Original Article

Thermosensitive liposomal doxorubicin plus radiofrequency ablation increased tumor destruction and improved survival in patients with medium and large hepatocellular carcinoma: A randomized, double-blinded, dummy-controlled clinical trial in a single center

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ABSTRACT

Background: Lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox) consists of doxorubicin encapsulated contained within a heat-sensitive liposome.

Aims and Objectives: We sought to evaluate whether the use of combined radiofrequency ablation (RFA) and LTLD would result in larger coagulation volume and longer overall survival (OS) compared with the use of RFA alone in patients with 3–7 cm unresectable hepatocellular carcinoma (HCC).

Materials and Methods: Between 2010 and 2012, 22 HCC patients were randomly assigned to one of two treatments in our center: (1) ultrasound-guided percutaneous RFA plus intravenous (IV) infusion of LTLD (combination, $n = 11$) or (2) RFA plus IV dummy (RFA, $n = 11$). Four patients withdrew from the study, and the remaining 18 patients entered the final analysis. There were 14 male and 4 female patients with an average age of 61.1 ± 9.3 years (range: 40–73 years). The average tumor size was 4.2 ± 1.0 cm (range: 3.1–6.1 cm). One-month enhanced computed tomography was used to evaluate the ablation efficacy and coagulation volume after RFA. Regular follow-up after RFA was performed to assess toxicity, local response rates, and OS rates.

Results: A major complication (empyema) occurred in one case in the combination group. Combination treatment region did not induce any additional toxicity beyond doxorubicin. The primary ablation success rate was 93.3% (14/15 tumors) in the combination group and 77.8% (7/9 tumors) in the RFA group ($P = 0.308$). The difference in coagulation volume between pre- and post-RFA in the combination group was significantly larger than that of the RFA group (105.7 ± 73.8 cm^3 vs. 37.3 ± 8.5 cm^3, $P = 0.013$). The follow-up period ranged from 11 to 80 months (average: 49.1 ± 24.8 months). The local progression rate was 6.7% (1/15 tumors) in the combination group and 22.2% (2/9 tumors) in the RFA group. The mean OS for the combination group was 68.5 ± 7.2 months, which was significantly greater compared with the RFA group (46.0 ± 10.6 months, $P = 0.045$).

Conclusions: RFA with heat target delivery chemotherapy facilitated better tumor coagulation necrosis without additional toxicity. This combined treatment may improve the clinical efficacy of RFA or free doxorubicin and prolong survival in patients with medium to large HCC.

KEY WORDS: Combination treatment, doxorubicin, hepatocellular carcinoma, radiofrequency ablation, thermosensitive liposomal doxorubicin

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world, causing

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nearly 782,500 new cases and 745,500 deaths every year.[1] Locoregional therapy, such as radiofrequency ablation (RFA), has been widely performed for the treatment of small HCC because it is minimally invasive, safe and effective.[2-4] RFA has shown satisfactory survival and effectiveness in small HCC in many clinical studies and is currently one the most widely used ablative techniques for unresectable HCC.[5-7] However, current RFA therapies have been less effective for treatment of larger HCC with local recurrence in 65% and 75% of tumors >3.5 and 5 cm in diameter, respectively.[8] Most often, tumor foci as inadequately treated residual tissue remain given the biophysical limitations of the procedure, such as perfusion-mediated tissue cooling, that prevents uniform heating of the entire tumor volume to a temperature sufficient for inducing coagulation necrosis (50°C–60°C).[9,10] Hence, strategies to increase the volume of induced tumor destruction are urgently required.

In an attempt to overcome the limitations of current therapy, combining RFA with other adjuvants, such as percutaneous ethanol injection, saline infusion, and embolization, are being investigated to promote larger zones of contiguous tissue destruction and improve survival.[11,12] An alternate approach is a combination of therapies with RFA and chemotherapeutic adjuvants. We and others worked on different adjuvant agent using nanoparticles to improve drug delivery and local efficacy to the tumor.[13,14] Many drugs, such as arsenic trioxide,[15] paclitaxel,[16] sorafenib,[17] doxorubicin[18] and quercetin,[19] have been used in thermal ablation to enhance tumor cell death in the peripheral or transitional zone via different mechanisms. On the basis of this synergistic antineoplastic effect, it has been demonstrated that combined RFA and systemic liposomal doxorubicin increases intratumoral accumulation of doxorubicin, increases coagulation necrosis, reduces tumor growth and increases animal survival in tumor model studies and a pilot clinical study.[20-23]

Among the various nanoparticle drug delivery systems, temperature-sensitive liposomes attract great attention as they can focally release chemotherapeutic drugs at high concentrations and elevated temperatures.[24] In our previous animal study, a 15-fold increase in intratumoral concentration was achieved after the combined intervention of intravenous (IV) lyso-thermosensitive liposomal Vinorelbine.[14] These results suggest a clinical role for this type of adjuvant therapy, namely, combining systemic chemotherapy with RFA.

One clinical study reported the efficacy of IV pegylated liposomal doxorubicin (PLD) for RFA in patients with small HCC.[25] However, the key limitation for RFA therapy was >3 cm tumors. In this paper, we performed a randomized, dummy-controlled clinical trial in patients with medium to large sized HCC. The primary objective of this study was to evaluate the safety and potential toxicity of RFA combined with Lyso-thermosensitive liposomal doxorubicin (LTLD) under ultrasound guidance in inoperable 3–7 cm HCC patients. The secondary objective was to evaluate a novel image-guided RFA combined therapy to improve tumor necrosis efficacy and prolong overall survival (OS) [Figure 1].

MATERIALS AND METHODS

The clinical study was sponsored by Celsion Corporation and registered with ClinicalTrials.gov (NCT00617981). The protocol was approved by the institutional review board and independent ethics committee in our hospital. In addition, patients wrote informed consent before they were enrolled in the study and underwent any study-related procedures.

