

Clinical application of gelatin sponge microparticles-transcatheter arterial chemoembolization combined with synchronous antigen-presenting dendritic cell sequential reinfusion for treatment of advanced large liver cancer

A single-center, prospective, non-randomized, controlled trial

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Abstract

To assess the clinical efficacy and safety of gelatin sponge microparticles-transcatheter arterial chemoembolization (GSMs-TACE) plus synchronous antigen-presenting dendritic cell (DC) sequential reinfusion for advanced large liver cancer (LC).

Patients with large LC were assigned to the experimental (combined sequential DC therapy) or control group. All patients received standardized GSMs-TACE. In the experimental group, 60 mL of peripheral blood was collected for in vitro culture of DCs (10–14 days). Then, intravenous reinfusion was conducted 3 times within 10, 20, and 30 days after surgery. Adverse reactions during the treatment were recorded and evaluated. The overall survival, transcatheter arterial chemoembolization frequency, and physical score (PS) were calculated.

The median survival time of the experimental group was significantly longer than that of the control group. There were significant differences in median progression-free survival between the 2 groups ($P < .05$) and the objective effective rate at 1 and 6 months and 1 year ($P < .05$), but not 2 years ($P > .05$). The PSs of 2 groups were significantly improved at 1 month after GSMs-TACE, with more obvious improvement in the experimental group ($P < .05$).

GSMs-TACE plus synchronous DC sequential reinfusion significantly prolonged the median survival time, improved the tumor response rate and PS, prolonged progression-free survival, and reduced intervention frequency. GSMs-TACE plus synchronous DC sequential reinfusion treatment is suitable for comprehensive treatment of patients with advanced larger LC in China.

Abbreviations: BCLC = Barcelona Clinic Liver Cancer, CIK = cytokine-induced killer cell, CT = computed tomography, DC = dendritic cell, ECOG = Eastern Cooperative Oncology Group, GSMs-TACE = gelatin sponge microparticles-transcatheter arterial chemoembolization, HCC = hepatic cell carcinoma, IL = interleukin, LC = liver cancer, MDSCs = myeloid-derived suppressor cells, OS = overall survival, PD-1 = programmed cell death protein 1, PS = physical score, TACE = transcatheter arterial chemoembolization, Tregs = regulatory T cells.

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The study was approved by the Ethics Committee of Affiliated Zhongshan Hospital of Dalian University. All experiments were performed in accordance with relevant guidelines and regulations. The informed consent was obtained from all patients.

The written informed consent for publication was obtained.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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1. Introduction

Current treatments for liver cancer (LC) include surgical resection, liver transplantation, ablation therapy, and transcatheter arterial chemoembolization (TACE). Of these, surgical resection remains the preferred method for primary LC. However, in China, more than 80% of patients are ineligible for surgery at the time of diagnosis. Hence, TACE has become the standard treatment modality for advanced LC in China.^[1] TACE combined with sorafenib and other comprehensive treatments can further improve the survival of patients with advanced LC, although treatment is limited by drug costs, complications, and less than ideal long-term outcomes.^[2]

The combination of in vitro generated dendritic cells (DCs) and cytokine-induced killer cells (CIKs) is a widely applicable cellular approach for treatment of advanced LC. The aim of “green” DC-CIK immunotherapy is to isolate and amplify the peripheral blood immune cells of the patient in vitro and return the propagated DC-CIKs to the patient. Two meta-analytical data reviews have confirmed the efficacy and safety of TACE combined with DC-CIKs for treatment of LC.^[3,4] Microparticle embolic agents have become hotspots in TACE research in recent years, especially the application of embolic agents, such as drug-loaded microspheres and radioactive microspheres, which further increase the tumor response rate.^[5–9] However, most embolic agents are permanent. Previous studies have reported that treatment with gelatin sponge microparticles-transcatheter arterial chemoembolization (GSMs-TACE) promotes liquefaction and necrosis of tumors.^[10,11] The use of an absorbable embolic agent results in faster recovery of liver function with no increase in the incidence of related complications. Hepatic tumor necrosis after TACE is antigen-specific,^[12] which can activate anti-tumor-specific immune responses.

Preliminary clinical studies have reported the efficacy of GSMs-TACE combined with sequential DC transfusion at key time points after TACE. The aim of the present study was to prolong and maintain the positive immunization time after GSMs-TACE with the help of DC antigen presentation to increase the tumor response rate and improve the long-term survival of patients with large LC.

2. Methods

2.1. Study approval and patient consent

The study protocol was approved by the Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University (Dalian, China) and conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects described in the Declaration of Helsinki. Informed consent was obtained from all subjects prior to enrollment in this study.

2.2. Study cohort

The study cohort included 100 patients (68 males and 32 females; average age, 62.84 ± 6.51 years) with hepatic cell carcinoma (HCC) recruited from the Affiliated Zhongshan Hospital of

Dalian University from June 1, 2015 to March 1, 2017. The stage of HCC was determined using the internationally uniform Child–Pugh liver function scoring method and the Barcelona Clinic Liver Cancer (BCLC) staging criteria. The patients were assigned to the experimental (combined sequential DC reinfusion therapy) group (n=45) or control group (n=55).

