Expert Opinion

Lyso-thermosensitive liposomal doxorubicin: a novel approach to enhance efficacy of thermal ablation of liver cancer

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Background: Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer death worldwide. No more than 30% of HCC patients receive curative treatment. Factors limiting curative therapy include tumor size and degree of liver impairment. *Objective*: To develop a cure for medium (3.1 – 5.0 cm) and large (> 5 cm) tumors in seriously impaired livers. *Method*: Combine radiofrequency ablation (RFA) with lyso-thermosensitive liposomal doxorubicin (LTLD). *Results/conclusions*: RFA is used safely in patients with medium/large tumors and severe liver impairment; unclear tumor margins limit its curative efficacy. LTLD concentrates in the liver, where the anti-HCC chemotherapeutic, doxorubicin, is released into tumor margins by hyperthermia. RFA/LTLD can treat Child-Pugh class A-B patients with tumors up to 7 cm, a substantial increase in curable patients.

Keywords: hepatocellular carcinoma, lyso-thermosensitive liposomal doxorubicin, radiofrequency ablation

1. Introduction

Lyso-thermosensitive liposomal doxorubicin (LTLD, ThermoDox®, Celsion Corporation, Columbia, Maryland, USA) is the first heat-activated formulation of liposomal doxorubicin. Its unique property of heat-activated release of doxorubicin implies its potential use in enhancing cancer cell killing in thermal ablation of solid tumors. This review describes the development of LTLD and explains its use with radiofrequency ablation (RFA) to bring curative therapy to more hepatocellular carcinoma (HCC) patients.

2. Hepatocellular carcinoma

The estimated 2002 worldwide incidence of HCC was 641,000, making it the fifth most common solid tumor. The estimated 2002 global deaths from HCC were almost the same: 618,000. HCC is the fourth leading cause of death from cancer and the third most common in males [1]. One remarkable fact profoundly affects the incidence, geographic distribution, staging and treatment of HCC: about three-quarters of HCC cases occur in patients who already have chronic liver disease, such as hepatitis B or hepatitis C, often with a substantial degree of liver impairment [2]. In addition to viral hepatitis, the underlying chronic liver disease may be due to contaminated food (e.g., corn or peanuts with aflatoxin B_1), contaminated water (e.g., pond-ditch water with microcystin), or

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alcohol abuse [3]. HCC incidence is high in areas where hepatitis B is endemic, especially the Western Pacific region. Liver function is a crucial factor in HCC staging [4-6] and in HCC treatment decisions [5-7].

According to the Barcelona Clinic Liver Cancer (BCLC) [5,6], curative treatments for HCC include liver transplantation [8], surgical resection [9], percutaneous ethanol injection (PEI) and RFA. However, no more than 30% of HCC patients are considered suitable for these curative strategies because of tumor size (> 3 cm maximum diameter), multifocal tumors, vascular invasion, presence of extrahepatic metastases, and/or extensive liver impairment [10]. The incidence of extrahepatic tumors in patients with HCC is generally about 15%, including both metastases present at initial diagnosis of HCC and metastases found during follow-up [11,12].

3. Radiofrequency ablation

RFA has emerged as a successful therapy for small HCC lesions. In RFA, the tumor as well as a 'normal' zone of approximately 1 cm around it are ablated by hyperthermia [13]. Only about 2% of RFA patients with liver tumors experience major complications, such as intraperitoneal hemorrhage, intrahepatic abscesses, gastrointestinal wall perforations or hemothorax [14]. Minor complications, typically reported in ≤ 10% of patients, can include altered liver function tests, self-limited bleeding, effusions, pain, fever, infection and skin burn [15]. In a review of 3670 RFA cases, the overall complication rate was 8.9% and the overall mortality rate was 0.5%. RFA complication rates were 7.2% for percutaneous, 9.5% for laparoscopic, 9.9% for simple open and 31.8% for open combined with cryotherapy, hepatic or extrahepatic resection. Mortality rates were 0.5%, 0%, 0% and 4.5%, respectively [16].

Several literature reviews identified RFA as a promising therapy for small HCC tumors [17-19]. Randomized trials have now confirmed the effectiveness of RFA against HCC tumors ≤ 3 cm [20-22], ≤ 4 cm [23] and ≤ 5 cm [24,25]. RFA has been used safely in tumors up to 7 cm [26-30] or 8 cm [31]. For tumors > 3 cm, there is a greater propensity to leave viable tumor cells in the margins or clefts of overlapping ablation zones. This increases the possibility of incomplete ablation or a more rapid recurrence at the site of the original lesion as well as elsewhere within the liver at a site remote to the treatment area (due to vascular spread). Local recurrence rates after RFA for tumors ≤ 3 cm are reported to be ≤ 20% [20,21,32-34]; however, for tumors > 3 cm, local recurrence rates ≥ 40% are observed [35,36]. The BCLC criteria recommend RFA to cure patients with WHO performance status 0 and with portal hypertension and/or elevated bilirubin who have either i) a solitary tumor < 2 cm and no more than Child-Pugh class A liver dysfunction or ii) up to three tumors, each ≤ 3 cm, and no more than Child-Pugh class B liver dysfunction [5-6].

Randomized trials have compared percutaneous RFA with alternative treatments, namely percutaneous acetic acid injection (PAI) [20], surgical resection [24], PEI [20-23,25] and the combination of RFA and PEI [36-37] (Table 1). In overall survival among patients with tumors < 5.0 cm, RFA was found equivalent to surgery [24], to PEI alone [22,25], and to the RFA/PEI combination [36], and was found superior to PAI [20] and to PEI alone [20,21,23]. Combining all tumor sizes, RFA had overall survival inferior to RFA/PEI [37]. However, subgroup analyses show that PEI provides no additional benefit to RFA alone in small tumors (< 3.0 cm) [36,37] or in large tumors (> 5.0 cm) [37]; the RFA/PEI combination is superior specifically among patients with medium tumors (3.1 – 5.0 cm) [36,37].

In summary, RFA is safe among Child-Pugh class A or B patients. If the efficacy of RFA for HCC tumors > 3.0 cm could be increased, as by an adjuvant, it would be a formidable curative modality.

4. Doxorubicin

Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic. The recommended single-agent dose of doxorubicin HCl for injection (doxorubicin, Adriamycin®, Bedford Laboratories, Bedford, Ohio, USA) is $60 – 75$ mg/m² intravenously (IV) in 3-week cycles. Myelosuppression and cardiotoxicity (congestive heart failure) are dose-limiting. Since the metabolism and excretion of doxorubicin occur predominantly by the hepatobiliary route, its toxicity can be enhanced by hepatic impairment, and dose reduction is recommended when hyperbilirubinemia is present [38,39].