Patients

The enrollment criteria for this trial were as follows: >18 years; one to four unresectable HCC lesions; at least one lesion was

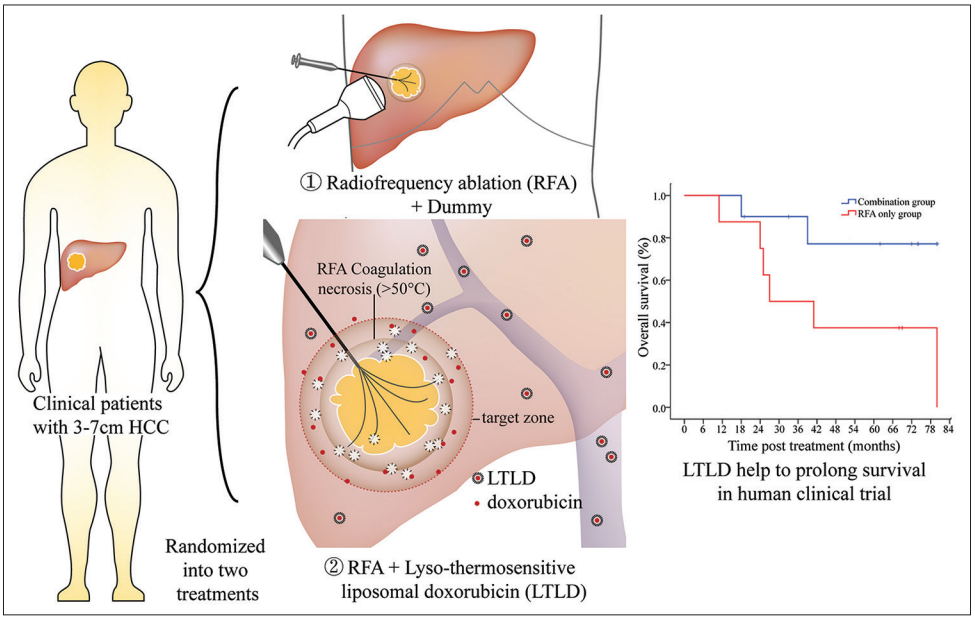

Figure 1: The schematic diagram for study hypothesis

3.0–7.0 cm in diameter; anticipated ablation volume could not be greater than the removal of three hepatic segments or 30% of total liver volume; and subjects were randomized without a biopsy if they met American Association for the Study of Liver Disease (AASLD) criteria for the diagnosis of HCC.[26] Such patients were required to have a biopsy during the RFA procedure unless the biopsy was not possible or was contraindicated. Subjects not meeting AASLD criteria needed a biopsy to confirm HCC prior to randomization. Subjects also had to be Child-Pugh Class A or B without current encephalopathy or ascites. Left ventricular ejection fraction (LVEF) had to be ≥50%.

Subjects were excluded if they were scheduled for liver transplantation or had any prior HCC treatment, any prior exposure to doxorubicin, extrahepatic metastasis, any concurrent malignancy (except treated squamous cell carcinoma of the skin or basal cell carcinoma of the skin), portal or hepatic vein tumor invasion/thrombosis, INR >1.5 times the upper normal limit, platelet count (PLT) $<75{,}000/mm^3$, neutrophil (NEU) count $<1500/mm^3$, hemoglobin <10.0 g/dL, serum creatinine (Crea) ≥2.5 mg/dL, serum bilirubin >3.0 mg/dL, serum albumin (Alb) <2.8 g/dL, or any serious illness within the prior 6 months (e.g., congestive heart failure, myocardial infarction, life-threatening cardiac arrhythmia, or cerebral vascular accident).

Between 2010 and 2012, 22 HCC patients met the enrollment criteria and were randomly assigned to one of two treatments in our center: (1) ultrasound-guided percutaneous RFA plus IV infusion of LTLD ($n = 11$) or (2) RFA plus IV dummy ($n = 11$). Four patients withdrew from the study (PLT $<75{,}000/mm^3$ in one patient, and the other three patients committed other therapies before RFA), and 18 patients completed the follow-up [Figure 2].

Study design

This prospective study was a randomized, blinded, dummy-controlled trial of the efficacy and safety of RFA plus LTLD compared with RFA alone for treatment of unresectable HCC lesions of 3–7 cm in diameter. A computer-generated randomization scheme was prepared before accrual. Eligible subjects were randomized 1:1 to the two arms using a web-based interactive response technology system.

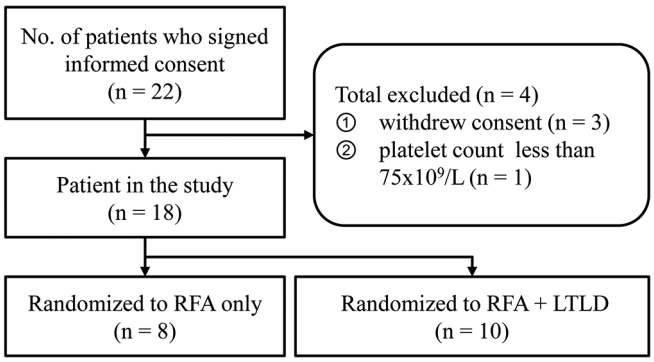

Figure 2: The flow chart for study enrollment

To prevent any hypersensitivity reactions to liposomes, subjects received a blinded premedication regimen. Twenty-four hours prior to the scheduled RFA procedure, subjects took 20 mg of oral overencapsulated dexamethasone (RFA + LTLD arm) or matching dummy capsule. Blinded IV premedication was administered within 30 min before starting the study treatment infusion. The RFA + LTLD arm received a steroid (e.g., 20 mg dexamethasone), H1 antihistamine (e.g., 50 mg diphenhydramine or 10 mg chlorpheniramine), and H2 antihistamine (e.g., 50 mg ranitidine or 20 mg famotidine). The control arm received dummy IV premedication of sodium chloride 0.9% or 5% dextrose in water (D5W). The IV bags and tubing during treatment was covered by masking materials to maintain the blind procedure.

Patients then received a similarly blinded 30-min IV infusion of either 50 mg/m^2 LTLD or D5W. RFA was initiated at minute 15 following the start of study drug infusion and was completed within 3 h after starting the infusion. The ablation procedure generally lasted for 45–60 min for one RFA session. The tumor size, shape, and border were obtained mainly using ultrasound scans, and enhanced computed tomography (CT)/magnetic resonance imaging was used as a reference.

