CLINICAL PROTOCOL

Study Title:	A Phase III, Randomized, Double Blind, Dummy-Controlled Study of ThermoDox® (Lyso-Thermosensitive Liposomal Doxorubicin-LTLD) in Hepatocellular Carcinoma (HCC) using standardized Radiofrequency Ablation (RFA) treatment time ≥ 45 minutes for solitary lesions ≥ 3 cm to ≤ 7 cm.
Protocol Number:	104-13-302
Protocol Version/Date:	**Version 2.0, 14-March-2014**
Prior Versions/Date:	Version 1.0, 13-January-2014
Investigational Product:	ThermoDox® (Lyso-Thermosensitive Liposomal Doxorubicin)
Development Phase:	3
Sponsor:	Celsion Corporation 997 Lenox Drive, Suite 100 Lawrenceville, NJ 08648 USA
IND Number:	66,827
EudraCT Number:	**2014-000934-53**
NCT Number:	**02112656**

[redacted]

LIST OF STUDY REPRESENTATIVES

The list of organizations below have responsibilities for oversight and conduct of the clinical study that will be identified in a transfer of responsibility document. Contact details are consistent at the time of the final protocol.

<table>
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Investigational Product Supplier	Sharp Clinical Supply Packaging 300 Kimberton Road Phoenixville, PA 19460 USA Telephone: [redacted]	Sharp Clinical Distribution 1041 W. Bridge Street Phoenixville, PA 19460 USA

Administrative changes during the study may not require amendment to the protocol. Future changes will be recorded in the transfer of responsibility document.

Coordinating Investigators

Mainland China	Chen Min Hua, M.D. Beijing University Cancer Hospital No. 52 FuCheng Road Haidian District Beijing, 100142, People's Republic of China
Asia/ Pacific	Ronnie T. Poon, M.D. The University of Hong Kong, Dept. of Surgery 3/F Block L Queen Mary Hospital 102 Pokfulam Road Hong Kong, People's Republic of China
Europe	Riccardo Lencioni, M.D. Ospedale Cisanello, University of Pisa Via Paradisa, 2 Pisa 56124, Italy
Responsible Investigators and Laboratories	FDA Form 1572 will capture list of Responsible Investigator's and Laboratories utilized for the trial.

RATIONAL FOR AMENDMENT CHANGES

Protocol Version 1.0 13-January-2014 to Version 2.0, 14-March-2014

Overview of Changes

The protocol amendment is issued to address regulatory comments received on 4-Mar-2014 from U.S. FDA. The overview of the changes include

1. Minor edits to the inclusion criteria to further characterize the RFA procedure duration ≥ 45 minutes and to the exclusion criteria increasing baseline platelet level counts to 75,000/mm^3
2. Standardize follow up visits to evaluate safety and PFS at consistent intervals.
3. Provide further guidance for consistency in reporting investigator determined PFS definitions in Section 12.0.
4. Administrative changes made throughout the protocol may not be detailed in 20.10 of changes

A table listing the changes in each protocol section is detailed in Appendix X of the protocol.

STATEMENT OF COMPLIANCE

The procedures set out in this study protocol are designed to ensure that Celsion and Investigator abide by the principles of the Guideline for Good Clinical Practice (GCP) of the International Conference on Harmonisation (ICH) and the Declaration of Helsinki in the conduct, evaluation and documentation of this study, required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312).
- EU Directive 2001/20EC of April 2001, in those countries where the directive is fully implemented

Before the start of the study, the study protocol, written informed consent form(s) and/or other appropriate documents will be submitted to the Independent Ethics Committee and/or the authorities, in accordance with local legal requirements.

In addition, any advertising materials must be approved by Celsion Clinical Affairs and an Independent Ethics Committee before use. The study will be conducted in accordance with US FDA, applicable national and local health authorities and the IEC requirements.

All study personnel are required to have proper training on study procedures and GCP guidelines on protection of subject interests, health and confidentiality.

SIGNATURE PAGE FOR SPONSOR

IND No. **66,827**

Study No. **104-13-302**

Title: A Phase III, Randomized, Double Blind, Dummy-Controlled Study of ThermoDox® (Lyso-Thermosensitive Liposomal Doxorubicin-LTLD) in Hepatocellular Carcinoma (HCC) using standardized Radiofrequency Ablation (RFA) treatment time ≥ 45 minutes for solitary lesions ≥ 3 cm to ≤ 7 cm.

This study protocol was reviewed internally and with Medical consultants and has been approved by the appropriate study protocol review committee. The information it contains is consistent with:

1. Investigator's Brochure with details of pharmacological and toxicological findings with the Investigational Product, and

2. The moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and Good Clinical Practice guidelines.

The Investigator will be supplied with details of any significant or new findings, including adverse events.

Approved by the following:

[redacted]
Senior Vice President and Chief Medical Officer
Celsion Corporation
997 Lenox Drive, Suite 100
Lawrenceville, NJ 08648 USA

_____ _____
Signature Date

SIGNATURE PAGE FOR INVESTIGATOR

IND No. **66,827**

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Title: A Phase III, Randomized, Double Blind, Dummy-Controlled Study of ThermoDox® (Lyso-Thermosensitive Liposomal Doxorubicin-LTLD) in Hepatocellular Carcinoma (HCC) using standardized Radiofrequency Ablation (RFA) treatment time ≥ 45 minutes for solitary lesions ≥ 3 cm to ≤ 7 cm.

I have carefully read this protocol and agree that it contains all the necessary information required to conduct the study. I agree to conduct this study as outlined in the protocol.