A 1988 review of 13 published trials of single-agent doxorubicin among 644 HCC patients found an objective response rate of 19% and median overall survival of 4 months [40]. In subsequent randomized trials comparing overall survival, single-agent doxorubicin has been found superior to no anticancer treatment [41], equivalent to combination chemotherapy (cisplatin, interferon α-2b, doxorubicin, and fluorouracil) [42], and superior to single-agent nolatrexed [43]. As a single agent, doxorubicin is active in Child-Pugh class A or B patients, but its lack of specificity to HCC lesions can lead to substantial systemic toxicity at therapeutic doses [41-43]. The relatively high incidence of severe toxicity of systemically administered doxorubicin in cirrhotic patients counteracts its benefit in tumor control, and overall systemic doxorubicin is not considered a beneficial treatment for HCC patients.

Intra-arterial (IA) administration of doxorubicin has been studied as a way to make the agent more liver-specific and less toxic overall. Clinical studies confirm that IA doxorubicin is active in HCC [44-47]. However, when doxorubicin is given intra-arterially in the presence of cirrhosis, both reduced efficacy against HCC [45] and increased hepatic toxicity have been reported [45,47].

Table 1. Randomized trials of radiofrequency ablation and alternative treatments for hepatocellular carcinoma.

Citation	Tumor(s)/maximum diameter	Treatments/patients	Local recurrence rate	Overall survival rate
Lin 2005 [20]	1 – 3 tumors, each ≤ 3.0 cm	RFA: 62 PEI: 62 PAI: 63	At 3 yrs, RFA 14%, PEI 34%, PAI 31% RFA < PEI, p = 0.012 RF < PAI, p = 0.017	At 3 yrs, RFA 74%, PEI 51%, PAI 53% RFA > PEI, p = 0.031 RFA > PAI, p = 0.038
Shiina 2005 [21]	1 – 3 tumors, each ≤ 3.0 cm	RFA: 118 PEI: 114	RFA 2%, PEI 11% RFA < PEI, p = 0.003	At 4 yrs, RFA 74%, PEI 57% RFA > PEI, p = 0.01
Brunello 2008 [22]	1 – 3 tumors, each ≤ 3.0 cm	RFA: 70 PEI: 69	NR*	At 3 yrs, RFA 63%, PEI 59% RFA ≈ PEI, p = 0.4754
Lin 2004 [23]	1 – 3 tumors, each ≤ 4.0 cm	RFA: 52 PEI: 52 PEI HD: 53	At 3 yrs, RFA 18%, PEI 45%, PEI HD 33% RFA < PEI, p = 0.012 RFA < PEI HD, p = 0.037	At 3 yrs, RFA 74%, PEI 50%, PEI HD 55% RFA > PEI, p = 0.014 RFA > PEI HD, p = 0.023
Chen 2005 [36]	Solitary tumor ≤ 5.0 cm	RFA alone: 41 RFA/PEI: 45	At 2 yrs, RFA 43%, RFA/PEI 26% RFA > RFA/PEI, p = 0.0393 Subgroup analysis by tumor size ≤ 3.0 cm: RFA 34%, RFA/PEI 21% RFA ≈ RFA/PEI, p = 0.3679, 3.1 – 5.0 cm: RFA 55%, RFA/PEI 27% RFA > RFA/PEI, p = 0.0440	At 2 yrs, RFA 61%, RFA/PEI 74% RFA ≈ RFA/PEI, p = 0.6181
Chen 2006 [24]	Solitary tumor ≤ 5.0 cm	RFA: 71 SRS: 90	NR	At 4 yrs, RFA 68%, SRS 64% RFA ≈ SRS, NS
Lencioni 2003 [25]	Solitary tumor ≤ 5.0 cm or 1 – 3 tumors, each ≤ 3.0 cm	RFA: 52 PEI: 50	At 2 yrs, RFA 4%, PEI 38% RFA < PEI, p = 0.002	At 2 yrs, RFA 98%, PEI 88% RFA ≈ PEI, p = 0.138
Zhang 2007 [37]	Solitary tumor ≤ 7.0 cm or 1 – 3 tumors, each ≤ 3.0 cm	RFA alone: 67 RFA/PEI: 66	RFA 21%, RFA/PEI 6% RFA > RFA/PEI, p = 0.01	At 3 yrs, RFA 58%, RFA/PEI 76% RFA < RFA/PEI, p = 0.01 Subgroup analysis by tumor size ≤ 3.0 cm: RFA 77%, RFA/PEI 84% RFA ≈ RFA/PEI, p = 0.20, 3.1 – 5.0 cm: RFA 48%, RFA/PEI 78% RFA < RFA/PEI, p = 0.04, 5.1 – 7.0 cm: RFA 35%, RFA/PEI 53% RFA ≈ RFA/PEI, p = 0.42

All RFA procedures were percutaneous.

Studies are arrayed in order of tumor size.

*Rates of sustained complete response at 1 year were 66% for RFA and 36% for PEI (p = 0.0005).

HCC: Hepatocellular carcinoma; HD: High dose; NR: Not reported; NS: Not statistically significant; PAI: Percutaneous acetic acid injection; PEI: Percutaneous ethanol injection; RFA: Radiofrequency ablation; SRS: Surgical resection.

4.1 Liposomal doxorubicin

An alternative strategy to make doxorubicin more liver-specific is to encapsulate it in liposomes. A large fraction of the human cardiac output circulates through the liver and spleen, and liposomes are rapidly concentrated and cleared by these organs [48]. (The exception is pegylated liposomal doxorubicin, which has a prolonged plasma half-life of 55 h [49]).

It has been shown in a series of experiments that high local concentrations of liposomal doxorubicin will provide for an enhanced volume of coagulation necrosis and a larger ablation zone in both animals and humans by sensitizing peripheral tissue to irreversible thermal injury and effectively lowering the temperature threshold for thermally mediated tissue necrosis [50-57]. This suggests that a heat-sensitive formulation of liposomal doxorubicin would be optimal for use with hyperthermia.

4.2 Lyso-thermosensitive liposomal doxorubicin

LTLD selectively releases its doxorubicin contents when exposed to temperatures ≥ 39.5°C, which creates a large concentration gradient of doxorubicin around the zone of RFA-induced tumor cell necrosis [58]. The doxorubicin then kills tumor cells in the ablation margin, providing more successful treatment of HCC lesions > 3 cm in diameter than thermal ablation alone.