2.2.1. Inclusion criteria. HCC diagnosis according to the “Standards for the Diagnosis and Treatment of Primary Liver Cancer”; HCC diagnosis of BCLC grade B or C; large or massive LC with a single tumor maximum diameter of ≥ 5 cm; liver function Child–Pugh grade A or B, BCLC grade B or C, and Eastern Cooperative Oncology Group (ECOG) physical score (PS) of 0 to 2; expected survival time of > 3 months; biochemical examination showing normal coagulation function and no serious liver or kidney dysfunction, biliary system disease, lesions of important organs, or history of contrast agent allergy; GSMs-TACE before surgery, peripheral blood samples before and after surgery for clinical and scientific research, and informed consent; and no other anti-tumor treatments (including radiotherapy, radiofrequency ablation, targeted drugs, etc) or use of other immune-enhancing drugs throughout the study period.

2.2.2. Exclusion criteria. Diagnosis of chronic inflammatory disease; allergy to contrast agents, chemotherapy drugs, or anesthetics, or serious organ disease other than LC; refusal of GSMs-TACE or combined DC reinfusion; poor compliance, irregular follow-up; BCLC stage A or D, ECOG PS > 2 points; liver function Child–Pugh grade C; single tumor with a maximum diameter < 5 cm; incomplete clinical data; and history of other anti-tumor treatments or use of any immunosuppressant during treatment.

2.3. Standardized GSMs-TACE

Patients in the HCC group who met the inclusion criteria were treated with standard GSMs-TACE. The right femoral artery was successfully punctured via the Seldinger method for placement of a catheter for hepatic arteriography. Conventional angiography of the celiac artery and common hepatic artery was performed first. Combined with pre-operative imaging data, the tumor location, size, and whether the tumor staining was complete and consistent were judged according to the LC image after digital subtraction angiography. If necessary, supplemental angiography of the lateral vessels of the radial artery, superior mesenteric artery, right renal artery, internal thoracic artery, and left gastric artery was performed to assess arterial blood supply to the tumor. The chemotherapeutic agent pirarubicin was diluted in 30 mL of water per injection for a microparticle suspension of GSMs (150–350 μm , 350–560 μm). Under guidance of digital subtraction angiography, the microparticle suspension was slowly injected into the artery supplying the tumor. Embolization was terminated upon stagnation of blood flow to the hepatic tumor and complete disappearance of tumor staining on the angiographic display. Conventional administration at 3 to 5 days after surgery included acid inhibition, liver protection, fluid replacement, and symptomatic treatment.

2.4. DC preparation

Peripheral blood collection methods: On the day of blood collection, patients had a light diet, the disposal room was disinfected to meet blood collection standards, and peripheral blood was collected under aseptic conditions. The syringes used for blood collection contained 2 mL of heparin and were shaken at a uniform speed to avoid blood clot formation and cell rupture. After blood collection, pressure was immediately applied to the puncture point for 10 to 15 minutes and the collected blood was placed in sterile containers and sent to the central laboratory.

Reagents and instruments: Roswell Park Memorial Institute 1640 medium was purchased from Gibco (Carlsbad, CA), fetal bovine serum from Tianjin Haoyang Biological Products Technology Co., Ltd. (Tianjin, China), methylthiazolyldiphenyl-tetrazolium bromide from Sigma-Aldrich Corporation (St. Louis, MO), and lymphocyte separation solution (density, 1.08 ± 0.07 g/mL) from Beijing Solaibio Science & Technology Co., Ltd. (Beijing, China). In the experimental group, 60 mL of peripheral blood were collected before TACE and after the first and second reinfusion into a tube containing lymphocyte separation solution (1:1 v/v) and centrifuged at 3000 rpm for 30 minutes. Peripheral blood mononuclear cells were collected, washed 3 times with physiological saline, adjusted to 4×10^6 c/mL with Roswell Park Memorial Institute 1640 medium, and cultured under an atmosphere of 5% CO₂/95% air for 2 hours at 37°C. After discarding the non-adherent cells, recombinant human granulocyte macrophage colony stimulating factor and recombinant human interleukin (IL)-4 were added at final concentrations of 200 ng/mL and 500 U/mL, respectively, and the adherent cells were cultured under an atmosphere of 5% CO₂/95% air for 4 days at 37°C. Afterward, recombinant human tumor necrosis factor α was added at a final concentration of 200 U/mL (factor F) and the culture was continued for an additional 4 days. Then, following the addition of 100 μ L (factor E) of LC antigen cell lysate, the culture was continued for another 4 days. Afterward, the culture supernatant was discarded and the adherent cells were washed 3 times with Hank's balanced salt solution, then scraped, collected, and washed 3 times with Hank's balanced salt solution. DC preparation was conducted by the staff of the Central Laboratory of Affiliated Zhongshan Hospital of Dalian University.

2.5. DC reinfusion and pre-treatment

DCs were isolated from 60 mL of peripheral blood and reinfused over a period of 2 consecutive days. The reinfusion process was generally completed within 30 minutes. The patients' vital signs were closely monitored for 24 hours after reinfusion and complications were recorded and symptomatic treatment was initiated as appropriate.