All randomized subjects were followed for safety and OS. Triphasic contrast CT imaging studies were used to assess the effectiveness of the ablation therapy. CT scans were obtained at every visit starting at 1 month after study treatment and at months 1, 3, 5, 7, 9, and 12 and every 3 months subsequently until subject discontinuation. Subjects with incomplete ablations were retreated according to the treatment assigned at randomization if they continued to meet eligibility criteria. These subjects were rescreened. If a successful ablation was not achieved after the completion procedure, the subject was considered a treatment failure. No more than 6 total RFA procedures per subject were allowed.

Safety data were collected from the time of informed consent until discontinuation or the end of the study. In our study, RFA-related morbidity was defined as any complication within 2 weeks of each session of RFA. RFA-related mortality was defined as death from a complication within 2 weeks of each session of RFA. Assessment of adverse events (AEs) began at the time of the signing of informed consent and continued until 1 month following subject discontinuation.

Radiofrequency ablation equipment and procedure

In the present study, RFA was conducted by two radiologists (M. H. Chen, K. Yan, both have more than 10 years of experience in ultrasound-guided tumor ablation). One type of RFA system of Model 1500X (RITA Medical Systems, Mountain View, Calif, United States) was used. The Model 1500X system consists of a 460-KHz generator unit that is capable of delivering a maximum

power of 200 W through a 14-gauge, 15-cm long electrode. The electrode contains nine hook-shaped needles that can be deployed from the applicator shaft. A sphere-like coagulation area of 2.0–5.0 cm in diameter can be produced by one circle in 20 min. Larger tumors (>3.5 cm in diameter) were treated by multiple overlapping ablations depending on the tumor size and shape.[27] Individual RFA protocols, i.e., hydrodissection aimed at avoiding damage to different adjacent structures, were used for tumors adjacent to the bowel, diaphragm and gallbladder given the restricted safety margin. The multiple-tined electrode was usually deployed parallel to vessels to avoid damage to tumors adjacent to large vessels.

Real-time Aloka ultrasound systems (Aloka α-10, Tokyo, Japan) and GE systems (Logic-L9, GE, United States) were used for scanning with 3.5–5.0 MHz convex probes with needle guide devices for all ablation procedures. Track ablation was performed when withdrawing the RFA electrode. The patient was under conscious sedation during the procedure, and an anesthetist monitored the patient's vital signs. Moderate sedation anesthesia was induced with the IV administration of 2.5–5.0 mg midazolam (Roche; Basel, Switzerland) and 50–100 μg fentanyl (Fentaini; Renfu, Yichang, China). Some patients with tumors adjacent to the diaphragm, hepatic hilum or ligament felt obvious local pain and right shoulder pain when the ablation was extended. IV infusion of propofol (Diprivan; Zeneca, Macclesfield, United Kingdom) (1–2 mg/kg) was used to temporarily enhance anesthesia.

The patients were conscious when the electrode was placed. Their vital signs, such as blood pressure, heart rate, respiration rate, and oxygen saturation, were continuously monitored during the procedure. These patients were moved to inpatients rooms 1–2 h after treatment if there was no evidence of active bleeding visible on the ultrasound scans. Generally, the patients were hospitalized for 1–3 days after the RFA procedure.

Study evaluations

The physical, imaging and laboratory examinations [Table 1] were performed 2 weeks prior to the treatment and perioperative period; days 1, 7, and 28 posttreatment; and months 1, 3, 5, 7, 9, and 12. After month 12, the examinations were repeated every 3 months.

Imaging strategy and analysis

Patients had contrast CT imaging studies of the chest, abdomen, and pelvis within 14 days before treatment to confirm the presence of evaluable HCC and at day 28 posttreatment to confirm complete initial ablation. CT parameters for all studies included a 5-mm slice thickness through the liver for unenhanced images and for images obtained 30 s, 60 s, and 2 min after IV administration of contrast material (i.e., during the hepatic arterial, portal venous, and equilibrium phases of liver contrast enhancement, respectively). Contrast material (1.5 ml/kg of Omnipaque; GE, USA) was administered at a rate of 3.5 ml/s. Imaging data were subject to further processing for volumetric assessment. Briefly, the margin of the tumor or radiofrequency-induced thermal lesion was carefully traced using the paintbrush option on all axially acquired images. The tumor area on each axial image was automatically calculated, and the tumor volume was calculated.

The volume of the radiofrequency-induced thermal lesion was evaluated by investigators who were unaware of patient treatment and when the scan was obtained in relation to the RFA therapy. All protocol-specified CT images were read and assessed by a radiologist (CK with more than 15 years of experience in liver CT imaging) blinded.

Statistical analysis

The significance of differences in the baseline characteristics and treatment results was assessed by the Chi-squared test, Fisher's exact probability test, and independent-sample *t*-test. The difference in tumor volume shown on scans obtained before RFA and on scans obtained 4 weeks later was compared between the two study groups using parametric (paired Student's *t*-test) and nonparametric (Wilcoxon's rank sum) tests. OS was measured by time from randomization to death or the end of the study. Survival curves were evaluated using a Kaplan–Meier model and were compared using the log-rank

Table 1: Schedule of study evaluations

Procedure/Test	Screening Windows		treatment		Follow-up				
	-28 Days	-14 Days	-48 hr	Post-RFA	Day 7	Day 14	Month 1	Month 3, 5, 7, 9, 12	After month 12 every 3 months
Informed Consent	X								
Demographics and medical history	X								
Physical exam	X		X	X	X	X	X	X	X
Vital signs	X		X	X	X	X	X	X	X
CT of chest, abdomen, pelvis		X					X	X	X
ECG	X			X		X	X	X	X
ECHO	X						X		
Pre-medication			X						
CBC with differential		X	X	X	X	X	X	X	X
Serum chemistry and UA		X	X	X	X	X	X	X	X

Notes: ECG=Electrocardiograph; ECHO=Echocardiography; CBC=Complete blood counts; UA=Urine analysis; RFA=Radiofrequency ablation

test. $P < 0.05$ was considered statistical significance. SPSS statistical analysis software (SPSS, Chicago, IL, USA) was used to performed statistical analysis.