The active drug in LTLD is doxorubicin hydrochloride. Doxorubicin consists of a naphthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to an amino sugar, daunosamine. The chemical formula is $C_{27}H_{29}NO_{11}$ HCl and the molecular weight is 579.99 Daltons [58].

LTLD combines doxorubicin with lyso-thermosensitive liposomes that are made from three synthetic phospholipids: DPPC (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine), MSPC (1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine), and DSPE-MPEG-2000 (1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxypolyethyleneglycol-2000). LTLD is manufactured as stable doxorubicin-loaded liposomes, which are stored as a frozen solution [58].

5. RFA/LTLD mechanism of action

In RFA, imaging techniques such as ultrasound, computed tomography or magnetic resonance are used to help guide a needle electrode into a tumor. By exerting radio-energy with frequency of approximately 460 – 480 kHz, ionic agitation and frictional heat in the surrounding tissue occur, which leads to cell death and coagulation necrosis [59].

Doxorubicin's cytotoxic mechanism of action is thought to be related to its ability to bind to DNA and inhibit nucleic acid synthesis [39]. Doxorubicin binds to nucleic acids, presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix. The interaction of doxorubicin with topoisomerase II to form DNA cleavable complexes seems to be an important mechanism of doxorubicin cytocidal activity. Doxorubicin binds to cell membranes as well as plasma proteins [58].

LTLD is administered intravenously and, because it is a liposome, rapidly concentrates in the liver. Since liposomal doxorubicin is a larger particle than free doxorubicin, it is more than 1000 times less permeable across normal blood vessels than free doxorubicin, offering less potential for systemic toxicity [48]. During RFA/LTLD therapy, cytocidal heat is directed to a tumor. When heat-sensitive liposomes encounter a temperature of 39.5°C or above, their doxorubicin is released into the heated area [58]. The released doxorubicin remains stable up to 73°C. *In vitro* studies have repeatedly shown enhancement of cell killing when doxorubicin is combined with hyperthermia compared with doxorubicin without hyperthermia [60-71]. This enhancement has been attributed to the ability of hyperthermia to increase intracellular retention of chemotherapeutic agents by upregulating their influx [62,63].

6. RFA/LTLD preclinical studies

Twenty-one dogs (18 with sarcoma, 3 with carcinoma) were treated with LTLD and microwave hyperthermia in a Phase I dose-escalation study. The LTLD was given (0.7 – 1.0 mg/kg = 14 – 20 mg/m²) IV over 30 min. Three treatments, given 3 weeks apart, were scheduled. Grade 4 neutropenia and acute death secondary to liver failure, possibly drug-related, were the dose-limiting toxicities. The maximum tolerated dose was 0.93 mg/kg. Other toxicities, with the possible exception of renal damage, were consistent with those observed following free doxorubicin administration. Of the 20 dogs that received ≥ 2 doses of LTLD, 6 (30.0%) had a partial response and 12 (60.0%) had stable disease. Tumor drug concentrations at a dose of 1.0 mg/kg averaged 9.12 – 6.17 ng/mg tissue [72].

7. RFA/LTLD pharmacokinetics

Figure 1 shows pharmacokinetic data for six liver cancer patients treated with RFA/LTLD in a Phase I study. The major portion of exposure to LTLD (about 95% of the liposomal doxorubicin plasma $AUC_{0-\infty}$) occurred during the first 6 h after the infusion, establishing this time period as optimal for application of RFA. Most of the free doxorubicin exposure also occurred during the first 6 h (87.9% of the free doxorubicin $AUC_{0-\infty}$). Free doxorubicin represented 43.6% of the total doxorubicin $AUC_{0-\infty}$. For both liposomal and free doxorubicin, maximum plasma concentration occurred just before the end of the 30-min infusion [58,73].

8. RFA/LTLD clinical safety

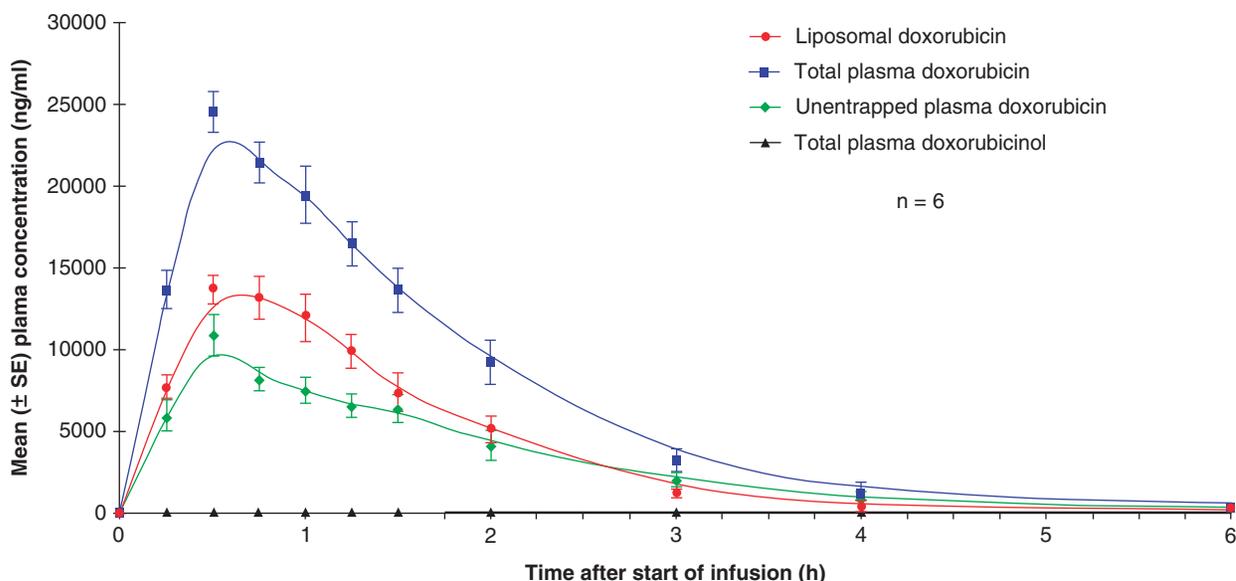In a Phase I trial among 24 subjects who received RFA/LTLD for HCC or metastatic liver cancer, the maximum

Figure 1. LTLD 50 mg/m² : human plasma clearance (Mean ± SE) in first 6 h [58].

tolerated dose (MTD) of LTLD was determined to be 50 mg/m², based on two dose-limiting toxicities (grade 3 alanine aminotransferase increase, grade 4 neutropenia) at a dose of 60 mg/m² [73].