The patients were infused with the DC suspension once per day for 2 consecutive days. At 30 minutes prior to infusion, the patients received an intramuscular injection of 5 mg of dexamethasone. DC infusion was conducted with a blood transfusion device and generally completed within 30 minutes. The second DC infusion was conducted with the same method the next day.

2.6. Evaluation of efficacy and observation of adverse reactions

- (1) Computed tomography (CT) was performed at 4 days after GSMs-TACE and enhanced CT/magnetic resonance imaging

once per month after surgery to monitor the size of the lesion, degree of necrosis, and formation of new lesions. All patients underwent abdominal CT/magnetic resonance imaging every 1 to 2 months after surgery to observe tumor progression and the tumor response to determine whether further intervention was needed. According to the International Solid Tumor Efficacy Evaluation Criteria (mRECIST 1.1), the "survival liver tumor" was used to evaluate the response of hepatic tumors.

- (2) Patients in the experimental group received combined synchronous DC sequential therapy and monitored for adverse reactions, such as allergy, shock, fever, and rash.
- (3) All adverse reactions to treatment were recorded and evaluated in accordance with the adverse drug reaction evaluation criteria.
- (4) The number of TACE procedures, median survival, and overall survival (OS) at 6, 12, and 24 months were recorded.
- (5) The ECOG and Karnofsky performance status scores of each HCC patient at corresponding time points were recorded.

2.7. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 19.0. (IBM Corporation, Armonk, NY). Measurement data are presented as the mean \pm standard deviation ($\bar{x} \pm s$). Count data were analyzed with the chi-square (χ^2) test and are presented as a rate (%). The *t* test was used for comparisons between the 2 groups, while variance analysis or the rank sum test was used for comparisons among 3 or more groups. The Pearson χ^2 test was performed with $\alpha = 0.05$ as the test level. A probability (*P*) value of $< .05$ was considered statistically significant.

3. Results

Of the 100 subjects recruited for this study, 55 were assigned to the control group and 45 to the experimental group, who received GSMs-TACE followed by combined DC sequential therapy. There were no significant differences in sex, age, alpha-fetoprotein, liver function grade, tumor size, tumor number, or portal vein tumor thrombus between the 2 groups ($P > .05$) (Table 1).

3.1. Tumor response to treatment

The objective effective response rates of tumors in the experimental group at 1 and 6 months and 1 and 2 years were 95.6%, 85.7%, 70.3%, and 72.0%, respectively. There were significant differences in the objective effective response rates at 1 and 6 months and 1 year between the experimental and control groups ($\chi^2 = 5.377, 4.896, \text{ and } 5.199; P = .026, .022, \text{ and } .020$, respectively), but not in the objective effective response rate of the tumors ($\chi^2 = 2.806, P = .090$) (Table 2). Representative cases of the experimental group are shown in Figures 1 to 3.

3.2. Median survival time of the experimental and control groups

Until March 1, 2019, the mean follow-up period was 21.7 (range, 6–32) months. There were significant differences in median survival time between the experimental and control groups (25.0 ± 5.8 vs 17.0 ± 2.4 , respectively, $\chi^2 = 4.228, P = .04$) and median progression-free survival (8 vs 5 months, respectively,

Table 1**General clinical information of patients in the experiment group and the control group.**

Clinical information	Total	Experimental group	Control group	P
Number	100	45	55	
Average age (yrs)	62.84±6.51	59.93±5.36	64.44±7.85	.17
Gender				.30
Male	68	33	35	
Female	32	12	20	
History of hepatitis				.97
Hepatitis B	68	30	38	
Hepatitis C	14	6	8	
Alcoholic liver disease	12	6	6	
None	6	3	3	
Child-Pugh grading				.66
A	70	30	40	
B	30	15	15	
BCLC staging				.88
B	67	31	36	
C	33	14	19	
Portal vein tumor thrombus (+/-)	22/78	10/35	12/43	.84
Mean maximum diameter of tumor (cm)	8.0	8.9	7.4	.37
Tumor size (cm)				.93
≥5, <10	66	29	37	
≥10	34	16	18	
Number of tumors				.54
Single lesion	76	36	40	
Multiple lesion	24	9	15	
AFP (ng/mL)				.76
≤400	27	11	16	
>400	73	34	39	

AFP = alpha-fetoprotein, BCLC = Barcelona Clinic Liver Cancer.

$\chi^2=4.274$, $P=.039$) (Fig. 4). There was also a significant difference in the mean number of interventions between the experimental and control groups (2.9 ± 0.8 vs 4.3 ± 1.1 , respectively, $P<.05$).

3.3. OS time of the experimental and control groups

Until March 1, 2019, there were no significant differences in OS rates at 6 and 12 months between the experimental and control groups (93.3%/90.9% vs 82.2%/63.6%, respectively, $P>.05$), but there was a significant difference at 24 months (55.6% vs 28.1%, respectively, $P<.05$) (Fig. 5, Table 3).

