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Treatment With Stem Cell Differentiation Stage Factors of Intermediate-Advanced Hepatocellular Carcinoma: An Open Randomized Clinical Trial

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There is no standard treatment for patients with advanced hepatocellular carcinoma (HCC). We developed a product containing stem cells differentiation stage factors (SCDSF) that inhibits tumor growth in vivo and in vitro. The aim of this open randomized study was to assess its efficacy in patients with HCC not suitable for resection, transplantation, ablation therapy, or arterial chemoembolization. A total of 179 consecutive patients were enrolled. We randomly assigned the patients to receive either SCDSF or only conservative treatment. Primary end points were tumor response and survival. Secondary end points were performance status and patient tolerance. Randomization was stopped at the second interim analysis (6 months) of the first 32 patients recruited when the inspection detected a significant difference in favor of treatment ($p = 0.037$). The responses to the therapy obtained in 154 additional patients confirmed previous results. Evaluation of survival showed a significant difference between the group of patients who responded to treatment versus the group with progression of disease ($p < 0.001$). Of the 23 treated patients with a performance status (PS) of 1, 19 changed to 0. The study indicated the efficacy of SCDSF treatment of the patients with intermediate-advanced HCC.

Key words: Biological response modifiers; Embryo; Stem cells; Differentiation factors; Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is a common tumor whose incidence is increasing worldwide, and it represents the third most frequent cause of cancer-related deaths (1). HCC usually affects patients with chronic liver disease, mainly of viral origin. According to the stage, one disease will prevail over the other. When the tumor is at an early stage, 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 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, because it becomes multicentric with time.

These patients are usually candidates to receive only noncurative regional intra-arterial treatment or systemic treatment. The former treatment, generally employed in less advanced (usually intermediate) stages, has demonstrated some benefit in a meta-analysis of seven randomized controlled trials, but only in well-selected patients (3). The latter, generally performed in more advanced stages, has evidenced the lack of usefulness in terms of survival. In particular, the results of treatments with interferon (4), tamoxifen (5), antiandrogen (6), octreotide (7,8), or chemotherapeutic agents (9–12) have been disappointing except for some anecdotal cases (13). Moreover, systemic chemotherapy is associated with relevant toxicity and should be used with caution in cancer patients with liver impairment.

In this context, it is very important to investigate new therapeutic approaches against HCC in stages untreatable with traditional therapies.

The results of a series of patients affected by HCC

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and treated with a product that contains stem cell differentiation stage factors (SCDSF), taken from fish embryos during the stage of cell differentiation, in which the embryonic totipotent stem cells are differentiating into the pluripotent adult stem cells (50% epiboly stage) are herein reported. The experimental data, which allowed us to conceive and prepare the product, are based on the evidence obtained from studying the interactions between tumor cells and embryonic tissues. Such studies suggested that tumor development in an embryo is reduced or suppressed when processes of cell differentiation are in progress. In fact, previous studies demonstrated that tumor growth can be delayed or even suppressed by factors present in the embryos of ovipara and in the pregnant uteri of mammals in both in vivo and in vitro experiments (14–17).

Further studies were carried out in order to address which cell regulation pathways are involved in the embryo in this mechanism of tumor growth inhibition. It was demonstrated that key role cell cycle regulator molecules, such as p53 and pRb, are involved through transcriptional and posttranslational events. Changes in the expression levels of p53 after treatment of cells with embryonic substances taken during precise stages of cell differentiation (18), as well as changes in the phosphorylation pattern of pRb, were observed. The latter changes led to a modification of the hyperphosphorylated/hypophosphorylated pRb forms ratio within the whole population of treated cells (19). To study the apoptotic and differentiation events, further experiments were carried out. Western blot analysis, performed on Caco2 cancer cells, revealed that treatment with SCDSF induces caspase-3 activation, mainly by increasing the release of E2F-1, leading to c-Myc overexpression and activation of p73 apoptotic-dependent pathway (Cucina, A.; Biava, P. M.; D'Anselmi, F.; et al., submitted to *Cell Death and Differentiation*). On the basis of these studies, a new vision of cancer, related to a complexity model, was proposed (20) and a product that contains SCDSF was prepared by P. M. Biava.

The aim of the study was to evaluate whether treatment with SCDSF could obtain a regression of disease in patients affected by HCC untreatable with traditional therapies and whether survival of treated patients was significantly different in relationship to the different response to treatment.