So far, 55 patients with HCC or prostate or breast cancer have been treated with LTLD and hyperthermia (**Table 2**). The most common adverse events (affecting ≥ 10% of patients) were AST/SGOT abnormalities (50.9%), ALT/SGPT abnormalities (50.9%), alopecia (38.2%), neutropenia (32.7%), fatigue (25.5%), hemoglobin decreased (23.6%), nausea (21.8%), leukopenia (20.0%), hypoalbuminemia (18.2%), abdominal pain (18.2%), thrombocytopenia (16.4%), hyperbilirubinemia (16.4%), anorexia (14.5%), hypernatremia (12.7%), vomiting (12.7%), weight loss (12.7%), dyspnea (12.7%), hyperkalemia (10.9%) and constipation (10.9%). The most common grade 3+ adverse events (affecting ≥ 5% of patients) were AST/SGOT abnormalities (40.0%), ALT/SGPT abnormalities (32.7%), neutropenia (29.1%), leukopenia (12.7%), and lymphopenia (9.1%). There were a total of 16 serious adverse events, each occurring only once [58].

Very few of the abnormalities in liver function were associated with LTLD. The most common drug-related adverse events (affecting ≥ 5% of patients) were alopecia (36.4%), neutropenia (32.7%), leukopenia (20.0%), hemoglobin decreased (18.2%), fatigue (14.5%), nausea (10.9%), thrombocytopenia (9.1%), decreased ejection fraction (9.1%), anorexia (7.3%), taste alteration (5.5%) and fever (5.5%) [55]. These drug-related events are consistent with the adverse event profile of free doxorubicin [38,39]. All of the ejection fraction decreases were drug-related but none was serious or grade 3+; all occurred in a closed prostate cancer study [58].

9. RFA/LTLD clinical efficacy

In a Phase I dose-finding study, 24 patients (9 with HCC and 15 with liver tumors metastatic from 9 other primary sites) were treated with a single 30-min IV infusion of LTLD at 20, 30, 40, 50 or 60 mg/m², starting 15 min before RFA. RFA was performed percutaneously or surgically on a total of 28 tumors. Half of the treated tumors were 3.8 – 6.5 cm in diameter [73,74].

Treatment failure was operationally defined as objective disease progression and/or initiation of an alternative anticancer therapy. There was a statistically significant (p = 0.0380) LTLD dose-response effect: median TTF for patients receiving at least the MTD of 50 mg/m² was 374 days while that for patients receiving less than 50 mg/m² was 80 days (**Table 3**). This is consistent with the hypothesis that LTLD significantly increases RFA efficacy.

10. Conclusion

LTLD concentrates in the liver and releases doxorubicin wherever heated by RFA. Doxorubicin improves the efficacy of RFA by its tumoricidal effect in the heated ablation margins, while RFA improves the efficacy of doxorubicin, possibly by upregulating its influx into HCC cells. Phase I data show that, among Child-Pugh class A-B patients with medium or large tumors, RFA/LTLD is safe and demonstrates a statistically significant dose-response effect.

11. Expert opinion

Percutaneous RFA is attractive in HCC because, compared with surgery, it is a less invasive treatment with maximal

Table 2. Frequency listing of adverse events, 'occurring in at least 5% of patients (liver, breast, or prostate cancer) treated with LTLD using targeted application of heat, either by RFA or microwave (as of April 2008)' [58].

System organ class/adverse event	Lyso-thermosensitive liposomal doxorubicin (LTLD) (n = 55)			
	Any AE n (%)	Grade 3 or more n (%)	Drug-related n (%)	Serious AE n (%)
Metabolic/laboratory				
AST, SGOT (serum glutamic oxaloacetic transaminase)	28 (50.9)	22 (40.0)	1 (1.8)	0 (0.0)
ALT, SGPT (serum glutamic pyruvic transaminase)	28 (50.9)	18 (32.7)	1 (1.8)	0 (0.0)
Albumin, serum – low (hypoalbuminemia)	10 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)
Sodium, serum – high (hypernatremia)	7 (12.7)	1 (1.8)	0 (0.0)	0 (0.0)
Potassium, serum – high (hyperkalemia)	6 (10.9)	2 (3.6)	0 (0.0)	0 (0.0)
CPK (creatine phosphokinase)	5 (9.1)	1 (1.8)	0 (0.0)	0 (0.0)
Alkaline phosphatase	4 (7.3)	1 (1.8)	0 (0.0)	0 (0.0)
Glucose, serum – high (hyperglycemia)	3 (5.5)	1 (1.8)	0 (0.0)	0 (0.0)
Phosphate, serum – low (hypophosphatemia)	3 (5.5)	2 (3.6)	0 (0.0)	0 (0.0)
Potassium, serum – low (hypokalemia)	3 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)
Blood and lymphatic system disorders				
Neutropenia/granulocytopenia (ANC/AGC)	18 (32.7%)	16 (29.1%)	18 (32.7%)	0 (0.0%)
Hemoglobin decreased	13 (23.6%)	1 (1.8%)	10 (18.2)	0 (0.0%)
Leukocytes (decrease in total WBC)	11 (20.0%)	7 (12.7%)	11 (20.0%)	0 (0.0%)
Thrombocytopenia	9 (16.4%)	2 (3.6%)	5 (9.1%)	1 (1.8%)
Lymphopenia	5 (9.1%)	5 (9.1%)	1 (1.8%)	0 (0.0%)
Gastrointestinal disorders				
Nausea	12 (21.8%)	0 (0.0%)	6 (10.9%)	0 (0.0%)
Anorexia	8 (14.5%)	0 (0.0%)	4 (7.3%)	0 (0.0%)
Vomiting	7 (12.7%)	0 (0.0%)	2 (3.6%)	0 (0.0%)
Constipation	6 (10.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Diarrhea	4 (7.3%)	1 (1.8%)	2 (3.6%)	0 (0.0%)
Mucositis/stomatitis oral cavity	3 (5.5%)	0 (0.0%)	2 (3.6%)	0 (0.0%)
Taste alteration (dysgeusia)	3 (5.5%)	0 (0.0%)	3 (5.5%)	0 (0.0%)
Constitutional symptoms				
Fatigue (asthenia, lethargy, malaise)	14 (25.5%)	0 (0.0%)	8 (14.5%)	0 (0.0%)
Weight loss	7 (12.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Fever (without neutropenia: ANC ≥ 1 (1.8%)0 (0.0%) × 1 (1.8%)0 (0.0%)9/L)	5 (9.1%)	0 (0.0%)	3 (5.5%)	1 (1.8%)
Insomnia	3 (5.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Skin and subcutaneous tissue disorders				
Alopecia	21 (38.2%)	0 (0.0%)	20 (36.4%)	0 (0.0%)
Pain				
Abdomen NOS	10 (18.2%)	2 (3.6%)	0 (0.0%)	0 (0.0%)
Joint	5 (9.1%)	2 (3.6%)	0 (0.0%)	0 (0.0%)
Back	3 (5.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Bone	3 (5.5%)	1 (1.8%)	0 (0.0%)	0 (0.0%)
Extremity – limb	3 (5.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Table 2. Frequency listing of adverse events, 'occurring in at least 5% of patients (liver, breast, or prostate cancer) treated with LTLD using targeted application of heat, either by RFA or microwave (as of April 2008)' [58] **(continued).**