3.4. PS of the experimental and control groups

The PS at different follow-up time points was calculated according to the Karnofsky functional status scoring criteria. The PS of the 2 groups after GSMs-TACE had significantly improved, although improvement was significantly greater in the experimental group ($P<.05$). Although the PS of both groups had decreased at 6, 12, and 24 months, the PS of the experimental group was significantly greater than that of the control group. In particular, the PS of the experimental group increased significantly after the first intervention, and then decreased afterward, but the overall score remained >80 points (Fig. 6).

Table 2**Evaluation of tumor response of patients in the experimental and the control group after treatment.**

After treatment	CR		PR		SD		PD		Objective effective rate	
	N	%	N	%	N	%	N	%	N	%
1 mo experimental	28	62.2	15	33.3	2	4.4	0	0	43	95.6*
Control	31	56.4	16	29.1	8	14.5	0	0	47	85.5*
6 mo experimental	21	46.7	15	33.3	4	8.8	2	4.4	36	85.7*
Control	14	28.0	20	40.0	11	22.0	5	10.0	37	68.5*
1 yr experimental	13	35.1	13	35.1	8	21.6	3	8.1	26	70.3*
Control	6	17.1	13	37.1	8	22.8	8	22.8	19	54.3*
2 yrs experimental	7	28.0	11	44.0	5	20.0	2	8.0	18	72.0†
Control	3	18.8	8	50.0	2	12.5	3	18.9	8	68.8†

CR = complete remission, PR = partial remission, SD = stable disease, PD = progressive disease.

* $P<.05$.

† $P>.05$, compared between groups.

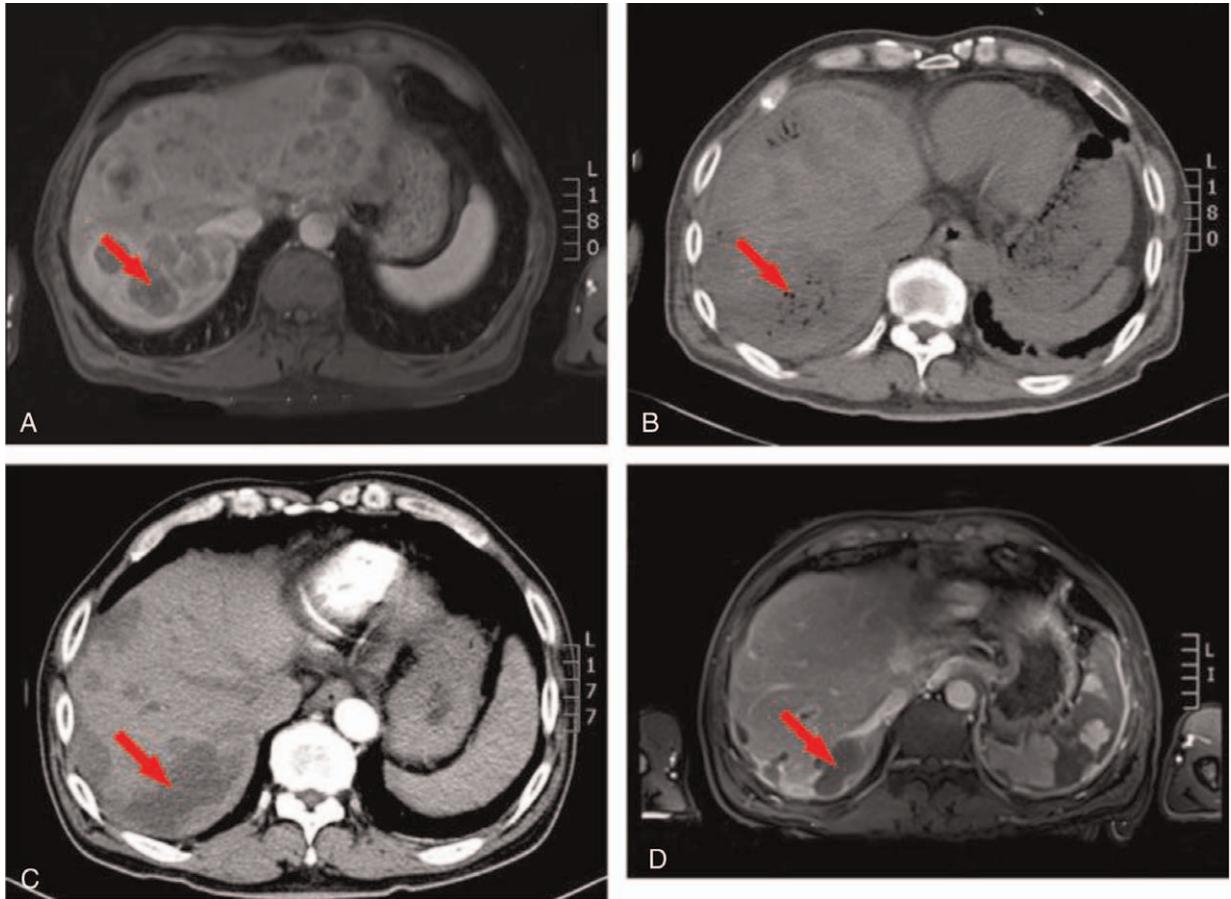


Figure 1. (A) The enhanced MRI image before interventional surgery showed diffuse nodular liver tumors in the liver (arrow); (B) Plain CT scan at 4 days after intervention showed obvious low-density changes (arrow); (C) Enhanced CT at 8 weeks after intervention showed that the range of tumors in the liver was significantly reduced, and there was no obvious enhancement (arrow); (D) 12 months after the first intervention, compared with pre-operative MRI, most of the liver tumors disappeared, and a few residual lesions showed no enhancement (arrow). CT = computed tomography, MRI = magnetic resonance imaging.