MATERIAL AND METHODS

Preparation and Characterization of SCDSG

A thousand embryos of zebra fish at the 50% epiboly stage were washed in distilled water and placed in a solution of 1000 ml of pure glycerine and 30% ethylic alcohol at the ratio 4:1. The embryos were treated with a turboemulsifier for 3 min and then were vacuum filtered through millipore 90-μm membranes and subsequently 10-μm membranes. A chromatographical (HPLC) and bidimensional gel-electrophoresis analysis showed that the concentration of the proteins was 400 μg/ml; 93% of these proteins had a molecular weight ranging from 10 to 20 kDa. This solution of proteins was then diluted with ethylic alcohol 30% at the ratio 1:10 and L-cysteine, L-methionine, lipoic acid, gamma linoleic acid, retinoic acid, vitamin B_6, thiamine, magnesium pidolate, and grape fruit extract (GSE) as preservative were added to prepare the product used for the clinical trial. The nutritional features for 100 ml of this product are: 130 kcal; 450 kJ; 1 g protein; 17 g carbohydrates; 0.1 g fat; 17 g polyalcohols; 56 mg thiamine; 100 mg vitamin B_6; 10 mg retinoic acid; 8 mg magnesium.

To study the efficacy and the stability of such a product some experiments on different tumor cell lines (A172 glioblastoma and ZR75.1 breast carcinoma) were performed. These experiments demonstrated that the efficacy of the product in reducing tumor growth was maintained at the temperatures ranging from 4°C to 40°C for 2 years (unpublished data).

Patients and Study Profile

An open randomized clinical trial was carried out, by the closed-envelope method with randomization in blocks of four (see Fig. 1, top), in two different hospitals (Clusone and Vimercate, Italy), enrolling a total of 179 consecutive patients affected by HCC with the clinical characteristics described below. An informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines adopted by our institutions in case of a clinical trial in which nutritional products are used, in accordance with Ethical Regulations for Human Experiments in our country and with the Helsinki Declaration of 1995.

The trial compared two groups of patients affected by HCC and untreatable by potentially local curative therapies or lobar intra-arterial therapies (i.e., with intermediate or advanced stage according to the classification proposed by the Liver Cancer Unit of Barcelona) (21). In fact, the patients were not eligible for other therapies, including liver resection or transplantation, because of the stage of the disease and alcohol injection or ablation with radiofrequency because of the size, number, or location of the lesions. They also had very advanced disease or presented physical impairment or other specific or general contraindications for chemoembolization, or lastly refused to undergo such an invasive procedure.

Because the patients were in an intermediate or advanced stage, we applied noninvasive diagnostic criteria for HCC as stated in the Conclusions of the 2000 EASL