System organ class/adverse event	Lyso-thermosensitive liposomal doxorubicin (LTLD) (n = 55)			
	Any AE n (%)	Grade 3 or more n (%)	Drug-related n (%)	Serious AE n (%)
Muscle	3 (5.5%)	2 (3.6%)	0 (0.0%)	0 (0.0%)
Chest/thorax NOS	3 (5.5%)	1 (1.8%)	0 (0.0%)	0 (0.0%)
Throat/pharynx/larynx	3 (5.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hepatobiliary disorders				
Bilirubin (hyperbilirubinemia)	9 (16.4%)	1 (1.8%)	0 (0.0%)	0 (0.0%)
Pulmonary/upper respiratory				
Dyspnea (shortness of breath)	7 (12.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Infections				
Urinary tract NOS	5 (9.1%)	1 (1.8%)	1 (1.8%)	1 (1.8%)
Hepatobiliary infection	3 (5.5%)	2 (3.6%)	1 (1.8%)	0 (0.0%)
Renal/genitourinary disorders				
Urinary retention	5 (9.1%)	0 (0.0%)	0 (0.0%)	1 (1.8%)
Bladder spasms	3 (5.5%)	0 (0.0%)	2 (3.6%)	0 (0.0%)
Incontinence, urinary	3 (5.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hemorrhage				
Urinary NOS	5 (9.1%)	0 (0.0%)	1 (1.8%)	0 (0.0%)
Cardiac general				
Decreased ejection fraction	5 (9.1%)	0 (0.0%)	5 (9.1%)	0 (0.0%)
Hypertension	4 (7.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hypotension	4 (7.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Musculoskeletal				
Muscle weakness – generalized	3 (5.5%)	0 (0.0%)	1 (1.8%)	0 (0.0%)
Lymphatics				
Edema – limb	4 (7.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Neurology				
Mood alteration: depression	5 (9.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dizziness	4 (7.3%)	0 (0.0%)	1 (1.8%)	0 (0.0%)

preservation of liver parenchyma, has fewer complications, causes less pain and requires fewer days in the hospital [24]. The key challenge with RFA is that its efficacy decreases as tumor size increases [36,37], prompting the hypothesis that an adjuvant is needed for the complete eradication of medium/large tumors. The addition of PEI has been shown in randomized trials to be beneficial in medium (3.1 – 5.0 cm) tumors [36-37] but not in large (> 5.0 cm) tumors [37]. Another approach in dealing with medium/large tumors is to use an open surgical RFA technique rather than a percutaneous RFA technique [31,75]. Advantages with open RFA include better tumor resolution and satellite nodule detection with intraoperative ultrasonography, unrestricted ability to place a cluster electrode, and free insertion of the electrode at different angles (with mobilization of the liver or tumor palpation, if necessary) [31]. As yet it is unclear whether these advantages result in improved patient outcomes. In a nonrandomized study, 35 RFA patients with tumors measuring 3.1 – 8.0 cm, 25 (71.4%) of whom were treated with the open technique, were compared with 51 RFA patients with tumors of < 3 cm. There was no difference in local recurrence rates or 1-year overall survival between the small and medium/large tumor groups [31]. In the RFA/LTLD Phase I study, patients treated with an open RFA technique had better TTF (median 188 days) than those treated percutaneously (median 80 days), but the

Table 3. Phase I RFA/LTLD time to treatment failure (TTF) by primary site, tumor size, RFA type, and LTLD dose.

Factor	Failed	Censored	MedianTTF	p-Value
			(days)	
Primary site				
Liver	7	2	355	0.2227
Other*	13	2	64	
Tumor size‡				
≤ 3.0 cm	6	2	156	0.4135
> 3.0 cm	14	1	86	
RFA type				
Open surgical	6	1	188	0.4315
Percutaneous	14	3	80	
LTLD dose				
< 50 mg/m^2	15	0	80	0.0380
≥ 50 mg/m^2	5	4	374	

*There were a total of nine other primary sites.

‡The maximum tumor diameter for one patient was not reported.

Notes: Source data are from the clinical study report [74] and were extracted as of 5 February 2008. Treatment failure is operationally defined as disease progression and/or initiation of an alternative anticancer therapy. This is a conservative way to assess efficacy, since the 15 patients with other primary sites might progress and/or begin alternative treatment for that primary or for a baseline metastatic site other than liver. Patients not experiencing treatment failure are censored as of their last reported date on-study. For each factor, TTFs are computed by the product-limit method [85] and compared by the two-tailed log-rank test [86]. No adjustments are made for multiple comparisons.

difference was not statistically significant (**Table 3**). The open surgical approach is a less attractive option for patients with higher morbidity, and there remains substantial room for improvement in efficacy by using an appropriate adjunct.

Adding LTLD to RFA is a rational approach to the problem of treating medium/large tumors. Other chemotherapeutics that have both some single-agent activity in HCC [76] and are more effective when used with hyperthermia, such as cisplatin [60,61,65] or fluorouracil [77-79], could be encapsulated in heat-activated liposomes. However, it is prudent to begin with doxorubicin for two reasons. First is the modest activity in HCC already shown by non-heat-activated liposomal doxorubicin as a single agent (objective response rate: 7 – 10%) [80,81]. Second is the increased efficacy of non-heat-activated liposomal doxorubicin when combined with hyperthermia [50-57]. An HCC case study reported substantial tumor regression with a combination of non-heat-activated liposomal doxorubicin and ultrasound hyperthermia [82].