RESULTS

Patient groups

In the 18 HCC patients who completed follow-up, there were 14 male and 4 female patients. The average of tumor size before treatment was 4.2 ± 1.0 cm (range 3.1–6.1 cm). The predominant cause of HCC in our study was hepatitis B viral infection (15 patients, 83.3%). There were no significant differences in demographic data between the two treatment groups [Table 2].

Toxicity evaluation

The direct parameters used to evaluate the toxicity of our treatment were clinical parameters, including vital signs, electrocardiograph and blood chemistry. Overall, all data indicated that LTLD in combination with RFA did not induce any unexpected deviation or additional toxicities compared with chemotherapy alone. There was no procedure-related and doxorubicin toxicity-related mortalities. The LVEF was 68.3% ± 6.4% and 71.8% ± 6.0% before and after treatments, respectively, in the combination group and was not significantly different compared with the RFA group (71.1% ± 5.8% vs. 70.4% ± 5.5%, $P > 0.05$).

One serious AE occurred during protocol therapy in the combination group. This patient had symptoms indicating pleural effusion, which necessitated hospitalization, and recovered after drainage. The most frequent possibly treatment-related AEs were hair loss, leukopenia, decreased NEU count, decreased hemoglobin level, fatigue, nausea, vomiting, abdominal pain, weight loss, and fever as described in Table 3. These events were registered as possibly related to protocol therapy. Since all the reported toxicities are expected side effects of doxorubicin, they were evaluated as chemotherapy related. All other AE were probably related to the progression of underlying disease.

Lab examination

In the combination group, complete blood count (CBC) examination showed that white blood cell (WBC) and NEU levels were increased immediately after RFA, reduced at day 14, and then increased again to the level before RFA at day 28. In the RFA group, WBC and NEU levels were similar before and after RFA. In both groups, the PLT and red blood cell (RBC) levels were similar before and after RFA [Figure 3].

Biochemistry tests demonstrated that serum alanine transaminase (ALT) and aspartate aminotransferase levels were increased immediately after RFA, reduced at day 14, and then returned to the level before RFA at day 28 in both groups. Alb and alkaline phosphatase (ALP) slightly decreased immediately after treatment but returned to the level before RFA at day 14 in both groups [Figure 4]. In the combination group, Crea levels increased immediately after RFA, was reduced at day 14, and then returned to the level before RFA at day 28. Thyroid stimulating hormone (TSH) levels decreased immediately after RFA, increased at day 14 and returned to normal levels at day 28. Crea and TSH levels were similar before or after RFA in the RFA group [Figure 5].

Post-α-fetoprotein (AFP) tests at 1 month after treatment returned to normal levels in 80% (8/10) of patients in the combination group and 62.5% (5/8) in the RFA group.

Ablation success and tumor ablative destruction volume

The primary ablation success rate was 93.3% (14/15 tumors) in the combination group and 77.8% (7/9 tumors) in the RFA group [Figure 6]. The second RFA treatment was performed in these patients, and all had second ablation success. According to 1-month enhanced CT, the induction of the maximum

Table 2: Baseline characteristics of HCC patients in the two groups

Variable	Combination group (*n*=10)	RFA only group (*n*=8)	*P*
Male/female	8/2	6/2	0.800
Age (years old)	64.9±5.9 (58-73)	56.3±10.8 (40-70)	0.324
Liver cirrhosis etiology			
Hepatitis B (%)	8 (80)	7 (87.5)	0.671
Hepatitis C (%)	1 (10)	1 (12.5)	
Alcohol abuse (%)	1 (10)	0	
Maximum diameter			
3-5cm (%)	6 (60)	7 (87.5)	0.196
5-7cm (%)	4 (40)	1 (12.5)	
Tumor number			
1(%)	6 (60)	7 (87.5)	0.391
2(%)	3 (30)	1 (12.5)	
3(%)	1 (10)		
Serum α-fetoprotein level (ng/ml)			
<200 (%)	9 (90)	5 (62.5)	0.228
200-400 (%)	0 (0)	2 (25)	
≥400 (%)	1 (10)	1 (12.5)	
Serum alanine aminotransferase level (U/ml)			
<40 (%)	7 (70)	7 (87.5)	0.148
41-79 (%)	0 (0)	1 (12.5)	
80-120 (%)	3 (30)	0 (0)	

Notes: HCC=Hepatocellular carcinoma; RFA=Radiofrequency ablation

Table 3: Adverse events after treatments in the two groups.

Adverse events Variable	Combination (*n*=10)	RFA only (*n*=8)	*P*
Hair lose*	9	0	<0.001
Leucopenia*	7	0	<0.001
Decreased neutrophil count*	7	0	<0.001
Decreased hemoglobin level	1	0	0.556
Fatigue	6	4	0.520
Nausea	3	1	0.382
Vomiting	1	0	0.556
Abdominal pain	6	4	0.520
Weight lose	1	1	1.000
Fever	3	2	0.814

*There was a significant difference between the two groups.
RFA=Radiofrequency ablation