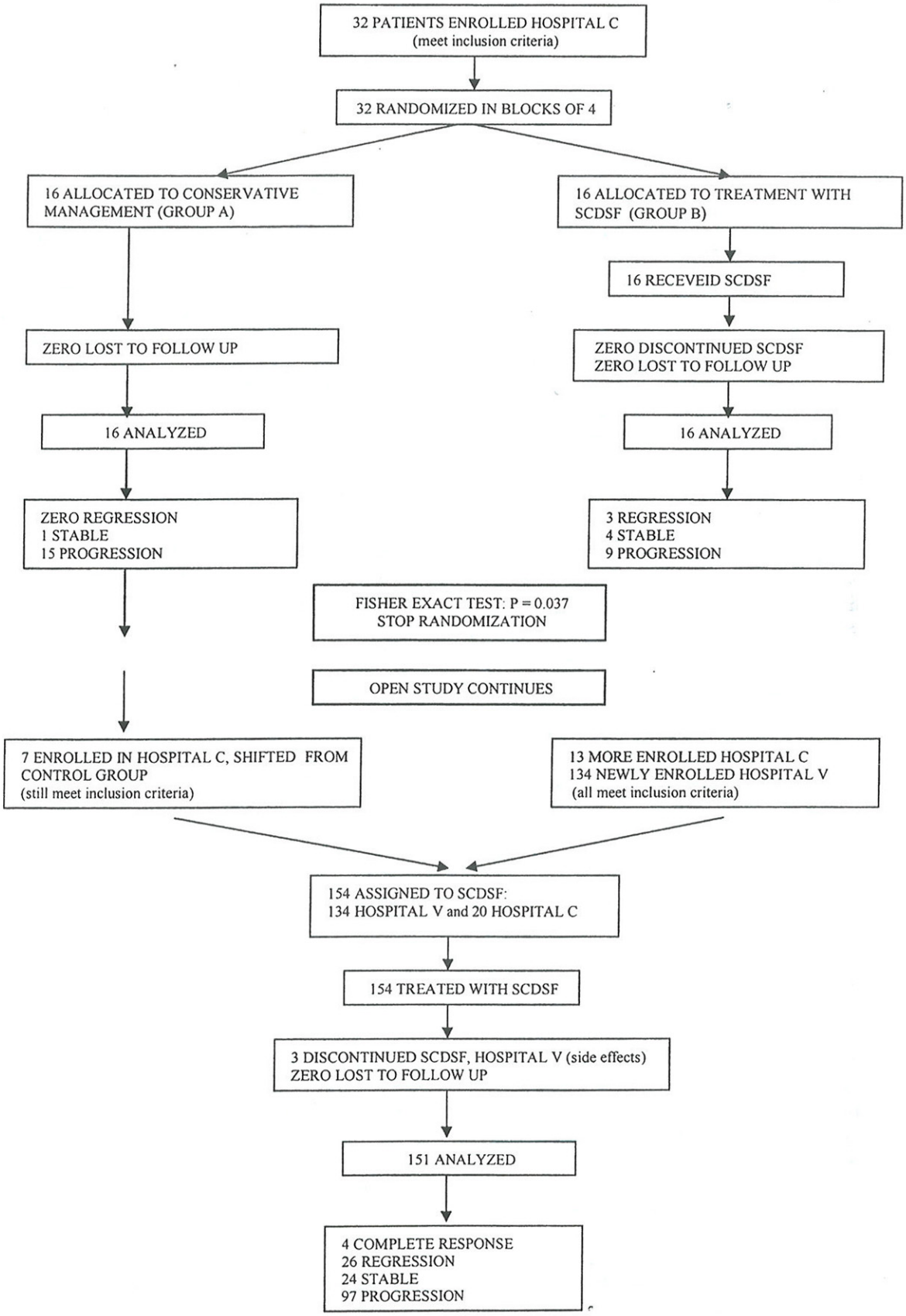

Figure 1. Study profile for stem cell differentiation stage factors (SCDSF).

Table 1. AFP and DCP Baseline and Posttreatment Levels After 6 Months in the Three Patients of the Treated Arm Presenting Regression of HCC

Patients	AFP Baseline	AFP Posttreatment	DCP Baseline	DCP Posttreatment
1	220	45	42	10
2	<20	<20	125	20
3	<20	<20	14	4

AFP, alpha-fetoprotein, ng/ml (normal value <20); DCP, des-gamma-carboxyprothrombin, ng/ml (normal value <2).

Conference on the Clinical management of HCC (22). Specifically, the diagnosis of HCC was established by the concomitant finding of two imaging techniques that showed a nodule larger than 2 cm with arterial hypervascularization, or by one positive imaging technique that showed hypervascularization associated with an alpha-fetoprotein (AFP) plasma concentration higher than 400 ng/ml. Diagnosis of liver cirrhosis was made on a clinical basis in most of the patients (clear endoscopic and/or ultrasound evidence of portal hypertension plus consistent alterations of serum liver parameters tested as indicated below). Exclusion criteria were: age over 80 years, uncontrolled liver disease, Child's class C, renal failure, terminal stage reflecting a life expectancy shorter than 4 months, or an ECOG performance status (PS) (23) greater than 2. The tumor stage was evaluated with spiral biphasic computerized tomography (CT) scan (arterial and portal phase) and ultrasound examination. Group A received no treatment and group B received SCDSF in an oral sublingual dose of 1 ml administered three times daily. The treatment was discontinued whenever the patient decided side effects had developed. The control group received only treatment for symptoms and complications, including vitamins, amino acids, and other nutritional integrators present in the composition of the product administered to group B, but without embryonic proteins.

The primary outcome measure was tumor response; in addition, the survival between the groups of patients with objective responses and stable disease in comparison with survival of the patients with progression was evaluated. The secondary outcome was the resultant performance status and patient tolerance. Tumor response was assessed on the basis of CT scans and the dosage of serum AFP or des-gamma-carboxy-prothrombin (DCP) (24). Both the markers were determined using the immunoassay method (AFP by Immunolite 2000, Medical Systems, Genova, Italy, and DCP by PIVKA 2, Roche, Monza, Italy). Complete response was defined as complete disappearance of tumor on imaging and normalization of tumor markers (when abnormal at baseline). Regression of disease was considered when the tumor was reduced in size and tumor markers, if augmented at baseline, decreased for a period lasting for at least 3 months. Stable disease was considered when no changes were obtained for a period lasting for at least 6 months, and progression of disease was considered when tumor size or tumor markers increased. Objective response was defined as the sum of complete response and regression of disease, sustained for at least 3 months. Treated and untreated patients were followed at intervals of 3 months, until the end of the study or death. Additionally, in all patients, the following serum tests were checked at baseline and during follow-up: bilirubin, transaminases, alkaline phosphatase, albumin, glucose, creatinine, prothrombin activity, blood cells and platelet count.