3.5. Adverse reactions and complications

The prevalence of postprocedural complications (i.e., fever, nausea, abdominal pain, and tumor embolism syndrome) may be related with tumor necrosis and absorption after intervention. Body temperature ranged from 37.5 to 39.5°C and gradually returned to normal within 5 to 7 days. All patients had transient

liver damage after intervention, which returned to pre-operative levels at 7 to 10 days after liver protection treatment. There were a total of 13 cases of acute cholecystitis between the 2 groups, likely due to ectopic embolization. Gelatin sponge particles of the absorbable microparticle embolization agent can promote complete remission after symptomatic treatment. There was no

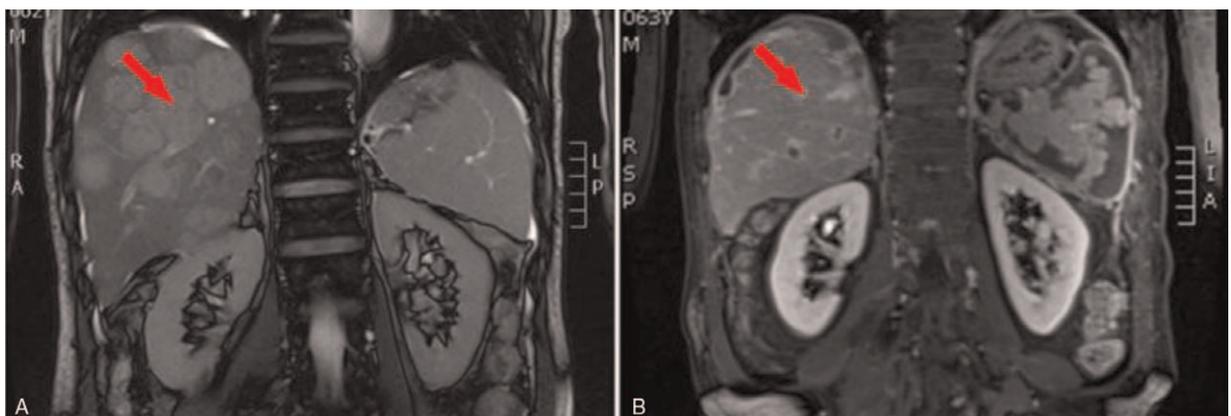


Figure 2. (A) The MRI coronal image before interventional surgery shows diffuse nodular liver tumors in the liver (arrow); (B) The MRI coronal image at 12 months after interventional surgery shows that most of the lesions disappeared (arrow). MRI = magnetic resonance imaging.