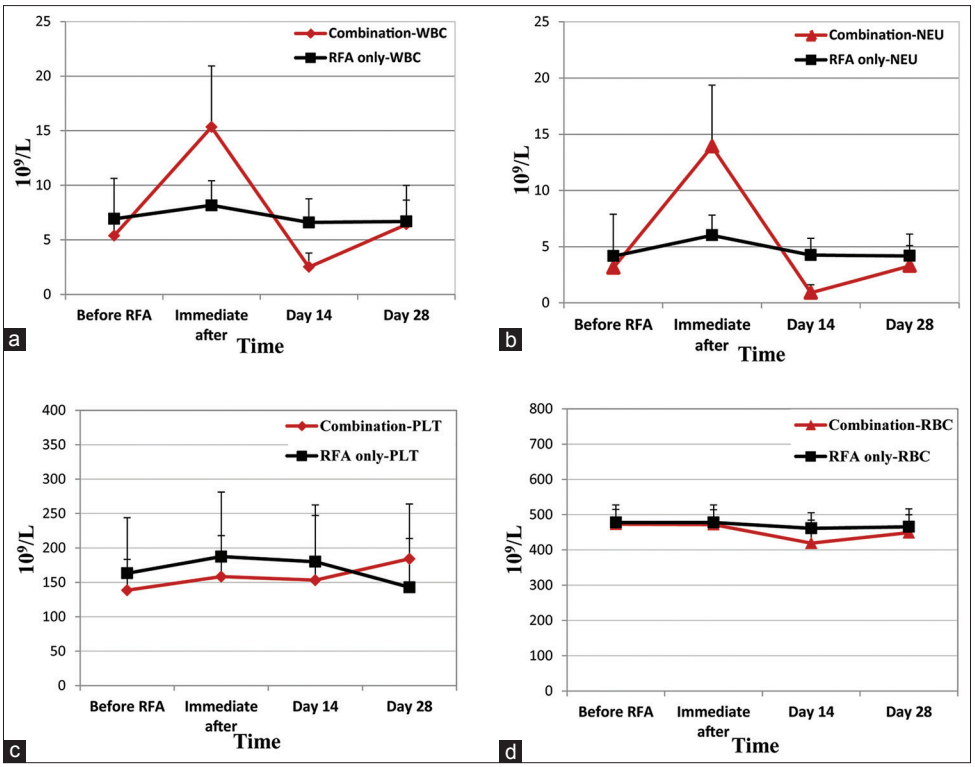

Figure 3: Comparison of complete blood count examination results during the treatment between the combination and radiofrequency ablation only groups. (a and b) In combination group (Red), the white blood cell and neutrophil level was increased immediate after radiofrequency ablation and dropped at day 14, and then increased again to the level before radiofrequency ablation at day 28. In radiofrequency ablation only group (Black), the white blood cell and neutrophil were similar before or after radiofrequency ablation. There was no significant difference at day 28 between the two groups ($P > 0.05$). (c and d) In combination group (Red) and radiofrequency ablation only group (Black), the platelet and red blood cell level were similar before or after radiofrequency ablation. There was no significant difference at day 28 between the two groups ($P > 0.05$)

Table 4: Comparison of difference in index tumor destruction volume pre and post RFA

Variable	Combination (*n*=10)	RFA only (*n*=8)	*P*
Tumor diameter (cm)	4.1±1.2	4.3±0.7	0.743
Tumor volume (cm³)	72.7±52.1	56.6±24.7	0.645
Ablative destruction diameter	5.7±1.4	5.0±0.4	0.193
Ablative destruction volume	178.4±96.2	93.9±27.1	0.044
Difference in ablative destruction diameter*	1.6±0.9	0.8±0.4	0.018
Difference in ablative destruction volume*	105.7±73.8	37.3±8.5	0.013

Note: *the difference in ablative destruction diameter and volume between pre and post-RFA in the combination group was significantly larger than that of RFA alone group (p=0.018, P=0.013). RFA=Radiofrequency ablation.

diameter of ablative destruction was greater than the initial tumor diameter (4.2 ± 1.0 cm vs. 5.4 ± 1.1 cm, $P < 0.001$). Significant increases in ablative destruction volume were noted for the tumor in the combination group compared with the RFA group on the 1-month CT (178.3 ± 96.2 cm³ vs. 93.9 ± 27.1 cm³, $P = 0.044$). In the combination group, the increase in the diameter of ablative destruction ranged from 0.5 to 3.7 cm in all 10 index tumors post-RFA compared with pre-RFA enhanced CT. In the RFA group, the increase in the diameter of ablative destruction ranged from 0.3 to 1.3 cm in all 8 index tumors ($P = 0.018$). In addition, the difference in volume between pre- and post-RFA in the combination group was significantly increased compared with the RFA group (105.7 ± 73.8 cm³ vs. 37.3 ± 8.5 cm³, $P = 0.013$) [Table 4].

Local tumor progression and metastasis

The mean follow-up period was 49.1 ± 24.8 months (range, 11–80 months). Local progression rate was 13.3% (2/15 tumors; 2, 23 months) in the combination group and 22.2% (2/9 tumors; 5, 18 months) in the RFA group ($P = 0.574$). Intrahepatic new lesion rate was 30% (3/10) in the combination group and 50% (4/8) in the RFA group ($P = 0.375$). One case developed extrahepatic metastasis in the combination group (lung) and the RFA group (pelvic cavity) separately. In the combination group, three patients (two with local progression and one intrahepatic new lesion) received retreatment with RFA, and the other two patients (two with intrahepatic new lesions) received transcatheter arterial chemoembolization (TACE). In the RFA group, two patients (one with local progression combined intrahepatic new lesions and one with intrahepatic new lesions) underwent retreatment with RFA, and the other three underwent TACE or supportive care only.

Overall survival

At the end of the follow-up (Jan 2017), two patients in the combination group (20%) and six in the RFA group (62.5%)