Statistical Analysis

The study had a sequential design to allow the trial to be stopped for ethical reasons if significant differences were detected at one determinate moment. To compare the frequency of the responses between treatment groups, we used contingency tables, utilizing chi-square distribution or the Fisher exact test, as appropriate according to the number of cases.

To compare baseline and posttreatment values of AFP and DCP, we employed one-tailed Student's *t*-test for paired samples and we use two-tailed Student's *t*-test to compare mean weight of the patients with different survival. In order to study possible predictive factors for response, a multivariate analysis of the variance was applied to the following parameters: age, sex, severity of liver disease, AFP, DCP, serum total bilirubin, size and number of tumors. The Kaplan-Meier method was used to determine the cumulative probability of survival between the objective response and stable disease versus progression. The log rank test was used for comparison of the survival curves. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Overall, between January 2001 and June 2003, 187 patients who fulfilled the inclusion criteria were assessed for eligibility; eight patients refused to take part in the study. Therefore, 179 patients were enrolled (123 men and 56 women; mean age, 64.9 years; range, 46–78). The study profile is shown in Figure 1. Follow-up ranged from 6 to 29 months (mean, 14.4). Survival was evaluated as of April 31, 2004.

A total of 137 (76.5%) patients presented an intermediate stage and 42 (23.5%) an advanced stage. PS was 0 (normal activity) in 156 patients (87.1%) and 1 (symptoms, but nearly fully ambulatory) in 23 (12.9%). Fifty-seven patients (31.8%) presented abnormal AFP and 113

(63.1%) abnormal DCP (23 of them presented with both the markers abnormal). Nine patients (5%) had both serum HCC markers within the normal range.

There were a mean of 3.63 (2.68 SD) and of 3.18 (2.9 SD) HCC nodules in the patients with advanced and intermediate stage disease, respectively. The mean size of HCC nodules was 4.49 (1.78 SD) cm in diameter in patients with advanced disease and 4.2 (7.8 SD) cm in diameter in patients with intermediate stage disease. In the advanced stage subgroup, 37 patients had tumors that invaded the portal venous system and 5 had metastatic disease outside the liver.

On the basis of serological viral markers, the etiology of the disease was hepatitis B virus in 37 (20.7%) cases and hepatitis C virus in 115 (64.2%). In 10 (5.6%) of the cases, both viruses coexisted as probable cofactors.