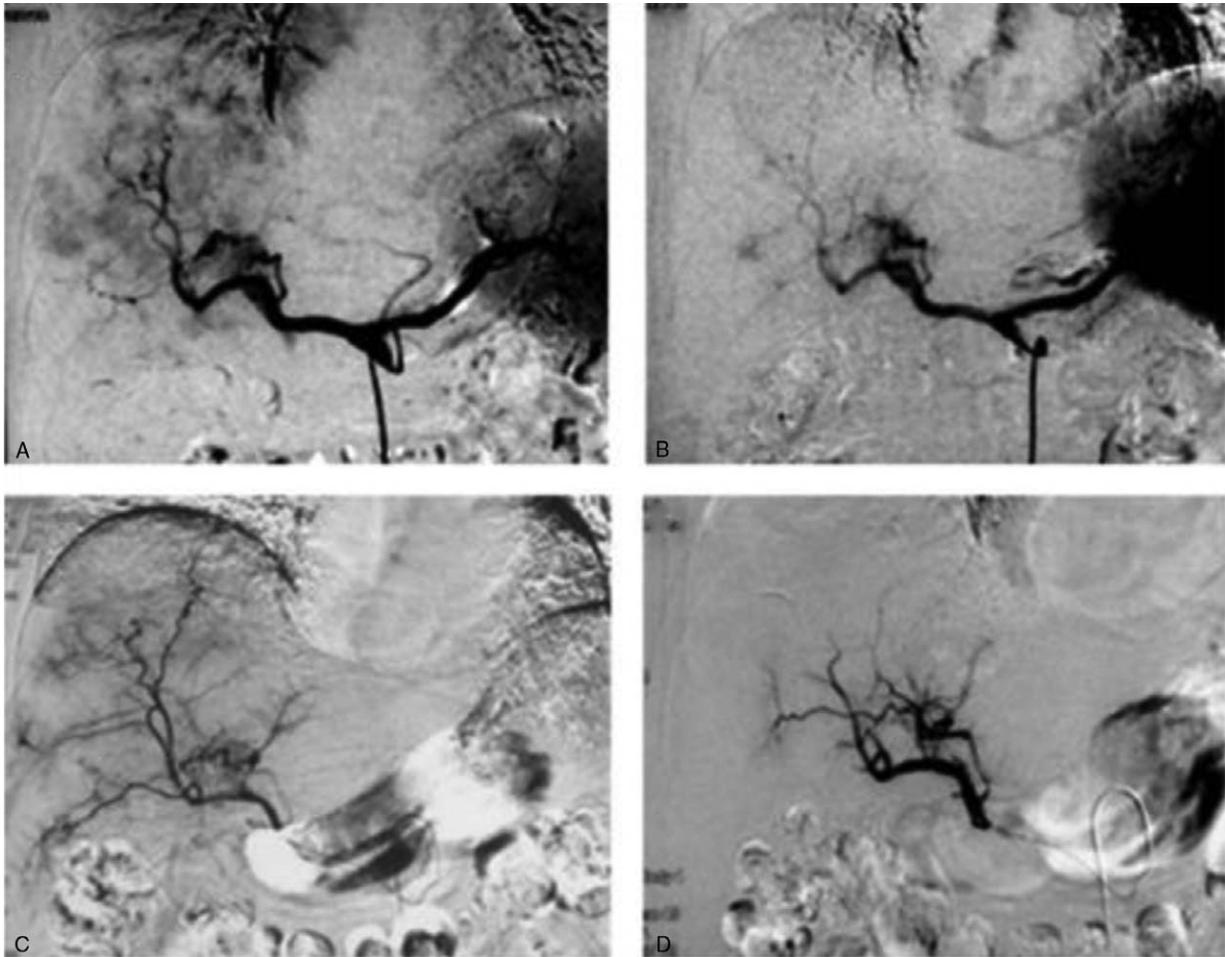


Figure 3. (A) Angiography during interventional surgery showed multiple nodular staining in the liver, blood is supplied by the branches of the hepatic artery; (B) Angiography after interventional embolization showed the tumor staining disappears; (C) Sequential DC reinfusion treatment after interventional surgery, and interventional angiography at 8 weeks after surgery showed no exact tumor staining. Compared with the first interventional angiography image, some suspected tumors have a small blood supply at the edge; (D) The distal branch of the hepatic artery was truncated, and the abnormally stained artery disappeared. Sequential DC reinfusion treatment was performed again after surgery. DC = dendritic cell.

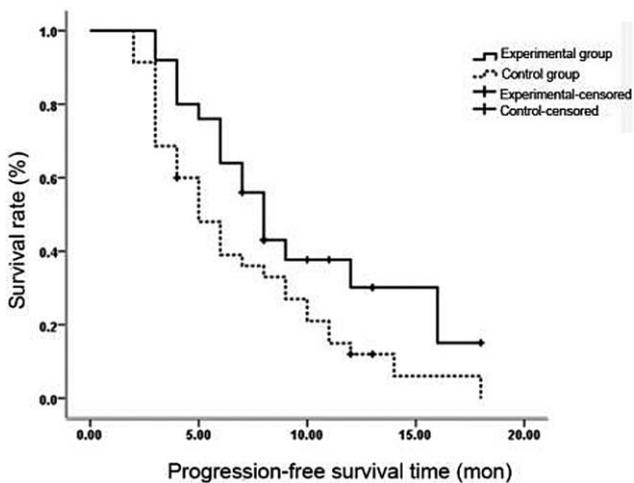


Figure 4. The progression-free survival time of 2 groups.

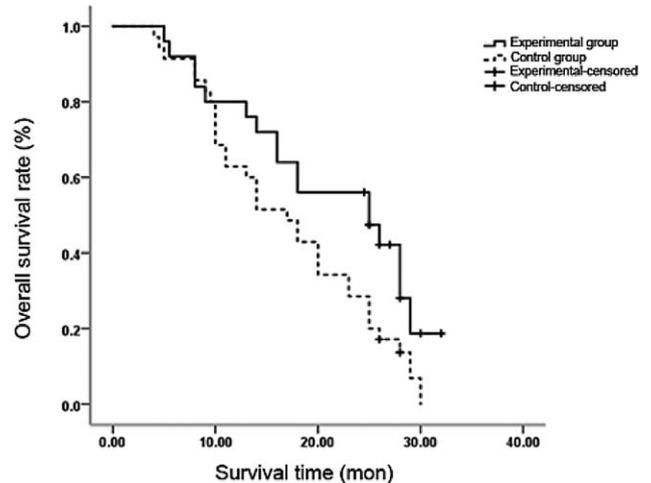


Figure 5. Comparison of overall survival time between 2 groups.

Table 3
Comparison of overall survival time and GSMs-TACE frequency between the experimental group and the control group.