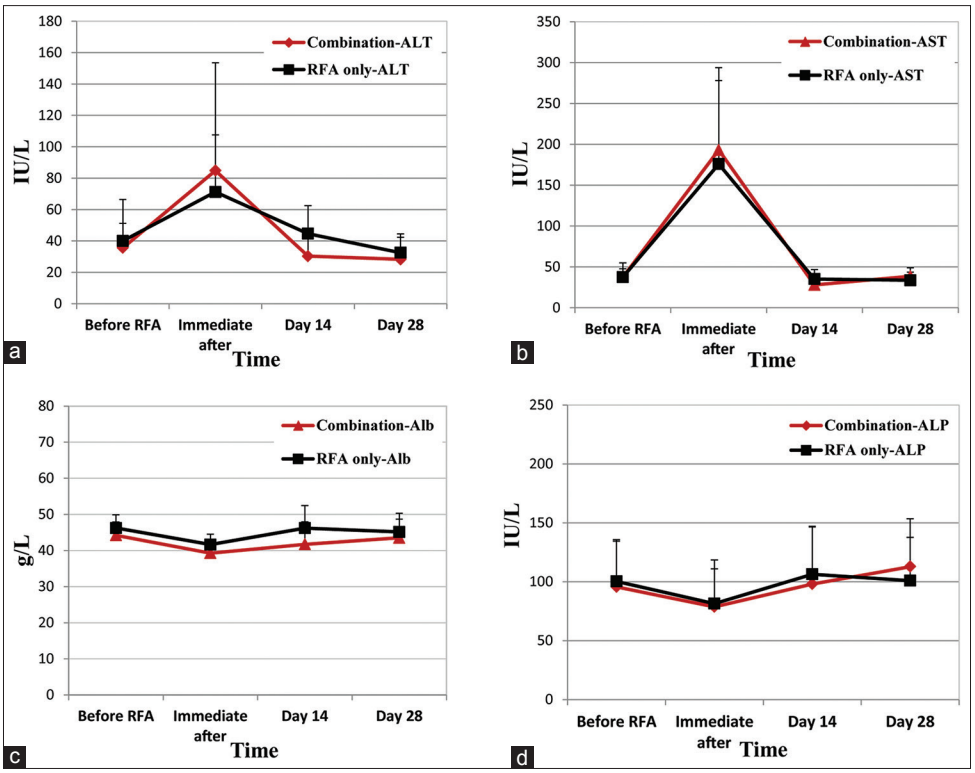

Figure 4: Comparison of liver function lab results during the treatment between the combination and radiofrequency ablation only groups. (a and b) In combination group (Red) and radiofrequency ablation only group (Black), the alanine transaminase and aspartate aminotransferase level was increased immediate after radiofrequency ablation and dropped at day 14, and then returned to the level before radiofrequency ablation at day 28. There was no significant difference at day 28 between the two groups ($P > 0.05$). (c and d) In combination group (Red) and radiofrequency ablation only group (Black), the albumin and alkaline phosphatase slightly decreased immediately after treatment but returned the level before radiofrequency ablation at Day 14. There was no significant difference at day 28 between the two groups ($P > 0.05$)

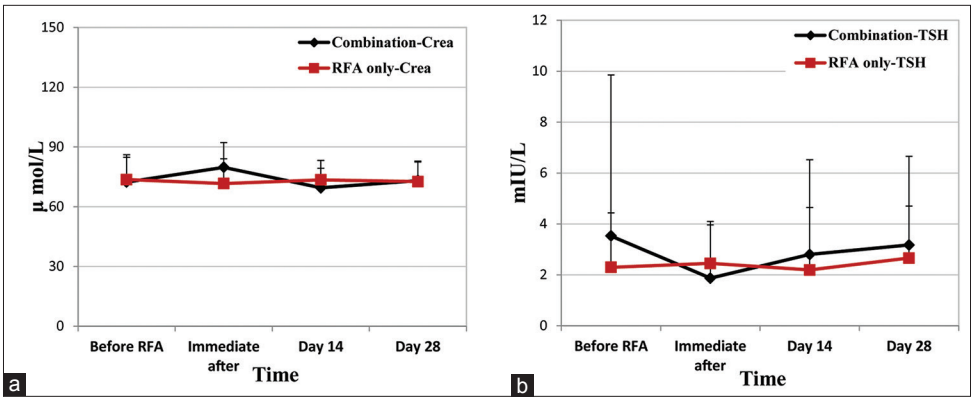

Figure 5: Comparison of kidney and thyroid function lab results during the treatment between the combination and radiofrequency ablation only groups. (a) In combination group (Red), the Creatinine level was increased immediate after radiofrequency ablation and dropped at day 14, and then returned to the level before radiofrequency ablation at day 28. In radiofrequency ablation only group (Black), the Crea level was similar before and after radiofrequency ablation. There was no significant difference at day 28 between the two groups ($P > 0.05$). (b) In combination group (red), the thyroid stimulating hormone level was decreased immediate after radiofrequency ablation but went up at day 14 and returned back at day 28. In radiofrequency ablation only group (black), the thyroid stimulating hormone level was similar before and after radiofrequency ablation. There was no significant difference at day 28 between the two groups ($P > 0.05$)

died. The two patients in the combination group died of tumor progression. In the RFA group, the cause of death was extrahepatic metastasis in one patient, bleeding of esophageal varices in one patient and tumor progressions in four patients. The probabilities of OS after 1, 3 and 5 years were 90%, 90% and 77.1%, respectively, in the combination group and 87.5%, 50.0% and 37.5% in the RFA group, respectively. The mean OS rate in the combination group was 68.5 ± 7.2 months, which was higher than that of the RFA group (46.0 ± 10.6 months, log-rank test, $P = 0.045$) [Figure 7].

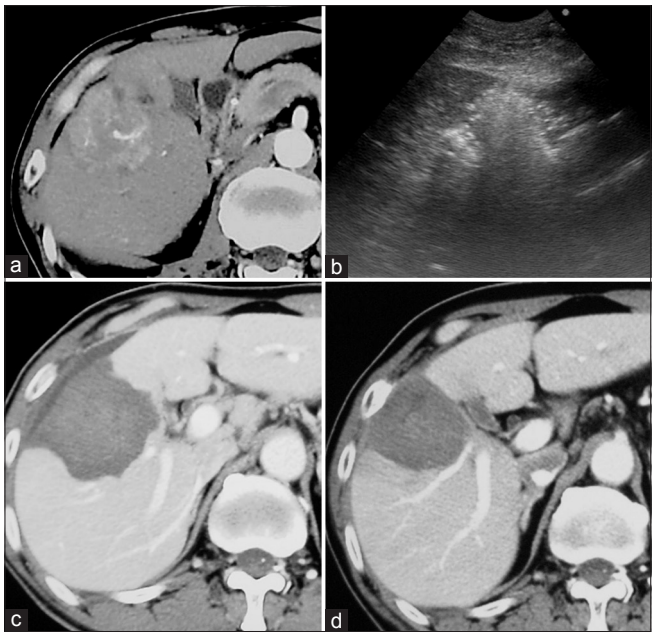

Figure 6: A 58-year-old male had Hepatitis B for 10 years. Hepatocellular carcinoma was found with α-fetoprotein 39.85 ng/ml. the biopsy before radiofrequency ablation showed the pathological diagnosis of hepatocellular carcinoma-II. (a) Enhanced computed tomography before treatment showed the tumor size was 6 × 5 cm. (b) Intravenous infusion of 50 mg/m^2 Lyso-thermosensitive liposomal doxorubicin and then radiofrequency ablation was initiated under ultrasound guidance. One month (c) and 15 months (d) follow up enhanced computed tomography showed the ablation area had no viability