Owing to its encouraging Phase I results, RFA/LTLD has been allowed to go directly into Phase III development. A randomized, double-blind, placebo-controlled multicenter trial has recently been initiated comparing RFA/LTLD with RFA alone among 600 patients with unresectable HCC. Child-Pugh class A or B patients are eligible, except that both ascites and encephalopathy are exclusionary. Eligible patients can have no more than four HCC lesions with at least one ≥ 3.0 cm and none > 7.0 cm in maximum diameter. However, if a patient has a large lesion (5.0 – 7.0 cm), any other lesions must be less than 5.0 cm. In both arms, RFA may be performed percutaneously, laparoscoplicly or surgically, per investigators' clinical judgment. Patients in the combination arm will receive a single 30-min IV infusion of LTLD at 50 mg/m^2, starting 15 min before RFA; the RFA-only arm patients receive a dummy infusion. Progression-free survival is the primary endpoint. Secondary endpoints include overall survival, time to local recurrence and time to a clinically significant deterioration in patient self-reported symptoms. To avoid confounding the effect of RFA/LTLD with those of tumor size and RFA technique, both randomization and statistical analysis will be stratified by largest lesion size (3.0 – 5.0 cm vs 5.1 – 7.0 cm) and type of RFA approach (percutaneous, laparoscopic or open surgical) [83,84]. RFA/LTLD treatment to cure medium/large HCC tumors is now being definitively tested by this randomized trial.

In addition to its role in HCC, LTLD may have potential clinical application in treatment of other malignancies such as breast cancer. A Phase I/II study has recently been initiated among patients with breast cancer recurrent at the chest wall. Six LTLD/microwave hyperthermia treatments will be administered at 21-day intervals. The Phase I portion will determine the maximum tolerated dose. The primary objective in the 100-patient Phase II portion is to determine the durable (lasting ≥ 3 months) complete local response rate.

In summary, LTLD is an innovative drug exploiting the heat sensitivity of liposomes in delivering a high dose of doxorubicin to the cancer site undergoing thermal ablation. Preliminary evidence shows that it is safe with better systemic toxicity profile than systemically administered free doxorubicin, and its efficacy is now being tested in clinical trials.

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Declaration of interest

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Revised Global Burden of Disease (GBD) 2002; Estimates. World Health Organization. Available from: http://www.who.int/healthinfo/bodgbd2002;revised/en/index.html [Last accessed 16 May 2008]

2. Johnson PJ, Williams R. Cirrhosis and the aetiology of hepatocellular carcinoma. J Hepatol 1987;4:140-7

3. Tang ZY, Curley SA. Liver cancer. 8th edition. In: Pollock RE, Doroshow JH, Khayat D, et al., editors. UICC Manual of Clinical Oncology, Hoboken, NJ: John Wiley & Sons, 2004. p.405-27

4. Okuda K, Ohtsuki T, Obata H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Cancer 1985;56:918-28

5. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907-17

6. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208-36

7. Pugh RN, Murray-Lyon IM, Dawson JL, et al. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9

8. Thomas MB, Zhu AX. Hepatocellular carcinoma: the need for progress. J Clin Oncol 2005;23:2892-9

9. Bruix J, Sherman M, Llovet JM, et al. Clinical Management of Hepatocellular Carcinoma. Conclusions of the Barcelona-2000 EASL Conference. J Hepatol 2001;35:421-30

10. Llovet JM, Di Bisceglie AM, Bruix M, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698-711

11. Uka K, Aikata H, Takaki S, et al. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. World J Gastroenterol 2007;13:414-20

12. Yang Y, Nagano H, Ota H, et al. Patterns and clinicopathologic features of extrahepatic recurrence of hepatocellular carcinoma after curative resection. Surgery 2007;141:196-202

13. Berber E, Herceg NL, Casto KJ, Siperstein AE. Laparoscopic radiofrequency ablation of hepatic tumors: prospective clinical evaluation of ablation size comparing two treatment algorithms. Surg Endosc 2004;18:390-6

14. Berber E, Ari E, Herceg N, Siperstein A. Laparoscopic radiofrequency thermal ablation for unusual hepatic tumors: operative indications and outcomes. Surg Endosc 2005;19:1613-17

15. Berber E, Siperstein AE. Perioperative outcome after laparoscopic radiofrequency ablation of liver tumors: an analysis of 521 cases. Surg Endosc 2007;21:613-18

16. Mulier S, Mulier P, Ni Y, et al. Complications of radiofrequency coagulation of liver tmours. Br J Surg 2002;89:1206-22

17. Galandi D, Antes G. Radiofrequency thermal ablation versus other interventions for hepatocellular carcinoma. Cochrane Database Syst Rev 2002;3:CD003046

18. Sutherland LM, Williams JA, Padbury RT, et al. Radiofrequency ablation of liver tumors: a systematic review. Arch Surg 2006;141:181-90

19. Garrean S, Hering J, Saied A, et al. Radiofrequency ablation of primary and metastatic liver tumors: a critical review of the literature. Am J Surg 2008;195:508-20

20. Lin SM, Lin CJ, Lin CC, et al. Randomized controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percuteanous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 2005;54:1151-6

21. Shiina S, Teratani T, Obi S, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122-30

22. Brunello F, Veltri A, Carucci P, et al. Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: a randomized controlled trial. Scand J Gastroenterol 2008;43:727-35

23. Lin SM, Lin CJ, Lin CC, et al. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or = 4 cm. Gastroenterology 2004;127:1714-23

24. Chen MS, Li JQ, Zheng Y, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 2006;243:321-8
• **Randomized trial showing that, for small/medium hepatocellular carcinoma, percutaneous radiofrequency ablation and surgical resection are not significantly different in overall survival; it also shows that percutaneous radiofrequency ablation is both less toxic and less costly than surgery.**

25. Lencioni RA, Allgaier HP, Cioni D, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235-40

26. Chen Q, Tong S, Dewhirst MW, Yuan F. Targeting tumor microvessels using doxorubicin encapsulated in a novel thermosensitive liposome. Mol Cancer Ther 2004;3:1311-17

27. Dodd GD 3rd, Frank Ms, Aribandi M, et al. Radiofrequency thermal ablation: computer analysis of the size of the thermal injury created by overlapping ablations. Am J Roentgenol 2001;177:777-82

28. Guglielmi A, Ruzzenente A, Battocchia A, et al. Radiofrequency ablation of hepatocellular carcinoma in cirrhotic patients. Hepatogastroenterology 2003;50:480-4

29. Curley SA, Izzo F, Abdalla E, Vauthey JN. Surgical treatment of colorectal cancer metastasis. Cancer Metastasis Rev 2004;23:165-82