Group	N	6-mo survival rate		12-mo survival rate		24-mo survival rate		Median survival time	GSMs-TACE frequency
		N	%	N	%	N	%		
Experimental	45	42	93.3	37	82.2	25	55.6	25.0±5.8	2.9±0.8
Control	55	50	90.9	35	63.6	16	28.1	17.0±2.4	4.3±1.1
X ² /t								9.309	7.131
P		0.197		3.369		15.458		.000	.000
		.657		.06		.000			

GSMs-TACE = gelatin sponge microparticles-transcatheter arterial chemoembolization.

case of gallbladder perforation or gangrene, no serious complications (i.e., acute liver failure, gastrointestinal ulcer or hemorrhage, liver abscess, and pulmonary embolism), and no case of acute liver injury or surgery- or chemotherapy-related deaths. There were no statistical differences in intervention-related adverse events between the 2 groups (Table 4).

Fever was the most common adverse reaction in the experimental group (24.4%, 11/45). All patients completed GSMs-TACE combined with synchronous DC sequential reinfusion therapy according to the experimental protocol. Of a total of 429 treatments, the overall incidence of fever was 11.2% (48/429). Fever after reinfusion of DC usually occurred in patients who were first reinfused and then treated with dexamethasone. The incidence of fever was significantly reduced by appropriate adjustment with DC culture reagent. No serious adverse events due to reinfusion were observed.

4. Discussion

LC is the third most cause of cancer-related mortality worldwide, accounting for about 780,000 deaths annually, with nearly 370,000 in China.^[13] Hepatitis B-related LC, which is associated with a relatively poor prognosis, accounts for approximately 90% of LC cases in China.^[14,15] The patients in this study had advanced disease at the time of diagnosis and were, therefore, ineligible for surgery. In addition to liver deficiency, medical expenses and lifelong medications limit the clinical application of liver transplantation.

Other than the relatively high prevalence of hepatitis B, China has an aging population. The incidence of LC in the elderly population has also attracted the attention of the health sector in China.^[16] GSMs combined with single chemotherapeutic drugs, first described in 2008, achieved good clinical efficacy for the treatment of advanced massive LC. A rabbit model of hepatic tumors was established by direct injection of VX2 cell suspension into the liver. A previous study of this model confirmed that the mechanism of drug-loaded sustained-release GSMs-TACE for the treatment of HCC is similar to that of drug-loaded microspheres, which is considered as one of the reasons for the good clinical efficacy.^[17] GSMs-TACE for treatment of LC with portal vein tumor thrombus achieved good clinical results.^[18] GSMs-TACE is also used for treatment of refractory LC, senile LC, and LC rupture.^[16,19]

TACE combined with the multi-target drug sorafenib has also been considered as a new treatment for advanced LC, opening a new era of targeted therapy for primary LC. However, the results of preliminary studies showed that the effect of the timing of sorafenib impacts patient prognosis.^[2,20,21] Current cellular immunotherapies include CIK, DCs, DC-CIK reinfusion, and chimeric antigen receptor-glypican-3 T-cell therapy.^[3,4,22] As novel immunological target preparations for the treatment of LC, blocking cytotoxic T lymphocyte-associated protein 4 and programmed cell death 1 (PD-1)/programmed cell death-Ligand 1 has entered the clinical stage, especially for patients with refractory LC with resistance to TACE and sorafenib.^[23] Angiogenesis inhibitors combined with immunotherapy can

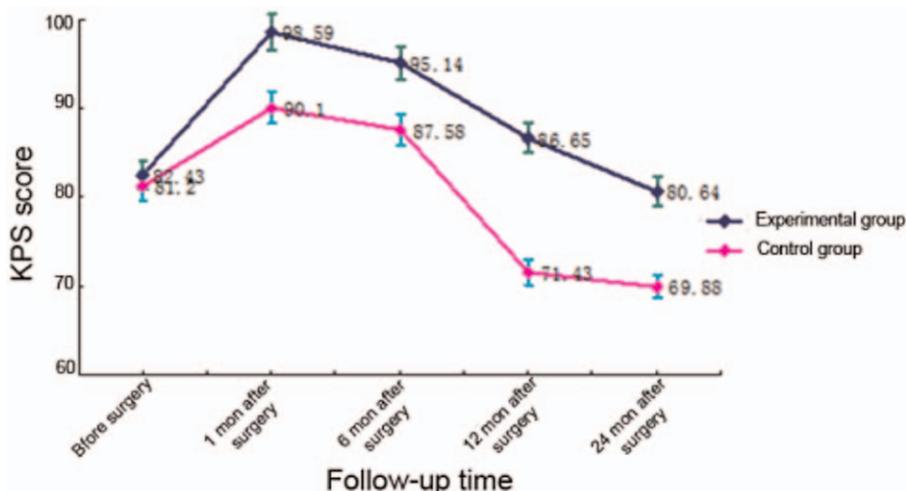


Figure 6. Trend chart of comparison of physical scores between the 2 groups.

Table 4
Comparison of incidence of adverse reactions between the experimental and the control group.