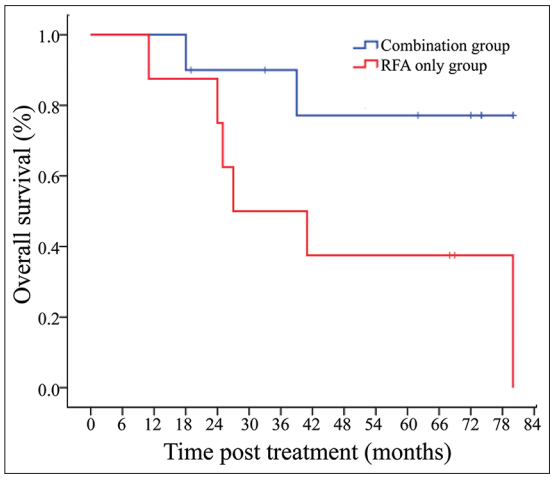

Figure 7: Comparison of overall survival after treatment in hepatocellular carcinoma patients. The probabilities of overall survival after 1-, 3- and 5-years were 90%, 90% and 77.1% in the combination group, and 87.5%, 50.0% and 37.5% in the radiofrequency ablation only group, respectively ($P = 0.045$)

DISCUSSION

Recently, local thermal ablation of liver tumors has drawn considerable attention in clinical works, and randomized control trials showed no significant differences in survival rates (overall or disease-free) after RFA or resection.[2,28,29] However, the RFA efficacy of >3 cm HCC has not broadly accepted in clinical practice given tumor recurrence after RFA. Despite promising results with RFA in monotherapy, the incidence of local and distant recurrence remains a challenging issue.[30] This finding suggests that there are residual patches of untreated tumor in a substantial but unknown number of cases; this result falls far short of our goal of completely eradicating all tumors treated by RFA. Therefore, current strategies have evolved from this need to increase the completeness of RFA destruction, even for small lesions. More recent avenues of investigation have focused on the potential gains in tumor destruction that can be achieved by combining tumor ablation with adjuvant chemotherapy or radiation. The role of combined locoregional treatment (RFA plus IV liposomal agents), especially for nodules >3 cm, should be assessed.[31]

Animal studies that combined RFA with adjuvant liposomal doxorubicin in a rat breast adenocarcinoma model have demonstrated significant increases in mean tumor coagulation diameter from combination RFA/Doxil therapy (13.1 mm) compared with RFA alone (6.7 mm).[32] Confirmatory studies performed in different models demonstrated similar gains in overall ablation-induced tumor coagulation. Similarly, increases in intratumoral drug accumulation with combination therapy were also observed.[21] These findings help explain why liposomal doxorubicin is likely to be complementary to RFA. The majority of the liposomes concentrated in a zone immediately peripheral to the area coagulated by RFA heating and were located within the region where nonlethal hyperthermia and increased destruction is observed.[33] Additionally, the patchy penetration of liposomes into the zone of coagulation implies infiltration of chemotherapy into the coagulated focus (possibly through residual patent vessels) that may improve the completeness of tumor destruction. As local benefit has been confirmed in numbers of animal studies, randomized controlled clinical trials are rare for the combination of RFA and LTLD for HCC patients. In this study, we evaluated whether the use of combined RFA and LTLD results in larger coagulation volume and longer OS compared with the use of RFA alone in patients with 3–7 cm HCC.

In a pilot clinical study,[22] Goldberg *et al*. showed increased tumor destruction for tumors treated with RFA and a long-circulating liposomal doxorubicin preparation (Doxil; ALZA Pharmaceuticals, Mountainview, CA, USA) compared with tumors treated with RFA alone. By comparison, increased tumor destruction at 2–4 weeks after ablation was observed for all lesions treated with combined Doxil and RFA ($P < 0.001$). This preliminary translational pilot study revealed that combined RFA and IV liposomal doxorubicin chemotherapy result in greater tumor destruction than RFA alone; these findings corroborate our study. In our study, CT showed an evolving zone of ablation that extended to include additional portions of the tumor and its margins 4 weeks after combined therapy. This increase was only observed in tumors exposed

to both therapies and differed markedly from the expected evolution of the RFA zone.

Our clinical data showed that LTLD significantly increased the tumor coagulation volume after RFA treatment and potentially improved the technical success rate. The increase in the longest diameter of ablative destruction ranged from 0.5 to 3.7 cm in the combination group and 0.3–1.3 cm ($P = 0.018$) in the RFA alone group. Additionally, the difference in volume between pre- and post-RFA in the RFA plus LTLD group was significantly larger than that of RFA alone group (105.7 ± 73.8 cm^3 vs. 37.3 ± 8.5 cm^3, $P = 0.013$). This finding suggested that administration of LTLD may help promote the destruction of the 0.5-to 1-cm safety margin that is necessary to adequately treat an entire tumor.

Several mechanisms have been postulated to explain this synergistic effect, including the following: (1) The synergy between chemotherapy and thermal ablation has reciprocal zones of efficacy.[34] The highest risk of chemotherapy resistance occurs at the center of the tumor because chemotherapy depends on drug penetration from the vascular network to the center of the tumor. In contrast, the margin of the tumor is where sublethal temperatures and reversible injury occur during thermal ablation, especially with the heat-sink effect. Hyperthermia as well as increased vasodilation and vascular permeability at the peripheral zone would increase drug deposition in this area, which would further increase the efficacy of chemotherapy. (2) Chemotherapy impairs cell repair mechanisms and causes cell apoptosis. Markers of oxidative and nitrosative stress also increase during combination therapy.[23] (3) The eradication of preexisting microscopic tumor foci that are undetected by imaging. (4) Similarly, increases in intratumoral drug accumulation with combination therapy were also observed.[32] Studies in both small and large animal models have demonstrated an up to 5.6-fold increase in intratumoral doxorubicin accumulation following RFA. Additionally, Moussa *et al*. reported that RF ablation induced morphologic changes in vessels within the ablation zone lasting 12–24 h after treatment. The addition of liposomal doxorubicin causes early vessel contraction and a reduction in periablational microvascular patency, which can improve the tumor inhibition rate.[35] Thus, this clinical study was consistent with the results of previous animal studies. Importantly, the RFA and LTLD combination proved to be an important new approach in the exciting frontier of tumor therapy.

