<2 cm), lesions, appeared in other hepatic segments. However this event was considered as regression, because it was not clinically relevant in relation to the period of observation compared to the observed regression of primary mass.

The main goal of this study was to confirm the possibility to improve the PS and to obtain cases of CR treating patients with SCDSF. The present retrospective cohort had a rate 13.1% of sustained CR, higher than that reported after the original RCT study.

However a recent RCT which compared 43 patients treated with SCDSF and 18 with megestrol, obtained better survival using megestrol with no CR in either treatment, while PS resulted better in patients treated with SCDSF [15]. Why did such a trial not obtain cases of CR after treatment with SCDSF? This trial recruited and randomized patients in two groups 2:1. In detail, its results were that at 3-month follow-up tumor burden was more frequently stable with megestrol (p=0.0017), while performance status was better with SCDSF (p=0.0017), and that at 6-month follow-up mortality was lower (p=0.0187) and long-term survival (p=0.025) more frequent with megestrol. The main explanation is that data were flawed by critical drawbacks in baseline characteristics, particularly regarding the different patients enrolled, i.e. with more advanced stage and then with poorer prognosis. In fact, the patients recruited in such a trial reached 1-year survival in 0.4% of cases treated with SCDSF, while in the original study [10] non responders reached 1-year survival in 48% of cases. From the original

a. **b.**

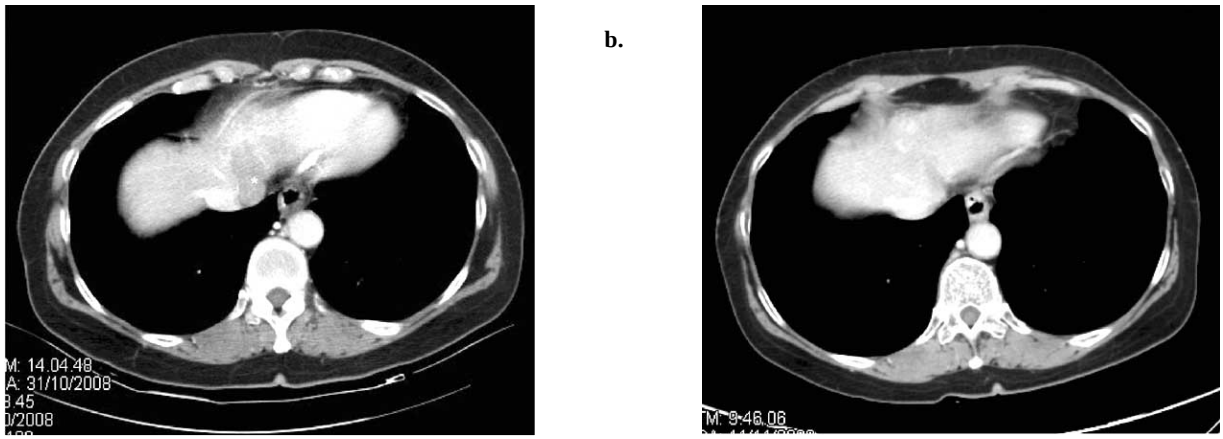

Fig. (5). a. CT scan during the portal phase performed prior to treatment shows a neoplastic thrombosis (asterisk) into the left hepatic vein reaching the vena cava. **b.** CT scan performed during the portal phase after therapy shows the disappearance of neoplastic thrombosis and the vein patency. The same pattern was confirmed along the follow-up.

trial and from the present study it emerged that SCDSF treatment usually needs some months to achieve valuable results, and never in already symptomatic patients. The same figure was observed in the present study for those patients with "terminal" stage who died before the first evaluation. This means that the majority of patients recruited into the trials was not comparable.

Of note, the present study also confirmed the possibility that some patients with objective response (1 out of 5 with CR) may present, even when the advanced tumoral presentation disappeared, new, small nodules, however not relevant to the poor prognosis expected without treatment.

HCC is molecularly heterogeneous, i.e. the underlying pathology that leads to its development may be different from patient to patient, and an agent may only exhibit efficacy in a subgroup of patients or even in only a portion of the same tumor. The fact could explain the cases presenting novel neoplastic foci even in presence of CR of the original tumor. Further studies are in progress to clarify the reasons for the different response behavior observed in different patients, in an attempt to establish, on the one hand, the more specific regulators for each kind of tumor and, on the other, the best selection criteria for optimal prediction of response. This means that studies should be continued in the fields of basic research to identify the specific networks of regulators for each type of HCC and in the field of clinical trials to confirm the efficacy of these networks in controlling tumor progression.

Meanwhile, the identification of key enzymatic steps in the growth control and angiogenesis pathways, resulting in specific chemical inhibitors and antibodies to several of the involved kinases, is stimulating a novel therapeutic approach for treating patients with HCC not to date considered suitable for codified therapies. Another challenge is that some mutations with a constitutionally active protein potentiate not one but several intracellular pathways. The two multitargeted kinase agents that are most advanced in clinical development are sorafenib and sunitinib. Sorafenib inhibits the Ras/Raf/MAP/ERK pathway, VEGFR-2 and -3, PDGFR-beta, KIT, RET and Flt-3 receptor tyrosine kinases. In a phase III trial, 602 patients from Europe, United States and Australia with "intermediate "(17.4%) or "advanced" (82.4%) stage and with Child-Pugh A class were randomized to receive either placebo or sorafenib at 400 mg twice a day [8]. Patients in the placebo arm had an overall survival of 7.9 months, whereas those in the sorafenib arm had an overall survival of 10.9 months (p=0.00058). No patients presented CR, while a partial response was documented in 7 of the sorafenib group (2%) and in 2 of the placebo group (1%). Drug-related adverse events reported by 10% of patients or more included principally hand-foot skin reaction and diarrhea. The multicenter RCT conducted in Asia-Pacific countries confirmed that the overall survival in the sorafenib arm was improved to 6.5 months in respect to 4.2 months of the placebo arm [9], without cases of CR. A phase II trial using sunitinib obtained promising results, useful for an ongoing RCT versus sorafenib [16]. Sorafenib, inhibiting tumor blood vessel development and tumor cell proliferation, is the first molecularly targeted therapy able to increase survival. Even though the drug has been approved both by the FDA and EMEA for the treatment of HCC, recently, because the cost is too high ($ 5.400/months for treatment) for the limited benefit sorafenib offers (about 2-3 months), the National Institute for Health and Clinical Excellence of London does not recommend its use [17]. Although the survival benefit is modest, sorafenib or other similar agents are proof-of-principle that targeting the different signaling pathways deregulated in HCC can be effective.

However, for the present, after the validation of the original trial, SCDSF treatment remains the only therapy able to obtain cases of CR.

Accordingly, the ideal strategy for "advanced" stage patients is to enroll them in RCTs testing sorafenib or similar drugs in combination with SCDSF in the attempt to improve survival and PS.

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