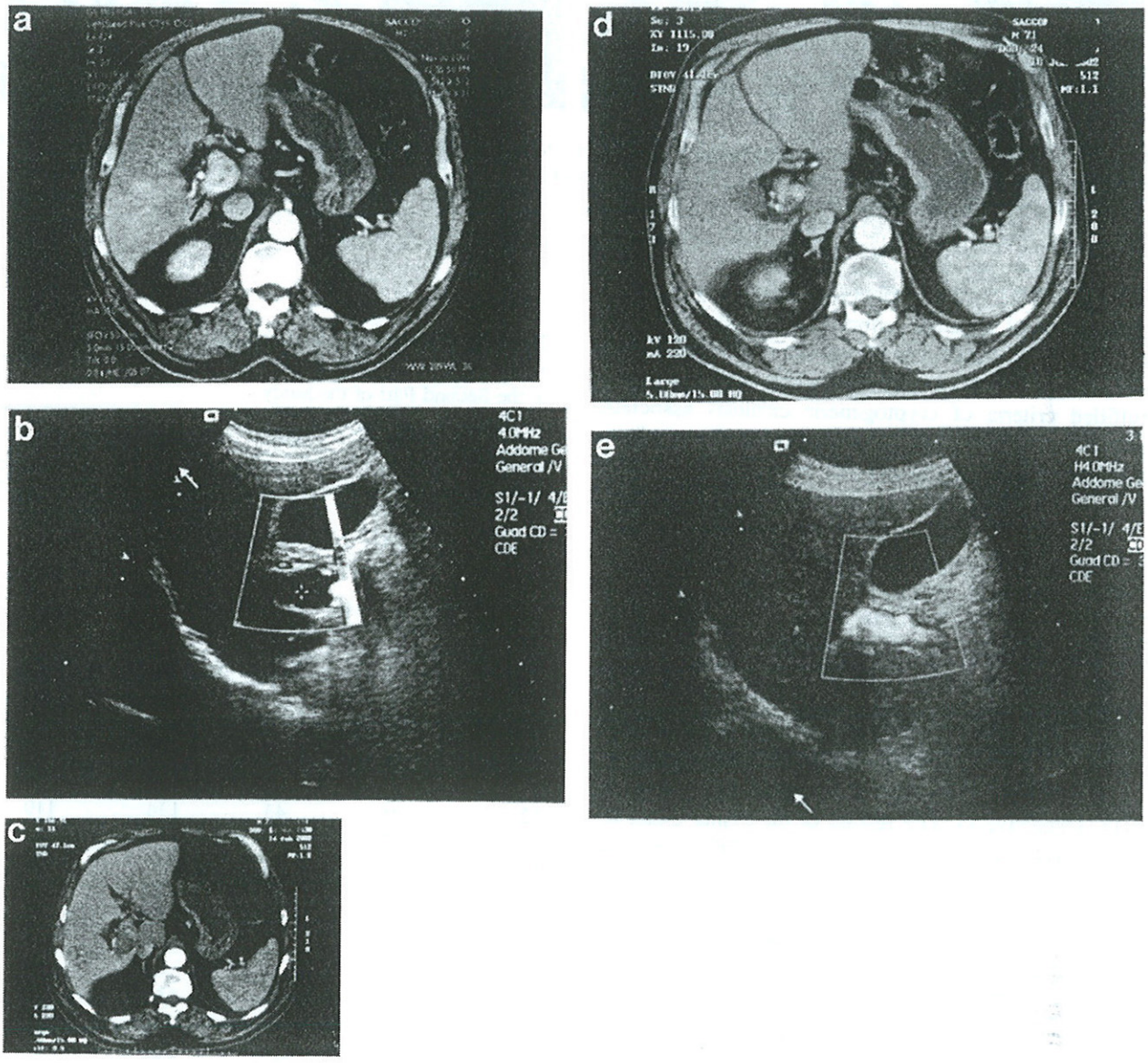

Figure 2. (a) Spiral CT scan during the arterial phase performed prior to treatment shows an advanced HCC of the right lobe. Scattered neoplastic hypervascularized areas are present in segment 7, and a hypervascularized thrombus (arrow) occupies the right portal branch and reaches the main trunk. (b) Power Doppler examination confirms the thrombosis (asterisk) inside the main portal vein. (c) Spiral CT scan during the arterial phase performed 3 months after treatment shows the disappearance of the neoplastic areas and evident reduction of the arterial supply inside the thrombus. (d) Spiral CT performed 6 months after treatment shows the shrinkage of the portal thrombus and confirms the disappearance of the HCC in the right lobe. The same pattern was confirmed 21 months after treatment. (e) Power Doppler examination confirms the shrinkage of the thrombosis and the patency of the main portal vein.

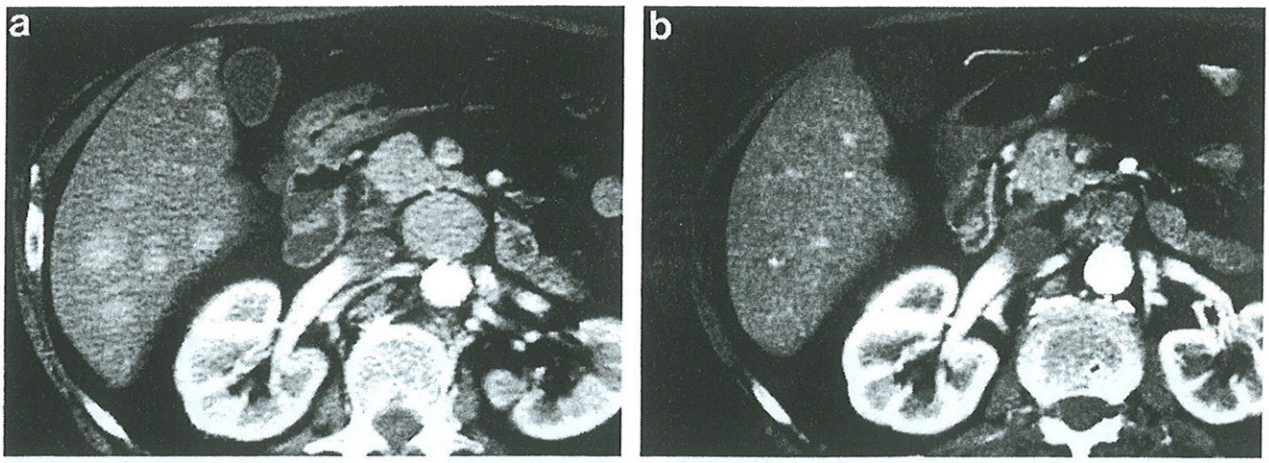

Figure 3. (a) Spiral CT scan during arterial phase performed prior to treatment shows several hypervascularized nodules of HCC in the right lobe. (b) Spiral CT scan performed 6 months after treatment shows the disappearance of the neoplastic hypervascularization inside the nodules. The pattern lasted for 12 months.