For clinically effective anticancer therapy, high concentrations and selective drug delivery to the tumor site are necessary.[36,37] The nontarget toxicity of chemotherapy is a major undesirable side effect that limits the dose and therapeutic window. Targeted drug delivery to the tumor site is also possible not only through the moiety but also external stimuli (e.g., temperature, light, magnetic field, and pulse) with stimulus-sensitive drug delivery systems.[38] Temperature-induced drug delivery using lyso-thermosensitive liposomes and RFA or high-intensity focused ultrasound is currently being investigated by a number of researchers.[39,40] To build on their work and to improve spatial distribution of the chemotherapeutic payload in our study, the phospholipid composition of thermosensitive liposomes was further modified to enable drug release at temperatures >41°C. This feature also made the liposomes more stable in body circulation compared with conventional liposomes.[41] Complete and fast drug release from LTLD above the solid ordered to liquid disordered phase transition temperature is facilitated by the presence of phosphatidylcholine. This notion may be predicted by the significant decrease in plasma total doxorubicin levels 1-h post-LTLD injection from 48.4% total plasma concentration to 0.7% total plasma concentration at 24 h.[42] Thus, we administered IV infusion 30 min before RFA and completed treatment within 3 h after infusion in this study to make full use of the high concentration of doxorubicin in plasma.

Key findings from this study also included the AE and toxicity evaluation for this new treatment strategy. The safety profile of RFA + LTLD was similar to the RFA alone arm, with the exception of reversible myelosuppression. This finding is not unexpected since free doxorubicin also causes myelosuppression.[43] Moreover, the safety results in this study showed no indication of increased cardiac toxicity with the addition of LTLD in contrast with the safety profile of free doxorubicin, which has a well-described increased risk of cumulative cardiotoxicity.[44] We did not find a significant difference in cardiac function between the two groups after treatment. In addition to evaluating cardiotoxicity, we evaluated a variety of lab tests at different time-points (before treatment, immediate posttreatment, 14 and 28 days posttreatment). Due to the enhanced anti-cancer effect, WBC and NEU levels were increased immediately after RFA, reduced at day 14 given the toxicity of chemotherapy, and then recovered at day 28. There was no significant difference at day 28 between the two groups. In both groups, PLT and RBC levels were similar before or after RFA. This result indicated that LTLD enhanced the inflammatory reaction in a short time and then decreased white cells. However, the toxicity of chemotherapy did not last a long time. CBC results returned normal levels approximately 1 month after treatment. Liver function lab tests also demonstrated that ALT, AST, Alb and ALP levels were increased immediately after RFA and returned to the level before RFA at day 28 in both groups. These data demonstrate that the combination therapy caused only a temporary liver function abnormality. In addition, Crea and TSH levels changed immediately after RFA and then returned to normal levels at day 28 in the combination group. There was no significant difference at day 28 between the two groups. These data indicated that in addition to the liver, the combination treatment did not influence the function of other important organs, including the kidney and thyroid gland. No procedure-related and doxorubicin toxicity-related mortalities were noted. Based on our preliminary clinical data, we think

that the combination treatment was safe for HCC patients, and the toxicity profile of RFA + LTLD was manageable.

In our study, the probabilities of OS after 13 and 5 years were 90%, 90% and 77%, respectively, in the combination group and 87.5%, 50.0% and 37.5%, respectively, in the RFA-only group ($P = 0.045$). As previously reported, OS depends strictly on complete tumor destruction and safe margin.[45,46] Consistently, larger ablative destruction with safe margin was a relevant surrogate end-point that should be planned and carefully assessed by doctors in all patients who undergo RFA.[47] The RFA plus LTLD OS rates were similar to the previous RFA combination study. Shibata *et al*.[4] reported that 1-, 2-, 3-, and 4-year survival rates were 100%, 100%, 84.8%, and 72.7% for the RFA-TACE groups, respectively. Another study evaluated the efficacy and safety of combined RFA and PEI with a multipronged needle in the treatment of medium (3.1–5.0 cm) and large (5.1–7.0 cm) HCC. The 1- and 2-year survival rates were 93.1% and 88.1%, respectively.[48] Thus, we believe that RFA-LTLD may be a similar effective combination technique for patients with HCCs (>3 cm) as RFA-TACE or RFA-PEI. TACE with RFA has also been shown to be superior to RFA alone in prolongation of patient survival.[11] The enhancing and beneficial effects are due to obstruction of the nourishing artery by the bead or other embolizing agents and the anti-tumor effect of the accompanying chemotherapeutic agents. IV administration of LTLD was also a simpler operation than TACE and made the combination procedure feasible in most medical centers. In general, RFA-LTLD treatment was as effective as RFA combination treatment, and a further study comparing RFA plus TACE with RFA plus LTLD and TACE plus LTLD is ongoing.

Although this study provided positive preliminary results, several important limitations should be mentioned. The small patient population in a single center was clearly insufficient to adequately define and accurately quantify the anticipated increase in treatment effect. Furthermore, given that a single administration of IV LTLD was used before RFA in combination therapy, the maximum benefit achievable with this single administration of chemotherapy is unknown. Additionally, a comparatively large scale of tumor size was selected for this clinical trial. We wondered if the efficacy benefit of combination therapy could be achieved by one-time chemotherapy in large tumor between 5 and 7 cm. Differences in the extent of treatment effect between tumors size from 3 to 7 cm after combination therapy could be further analyzed in the next study.

CONCLUSIONS

Our study showed that combined RFA and LTLD therapy can increase tumor destruction in specific types of hepatic tumors compared with RFA alone without producing serious side effects and potentially leading to longer OS. The ongoing study is likely to confirm the clinical benefit that can be achieved by this combined method in multicenter clinical trials. Nevertheless, in our opinion, these novel results showed great promise for RFA and IV LTLD therapy in fighting cancers.

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Conflicts of interest

There are no conflicts of interest.

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