30. Tateishi R, Shiina S, Teratani T, et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201-09

31. Poon RT, Ng KK, Lam CM, et al. Effectiveness of radiofrequency ablation for hepatocellular carcinomas larger than 3 cm in diameter. Arch Surg 2004;139:281-7

32. Vivarelli M, Guglielmi A, Ruzzenente A, et al. Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. Ann Surg 2004;240:102-7

33. Machi J, Bueno RS, Wong LL. Long-term follow-up outcome of patients undergoing radiofrequency ablation for unresectable hepatocellular carcinoma. World J Surg 2005;29:1364-73

34. Ng KK, Poon RT, Lo CM, et al. Analysis of recurrence pattern and its influence on survival outcome after radiofrequency

ablation of hepatocellular carcinoma. J Gastrointest Surg 2008;12:183-91

35. Mulier S, Ni Y, Jamart J, et al. Local recurrence after hepatic radiofrequency coagulation: multivariate meta-analysis and review of contributing factors. Ann Surg 2005;242:158-71

36. Chen MS, Zhang YJ, Li JQ, et al. Randomized clinical trial of percutaneous radiofrequency ablation plus absolute ethanol injection compared with radiofrequency ablation alone for small hepatocellular carcinoma. Zhonghua Zhong Liu Za Zhi 2005;27:623-5
• Randomized trial showing that, for medium HCC, RFA alone is significantly less effective than RFA plus PEI.

37. Zhang YJ, Liang HH, Chen MS, et al. Hepatocellular carcinoma treated with radiofrequency ablation with or without ethanol injection: a prospective randomized trial. Radiology 2007;244:599-607
• Randomized trial showing that RFA plus PEI is significantly more effective than RFA alone for medium HCC but not for either small HCC or large HCC.

38. Doxorubicin Product Information, Bedford Laboratories, Bedford, OH, USA. Available from: http://www.bedfordlabs.com/ products/inserts/ADR-P01.pdf [Last accessed 23 May 2008]

39. Product Monograph – Adriamycin: doxorubicin hydrochloride injection. Pfizer Canada Inc., Kirkland, Quebec, Canada, 2007. Available from: http:// www.pfizer.ca/english/our%20products/ prescription%20pharmaceuticals/default. asp?s=1&id=35&doc=enmonograph [Last accessed 16 June 2008]

40. Nerenstone SR, Inde DC, Friedman MA. Clinical trials in primary hepatocellular carcinoma: current status and future directions. Cancer Treat Rev 1988;15:1-31

41. Lai CL, Wu PC, Chan GC, et al. Doxorubicin vs. no antitumor therapy in an inoperable hepatocellular carcinoma: a prospective randomized trial. Cancer 1988;62:479-83
• Randomized trial showing that single-agent doxorubicin is superior to no anticancer treatment for HCC.

42. Yeo W, Mok TS, Zee B, et al. A randomized Phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst 2005;97:1532-8

43. Gish RG, Porta C, Lazar L, et al. Phase III randomized controlled trial comparing the survival of patients with unresectable hepatocellular carcinoma treated with nolatrexed or doxorubicin. J Clin Oncol 2007;25:3069-75

44. Ueoka H, Kuroda S, Ohnoshi T, et al. Intraarterial Adriamycin for patients with hepatocellular carcinoma and metastatic liver carcinoma. Gan To Kagaku Ryoho 1984;11:2579-84

45. Doci R, Bignami P, Bozzetti F, et al. Intrahepatic chemotherapy for unresectable hepatocellular carcinoma. Cancer 1988;61:1983-7

46. Kalayci C, Johnson PJ, Raby N, et al. Intraarterial adriamycin and lipiodol for inoperable hepatocellular carcinoma: a comparison with intravenous adriamycin. J Hepatol 1990;11:349-53

47. Tzoracoleftherakis EE, Spiliotis JD, Kyriakopoulou T, et al. Intra-arterial versus systemic chemotherapy for non-operable hepatocellular carcinoma. Hepatogastroenterology 1999;46:1122-5

48. Needham D, Ponce A. Nanoscale drug delivery vehicles for solid tumors: a new paradigm for localized drug delivery using temperature sensitive liposomes. In: Amiji M, editor. Nanotechnology in Cancer Therapy. Boca Raton, FL: CRC Press; 2006. p. 677-719

49. Bao A, Goins B, Klipper R, et al. Direct 99mTc labeling of pegylated liposomal doxorubicin (Doxil) for pharmacokinetic and non-invasive imaging studies. J Pharmacol Exp Ther 2004;308:419-25

50. Ahmed M, Monsky WE, Girnun G, et al. Radiofrequency thermal ablation sharply increases intratumoral liposomal doxorubicin accumulation and tumor coagulation. Cancer Res 2003;63:6327-33

51. Ahmed M, Goldberg SN. Combination radiofrequency thermal ablation and adjuvant iv liposomal doxorubicin increases tissue coagulation and intratumoral drug accumulation. Int J Hyperthermia 2004;20:781-802

52. Ahmed M, Lukyanov AN, Torchilin V, et al. Combined radiofrequency ablation and adjuvant liposomal chemotherapeutic: effect of chemotherapy agent, nanoparticle size, and circulation time. J Vasc Interv Radiol 2005;16:1365-71

53. Ahmed M, Liu Z, Lukyanov AN, et al. Combination radiofrequency ablation with intratumoral liposomal doxorubicin: effect on drug accumulation and coagulation in multiple tissues and tumor types in animals. Radiology 2005;235:469-77

54. Goldberg SN, Gazelle GS, Compton CC, et al. Treatment of intrahepatic malignancy with radiofrequency ablation: radiologic-pathologic correlation. Cancer 2000;88:2452-63

55. Goldberg SN, Kamel IR, Kruskal JB, et al. Radiofrequency ablation of hepatic tumors: increased tumor destruction with adjuvant liposomal doxorubicin therapy. Am J Roentgenol 2002;179:93-101

56. Dvorak J, Zoul Z, Melichar B, et al. Liposomal doxorubicin combined with regional hyperthermia: reducing systemic toxicity and improving locoregional efficacy in the treatment of solid tumors. J Chemother 2004;16(Suppl 5):34-6

57. Liu P, Xu LX, Zhang A. Enhanced efficacy of anti-tumor liposomal doxorubicin by hyperthermia. Conf Proc IEEE Eng Med Biol Soc 2006;1:4354-7

58. ThermoDox®, Lyso-Thermosensitive Liposomal Doxorubicin: Investigator's Brochure for ThermoDox® in Combination with Focused Heat for the Treatment of Solid Tumors. Columbia, MD: Celsion Corporation, 2008
•• Presents LTLD chemistry, mechanism of action, pharmacokinetics, pharmacodynamics, and safety data.