Twelve (6.7%) of the patients without serological evidence of viral hepatitis reported a high alcohol intake (an average of more than 80 mg/day) and 5 (2.8%) had cirrhosis of unknown origin. None of these latter cases fulfilled criteria of cryptogenetic cirrhosis associated with overweight (25). As regards the severity of cirrhosis, 139 (77.6%) of the patients were Child-Pugh class A and 40 (22.4%) were class B.

The first part of the trial (randomized) was stopped at the second (6 months) interim analysis, when the inspection detected significant differences in favor of treatment with SCDSF. The initial recruitment (at the Clusone Hospital) enrolled 32 patients (20 men and 12 women; mean age, 63.1; range, 50–76), 16 assigned to conservative management (group A) and 16 to SCDSF treatment (group B); each group presented 2 patients with a PS of 1 and with a similar proportion of patients in an advanced and in an intermediate stage (6/10 and 5/11, respectively). The results of the Fisher exact test demonstrated a statistically significant difference (con-

Table 2. AFP and DCP Baseline and Posttreatment Levels After 6 Months in Four Patients With Complete Response in the Second Part of the Study

Patients	AFP Baseline	AFP Posttreatment	DCP Baseline	DCP Posttreatment
1	1760	<20	32	<2
2	290	<20	18	<2
3	35	<20	24	<2
4	<20	<20	80	<2

AFP, alpha-fetoprotein, ng/ml (normal value <20); DCP, des-gamma-carboxyprothrombin, ng/ml (normal value <2).

Table 3. AFP and DCP Baseline and Posttreatment Levels After 6 Months in 26 Patients Presenting Regression of HCC in the Second Part of the Study

Patients	AFP Baseline	AFP Posttreatment	DCP Baseline	DCP Posttreatment
1	35000	170	22	20
2	17000	350	<2	<2
3	15600	4200	<2	<2
4	3600	1000	24	12
5	3500	800	32	12
6	1766	20	60	20
7	450	150	<2	<2
8	130	80	10	4
9	120	100	4	3
10	80	35	160	60
11	80	80	160	20
12	45	43	134	110
13	45	45	90	60
14	42	40	340	130
15	39	35	22	14
16	35	35	24	10
17	28	27	10	3
18	25	24	40	12
19	22	22	350	80
20	<20	<20	240	120
21	<20	<20	220	12
22	<20	<20	100	60
23	<20	<20	22	6
24	<20	<20	20	8
25	<20	<20	14	6
26	<20	<20	<2	<2

AFP, alpha-fetoprotein, ng/ml (normal value <20); DCP, des-gamma-carboxyprothrombin, ng/ml (normal value <2).

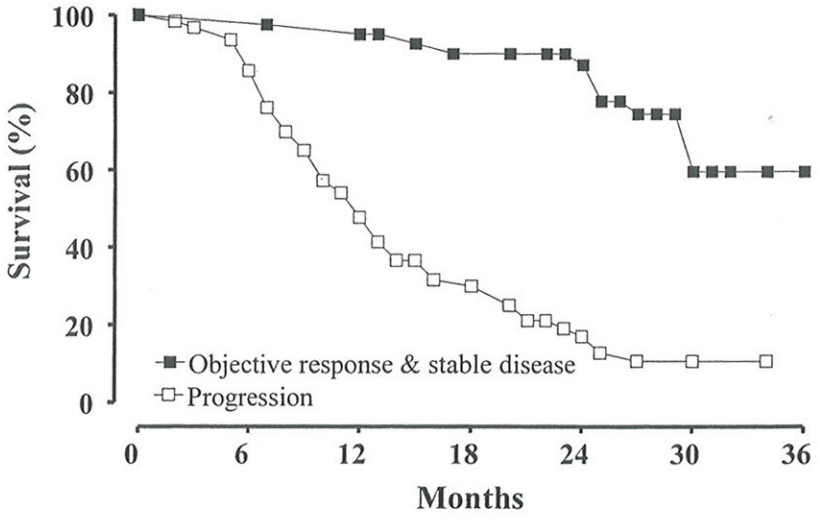

Figure 4. Survival curves of HCC patients treated with SCDSF presenting objective response and stable disease considered together and of patients presenting progression. Kaplan-Meier method and log rank test for statistical difference ($p < 0.001$).