Group	N	Leukocyte decline		Thrombocytopenia		Abdominal diarrhea		Poor appetite and fatigue		Stomach discomfort		Cholecystitis	
		N	%	N	%	N	%	N	%	N	%	N	%
Experimental	45	4	8.9	5	11.1	11	24.4	13	28.9	20	44.4	5	11.1
Control	55	5	9.1	8	14.5	16	29.1	17	30.9	22	40.0	8	14.5
χ^2		0.001		0.258		0.271		0.048		0.201		0.258	
<i>P</i>		.971		.611		.603		.826		.654		.611	

further increase the tumor response rate, thus demonstrating a greater advantage.^[24,25] A recent phase 1b study of rivastatinib in combination with PD-1 monoclonal antibody for the treatment of advanced LC reported an objective response rate of 60% and a disease control rate of 93.3%. However, this combination method is not only costly, but also associated with serious adverse events, such as explosive immune pneumonia.

GSMs-TACE induced changes to immunosuppressive cells, including regulatory T cells (Tregs), in hepatic tumors.^[26] This phenomenon can also be explained by the re-establishment of vascular endothelial growth factor levels at 7 days after GSMs-TACE intervention,^[27] and the immune microenvironment itself is a contradiction between immune attack and immune repair, which also can lead to recurrence and metastasis. Nevertheless, as an interventional treatment, GSMs-TACE can cause changes, even reversal, to the immune response, which cannot be achieved by other simple targeted drugs or immunosuppressive agents. The complexity of the immune microenvironment explains the low clinical efficiency and resistance of even newly developed drugs.^[28,29] Multi-target inhibitors or multiple approaches for treatment of advanced LC through different routes and mechanisms of action remain an insurmountable challenge. In consideration of the national financial status of China, combination approaches must be not only efficacious and safe, but also cost effective.

In the local micro-environment of hepatic tumors after GSMs-TACE-induced necrosis, lysed tumor cells release tumor antigens that may be more specific. As most or all tumor blood vessels are blocked after GSMs-TACE, tumor necrosis can significantly reduce the tumor burden. Specific antibodies against hepatic tumors are produced to further increase the production of repair factors, such as Tregs, thus leading to immune tolerance. Previous studies^[30–33] have shown that myeloid-derived suppressor cells (MDSCs) and Tregs can decrease DC levels, while increasing vascular endothelial growth factor levels. In addition, Tregs can inhibit specific immune functions of T cells by consuming IL-2 through high surface expression of cluster of differentiation 25 and the combination of cytotoxic T lymphocyte-associated protein 4 and cluster of differentiation 80/86 on the DC surface.

MDSCs produce IL-10, which decreases the antigen presentation ability of macrophages. MDSCs also produce cytokines, including interferon- γ , transforming growth factor β , and IL-10, which enhance the immune function of Tregs, leading to tumor cell escape, thereby promoting the recurrence of malignant tumors. Therefore, synchronous DC sequential transfusion after GSMs-TACE and supplementation of DCs during immune repair to exploit the powerful antigen presentation function of DCs can further promote necrosis and apoptosis of residual tumor cells,^[34] thereby maintaining and strengthening natural immuni-

ty. Recent studies have also shown that DC-CIK bioimmunotherapy after TACE is more beneficial for patients with LC with high expression of PD-1,^[35] suggesting that this combination can avoid the effect of PD-1 on T cells, and promote specific anti-tumor activities through the bypass pathway, thereby improving survival.

Changes to the immune microenvironment after GSMs-TACE can further improve the clinical efficacy of autologous synchronous DC sequential reinfusion. The results of this study showed that by prolonging the follow-up duration, differences in survival time between the 2 groups gradually appeared, as progression-free survival was improved by 3 months and 2-year survival was also greater than that of the control group. Also, prolonging of progression-free time significantly reduced the number of interventional treatments for LC patients and further protected liver function, which is especially important for patients with advanced large LC and those with cirrhosis. Autologous DC reinfusion is derived from self-immune cells cultured in vitro, which retain autoimmune specificity with no immune rejection, and complications are rare. In this study, the overall incidence of fever was 11.2%. Pre-treatment with dexamethasone or adjusting the proportion of reagents before reinfusion can significantly reduce the incidence of fever. The data also suggest that comprehensive treatment can significantly improve the patient's PS, thereby improving quality of life, which is basically consistent with previous reports.^[3,4] Although this study initially suggested the feasibility and safety of the proposed treatment strategy, the subjects were not randomly selected and the sample size was relatively small. Hence, further randomized controlled multicenter studies with larger samples are needed to further confirm the feasibility and effectiveness of the treatment. Although this study is a real-world study, due to the small sample size and retrospective study, there is obvious selection bias, and there is no joint study with other DC-related factors. Therefore, a prospective large sample comprehensive research is still needed to further explore the effect of this technology on immune factors such as DC.

In conclusion, the combination of DC sequential reinfusion following GSMs-TACE not only improved the medium- and long-term survival of patients with advanced large LC, but also improved quality of life with no increased incidence of related complications. Thus, the proposed treatment strategy should be applied clinically and further studies are warranted to elucidate the specific mechanism of action.

Author contributions

Zhao GS: responsible for clinical trial research and paper writing; Liu Y and Liu S: responsible for patient follow-up and data statistics; Li C, Wang RY, and Bian J: responsible for DC culture

and reinfusion process management; Zhu RP, Zhou J, and Zhang YW: responsible for project design and experimental implementation. All authors read and approved the final manuscript.

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