59. Ng KK, Lam CM, Poon RT, et al. Thermal ablative therapy for malignant liver tumors: a critical appraisal. J Gastroenterol Hepatol 2003;18:616-29

60. Herman TS, Sweets CC, White DM, Gerner EW. Effect of heating on lethality due to hyperthermia and selected chemotherapeutic drugs. J Natl Cancer Inst 1982;68:487-91

61. Herman TS. Temperature dependence of adriamycin, cis-diamminedichloroplatinum, bleomycin, and 1,3-bis(2-chloroethyl)-1-nitrosourea cytotoxicity in vitro. Cancer Res 1983;43:517-20

62. Bates DA, Mackillop WJ. Hyperthermia, adriamycin transport, and cytotoxicity in drug-sensitive and -resistant Chinese hamster ovary cells. Cancer Res 1986;46:5477-81

63. Nagaoka S, Kawasaki S, Sasaki K, Nakanishi T. Intracellular uptake, retention and cytotoxic effect of

adriamycin combined with hyperthermia in vitro. Jpn J Cancer Res 1986;77:205-11

64. Nitta M, Hisazumi H, Nakajima K. Combined cell killing effects of anticancer drugs and hyperthermia in vitro. Hinyokika Kiyo 1988;34:1525-8

65. Calabro A, Singletary SE, Tucker S, et al. In vitro thermo-chemosensitivity screening of spontaneous human tumors: significant potentiation for cisplatin but not adriamycin. Int J Cancer 1989;43:385-90

66. Yoshida M. Effect of adriamycin combined with 40 and 43 degrees C hyperthermia on cultured V79 cells and S-180 tumor and their uptake of the drug. Nippon Igaku Hoshasen Gakkai Zasshi 1991;51:1098-104

67. Uchibayashi T, Lee SW, Hisazumi H, et al. Studies of combined treatment of radiofrequency hyperthermia with anticancer agents or irradiation for invasive bladder cancer. Hinyokika Kiyo 1993;39:1209-13

68. Uchibayashi T, Lee SW, Kunimi K, et al. Studies of effects of anticancer agents in combination with/without hyperthermia on metastasized human bladder cancer cells in chick embryos using the polymerase chain reaction technique. Cancer Chemother Pharmacol 1994;35(Suppl):S84-7

69. Urano M, Begley J, Reynolds R. Interaction between adriamycin cytotoxicity and hyperthermia: growth-phase-dependent thermal sensitization. Int J Hyperthermia 1994;10:817-26

70. Hayashi S, Kano E, Tsuji K, et al. Modification of thermosensitivity and chemosensitivity induced by combined treatments with hyperthermia and adriamycin. Int J Mol Med 2001;8:417-22

71. Istomin YP, Zhavrid EA, Alexandrova EN, et al. Dose enhancement effect of anticancer drugs associated with increased temperature in vitro. Exp Oncol 2008;30:56-9

72. Hauck ML, Larue SM, Petros WP, et al. Phase I trial of doxorubicin-containing low temperature sensitive liposomes in spontaneous canine tumors. Clin Cancer Res 2006;12:4004-10

73. Poon R, Ng K, Yuen J, et al. Phase I study of ThermoDox (thermally sensitive liposomes containing doxorubicin) given prior to radiofrequency ablation for unresectable liver cancers [Abstract]. Mumbai, India: International Hepato-Pancreato-Biliary Association Conference, 2008

74. A Phase I dose escalation study of heat activated liposome delivery of doxorubicin and radiofrequency ablation of primary and metastatic tumors of the liver (Study 104-03-101). Celsion Corporation, Columbia, Maryland, USA, 2008 [Clinical Study Report]
•• Source of phase I RFA/LTLD efficacy data analyzed in this paper; also contains safety data.

75. Kuvshinoff BW, Ota DM. Radiofrequency ablation of liver tumors: influence of technique and tumor size. Surgery 2002;132:605-11

76. Curley SA, Levin B, Rich TA. Liver and bile ducts. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, editors. Clinical Oncology. New York: Churchill Livingstone, 1995

77. Mini E, Dombrowski J, Moroson BA, Bertino JR. Cytotoxic effects of hyperthermia, 5-fluorouracil and their combination on a human leukemia T-lymphoblast cell line, CCRF-CEM. Eur J Cancer Clin Oncol 1986;22:927-34

78. Shiiki S, Fuchimoto S, Iwagaki H, et al. Comparative antitumor activity of 5-fluorouracil and its prodrugs in combination with hyperthermia in vitro. Acta Med Okayama 1991;45:339-45

79. Maehara Y, Sakaguchi Y, Takahashi I, et al. 5-Fluorouracil's cytotoxicity is enhanced both in vitro and in vivo by concomitant treatment with hyperthermia and dipyridamole. Cancer Chemother Pharmacol 1992;29:257-60

80. Hong RL, Tseng YL. A Phase II and pharmacokinetic study of pegylated liposomal doxorubicin in patients with advanced hepatocellular carcinoma. Cancer Chemother Pharmacol 2003;51:433-8

81. Lind PA, Naucler G, Holm A, et al. Efficacy of pegylated liposomal doxorubicin in patients with advanced hepatocellular carcinoma. Acta Oncol 2007;46:230-3

82. Dvorák J, Zoul Z, Melichar B, et al. Pegylated liposomal doxorubicin in combination with hyperthermia in the treatment of a case of advanced hepatocellular carcinoma. J Clin Gastroenterol 2002;34:96-8

83. Phase III, randomized, double-blinded, dummy-controlled study of the efficacy and safety of ThermoDox® (thermally sensitive liposomal doxorubicin) in combination with radiofrequency ablation (RFA) compared to RFA-alone in the treatment of non-resectable hepatocellular carcinoma. Columbia, MD, USA: Celsion Corporation, 2007 [Protocol]
•• Will provide definitive test of RFA/LTLD therapy for medium/large HCC.

84. International Clinical Trials Registry. World Health Organization. Available from: http://www.who.int/trialsearch/advsearch.aspx; Study ID# NCT00617981 [Last accessed 4 June 2008]
•• Will provide definitive test of RFA/LTLD therapy for medium/large HCC.

85. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Statist Ass 1958;53:457-81

86. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 1977;35:1-39

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