sidering together objective response and stable disease) between the two groups, in favor of the treatment group ($p = 0.037$). In fact, in the treated group (B), 3 patients presented regression of disease (18.75%), 4 presented stable disease (25%), and 9 presented progression of disease (56.25%). In the control group A, none of the patients presented an objective response, 1 patient presented stable disease (6.25%), and 15 patients presented progression of disease (93.75%). In the control group A, none of the patients presented an improvement in PS, and in the treated group B the 2 patients with PS of 1 changed to PS 0. Improvement of general conditions was not related to tumor response. All the patients were alive at this period of observation. Table 1 shows the AFP and DCP baseline and posttreatment values after 6 months for the patients with regression of disease.

On the basis of these results, we discontinued the randomization and continued the study following only the treated group (Fig. 1, bottom). Furthermore, 154 additional patients were enrolled and treated (134 at the Vimercate Hospital and 20 at the Clusone Hospital, 7 of whom shifted from the control group). Treatment was discontinued in 3 patients (1.7%) owing to side effects after a period ranging from 15 days to 2 months. One patient presented diarrhea, one pain of the calves, and one itching. The results obtained in the 151 assessable patients substantially confirmed previous results. In fact, considering the patients completing the study and therefore analyzed, 4 patients (2.6%) presented a complete response, 26 patients (17.2%) regression of disease, 24 patients (16%) stable disease, and 97 patients (64.2%) progression of disease. In total, of the 167 treated and assessable patients (131 at the Vimercate and 36 at the Clusone Hospital), 33 (19.8%) presented an objective response [i.e., 4 (2.4%) presented a complete response and 29 (17.4%) regression of disease].

In the patients presenting an objective response, the pattern shown by CT scans was unusual: the typical neoplastic hypervascularization during the arterial phase decreased whereas the tumor maintained the baseline size, until completely disappearing when the response was complete (Fig. 2, Fig. 3). We called such a pattern the "vanishing effect."

Objective response ranged from 3 to 21 months (mean, 10.2). In 6 patients with intermediate stage disease, some new lesions, small in size (<2 cm), appeared during the objective response in other portions of the liver. We considered these cases as regression, because the appearance of the small nodules was not clinically relevant in relation to the period of observation compared with the observed regression of the primary mass.

Of the 23 patients with a PS of 1, 19 (82.6%) changed to PS 0.

Table 2 and Table 3 show the AFP and DCP baseline and posttreatment levels after six months of the 4 patients presenting complete response and the 26 patients presenting regression of disease, respectively. Excluding the cases where AFP was constantly normal, among the patients with regression of disease, the mean baseline and posttreatment levels were 4084.58 ng/ml (9033.7 SD) and 381.89 ng/ml (963.7 SD), respectively (CI: 95%; $p = 0.04$). Accordingly, for DCP we obtained a pretreatment and a posttreatment mean level of 95.36 ng/ml (107.1 SD) and 35.55 ng/ml (40.9 SD), respec-

tively (CI: 95%; $p = 0.0009$). The differences were thus statistically significant. In the cases of stable disease, the marker levels were: AFP—mean 502.05 ng/ml (1663.03 SD) at baseline, mean 437.62 ng/ml (957.58 SD) after 12 months, mean 458 ng/ml (1055.53 SD) after 24 months; DCP—mean 28.75 ng/ml (50.46 SD) at baseline, mean 29.11 ng/ml (49.30 SD) after 12 months, mean 21.00 ng/ml (37.39 SD) after 24 months. Tumor markers in the cases of progression were: AFP—mean 577.82 ng/ml (1409.63 SD) at baseline, mean 960.50 ng/ml (2225.42 SD) after 12 months, mean 2245.00 ng/ml (3441.75 SD) after 24 months; DCP—mean 62.70 ng/ml (141.32 SD) at baseline, mean 246.47 ng/ml (666.69 SD) after 12 months, mean 273.67 ng/ml (577.94 SD) after 24 months. The result of multivariate analysis of the variance was $F = 0.808$, with $p = 0.586$, considering the difference between the groups of objective response and stable disease versus progression in relation to the following variables: age, sex, stage of tumor, AFP, DCP, total bilirubin, number of nodules, tumor size. Therefore, none of these baseline variables were statistically significant by multivariate analysis as predictor factors for response.

Survival was evaluated as of April 31, 2004, for 160 patients. Seven patients were lost to follow-up.

Figure 4 shows the Kaplan-Meier survival curves for patients with an objective response and stable disease considered together and for patients with progression. The log rank test performed to compare the survival curves showed $p < 0.001$.

There were no significant differences in baseline mean weight of the patients with different survival rates. In fact, the baseline mean weight of the patients with objective response and stable disease was 64.9 kg (11.2 SD) and baseline mean weight of the patients with progression was 66.4 kg (11.4 SD) ($p = 0.437$).

DISCUSSION

Despite the fact that surveillance programs using ultrasound examination and AFP determination have been widely implemented, curative therapies can only be applied to less than 30% of patients with HCC (8). Excluding the few transplanted patients, the others are likely to present an intermediate and/or advanced disease, because HCC is multicentric with time. Some of these patients can benefit from intra-arterial chemoembolization (3). However, sooner or later all the patients, treated or untreated, become untreatable with regional therapies.

The clinical trial herein reported extends observations that SCDSF may be effective in terms of objective responses and stable disease (38%) and in terms of survival rate of such patients in the treatment of intermediate-advanced HCC. In fact, this open randomized trial demonstrated a statistically significant difference between treated and untreated patients before the stop of randomization. Furthermore, in the 151 additional and assessable patients, complete response was 2.6%, regression 17.2%, and stable disease 16%. Objective response ranged from 3 to 21 months (mean, 10.2).

The objective response rate of nearly 20% reached in the present study seems to be clearly reflected by the favorable trend of AFP and DCP (reaching statistical significance in the substantial subgroups) and by the impressive CT scan findings of decreasing or "vanishing" neoplastic hypervascularization. In addition, we observed a significant difference of survival rates between the group with objective response and stable disease versus the group with progression, whereas no predictive factors for response, such as age, sex, size or number of tumors, AFP, DCP, or serum bilirubin levels, emerged from multivariate analysis.

These results can be explained if we bear in mind that tumor cells, in a model recently presented (20), 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 (26), and receptors for embryonic regulators of cell differentiation on their surface. It was demonstrated in prior studies in our laboratory 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 (18,19). It was also demonstrated that the proteins with molecular weight ranging from 10 to 20 kDa contained in SCDSF enhance caspase-8 activation, with a concurrent significant normalization effect on the ratio of e-cadherin/β-catenin expression, two proteins directly involved in phenotype differentiation (Cucina, A.; Biava, P. M.; D'Anselmi, F.; et al., submitted to *Cell Death and Differentiation*).

In addition to these results, we also obtained an improvement in PS in 82.6% of the patients who presented an alteration of this clinical parameter. The amelioration of PS observed in many of the treated patients, not related to tumor response, could be explained by the action of the antioxidants and vitamins contained in the product, even though it cannot be excluded that the substances taken from zebra fish embryos may have played a role in improving the PS.

Actually, we obtained a very good level of concordance between the three fundamental end points in cancer treatment: response, PS, and survival.

In summary, there is no standard therapy for patients with intermediate HCC not responsive to intra-arterial chemoembolization or with advanced HCC. In the past, these patients have usually received antiestrogen ther-

apy, but after demonstration of its little benefit, such therapy is declining. On the basis of one controlled study (27), some patients are currently treated with megestrol, even after failing the determination for the presence of progesterone receptors on the biopsy specimen.

Newly emerging agents with promise include 90Y microspheres, antiangiogenesis agents, inhibitors of growth factors and their receptors, and K vitamins (28).

The findings from the present study indicate that SCDSF can be an effective treatment for HCC in an intermediate-advanced stage or in case of failure of any other therapeutic attempt. At the moment, we believe that SCDSF should be taken into consideration as a treatment option for HCC in an intermediate-advanced stage, particularly because of its ease of use, its high tolerability, and a negligible rate of side effects, which lead to a very high level of compliance. Such compliance is in turn enhanced by the beneficial effect of the product on the PS. The possibility to detect responders after only a few months of therapy allows early discontinuation of the treatment, unless the patients present a clear improvement in the PS.

Further studies are required to clarify the reasons for the different response behavior observed in different patients, in an attempt to establish the best selection criteria for optimal prediction of response, as well as to isolate the active principle(s) of SCDSF. In addition, double-blind randomized clinical trials, comparing SCDSF with the best conservative support or placebo, could be planned in the future to verify whether the objective response obtained with this compound favorably effects survival.

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