1. I understand that this study will not be initiated without approval of the appropriate Institutional Review Board/Research Ethics Board/Independent Ethics Committee (IRB/REB/IEC), and that all administrative requirements of the governing body of the Institution will be complied with fully.
2. Informed written consent will be obtained from all participating subjects in accordance with institutional guidelines, FDA requirements as specified in Title 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, the ICH Guideline for Good Clinical Practice, and the terms of the Declaration of Helsinki.
3. I will enroll subjects who meet the protocol criteria for entry.
4. I understand that I am responsible for medical supervision during the conduct of this protocol.
5. I understand that my signature on each completed Case Report Form indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from the Sponsor unless this requirement is superseded by the Food and Drug Administration, a Competent Authority of the European Union, China Food and Drug Administration or another Regulatory Authority.

Investigator Name	Signature	Date

Investigator Position	Investigator Address

Table of Contents

1. PROTOCOL SYNOPSIS

Title of Study	A Phase III, Randomized, Double Blind, Dummy-Controlled Study of ThermoDox® (Lyso-Thermosensitive Liposomal Doxorubicin-LTLD) in Hepatocellular Carcinoma (HCC) using standardized Radiofrequency Ablation (RFA) treatment time ≥ 45 minutes for solitary lesions ≥ 3 cm to ≤ 7 cm.
Protocol Number	104-13-302
Countries	Global
Study Centers	Up to 100 Centers
Study Period	Approximately 36-month enrollment with estimated 5-year follow up
Clinical Phase	Phase III
Objectives	The primary objective is to compare overall survival (OS) between patients receiving RFA plus ThermoDox versus RFA alone, using a standardized Radiofrequency Ablation (sRFA) treatment dwell time ≥ 45 minutes. The secondary objectives are to compare progression-free survival (PFS) and safety between patients receiving sRFA plus ThermoDox versus sRFA alone, using a standardized treatment dwell time ≥ 45 minutes.
Study Design	This is a randomized, double blind, dummy controlled multicenter trial to evaluate the efficacy and safety of ThermoDox plus sRFA compared to sRFA alone using standardized treatment dwell time ≥ 45 min. for solitary HCC lesions ≥ 3.0 cm to ≤7.0 cm. An sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. Eligible HCC patients will have a solitary lesion not amenable to curative resection consistent with clinical diagnosis of AASLD classification guidelines and will be candidates for RFA. Subjects will be randomly assigned to receive either standardized sRFA plus ThermoDox at 50 mg/m^2 or standardized sRFA plus a dummy infusion using a standardized RFA dwell time ≥ 45 minutes. Randomization and analysis will be stratified by maximum lesion diameter (3-5 cm versus > 5-7 cm) and RFA route (laparoscopic, open surgical, percutaneous). <u>Screening Period</u> Subjects will be evaluated up to 21 days prior to the RFA procedure date to establish eligibility for study treatment. Subjects must meet all the study inclusion/exclusion criteria prior to randomization to a treatment arm.

	Treatment Period Subjects who meet the eligibility criteria will be randomly assigned to either sRFA plus ThermoDox or sRFA plus dummy infusion using a web-based randomization system. The RFA procedure day will be Day 0 and subjects will return to the clinic Day 14 and Day 28 (+/- 3 days). Subjects with a complete ablation by imaging will continue in the follow up period described below. A subject who has an incomplete ablation is eligible for 1 retreatment procedure within 21 days after radiological imaging exam showing residual disease at Day 28. Subjects will be retreated only once with the same RFA equipment and treatment assigned at randomization. Baseline safety evaluations must meet the eligibility parameters prior to a retreatment. Subjects with a complete ablation after retreatment will be followed for both overall survival (OS) and progression-free survival (PFS). If after 2 ablations the subject has local, distant intrahepatic, or extrahepatic HCC, then the subject will be considered a treatment failure and will have met the PFS endpoint. The subject is still followed for OS every 3 months after progression. Among subjects who are not treatment failures, up to five repeat treatments are permitted to treat a recurrent lesion or to treat newly identified local or distant intrahepatic lesions at the Investigator's discretion after the PFS endpoint is reported and with agreement from the Sponsor. The subject must be eligible for retreatment consistent with the safety eligibility criteria and will be retreated with the same randomized treatment. Follow up visits are performed Day 14 and Day 28 (+/- 3 days) following the treatment. Subjects with bilirubin levels > 2.0 mg/dL and ≤ 3.0 mg/dL will return to the clinic on Day 7 for additional safety assessments. Exclusion concomitant medications are to be restricted through Day 28 following study treatment. Follow-Up Period Following study treatment, subjects will undergo CT or MRI imaging scans (chest, abdomen, and pelvis) at months 1, 5, 9, 13, 17, 21, and 25 (+/- 2 weeks), then at 6-month intervals (+/- 2 weeks) until radiological progression is seen. The same imaging modality and measurement of assessment should be used to characterize disease at baseline and during follow up for an individual patient. Investigator determined radiological progression must be observed and recorded prior to beginning alternate treatments for HCC. Post-progression treatments will be reported and the subject will continue to be followed for OS. To provide surveillance for any late hematologic, cardiac, or liver toxicity, the following additional safety assessments will be done: • Physical examination, assessment of vital signs, ECGs, CBC with differential, serum chemistry, PT/INR and urinalysis at Month 5, Month 9, and Month 13 (+/- 2 weeks) or at disease progression, whichever occurs first. • LVEF monitoring at Month 13 or at disease progression, whichever occurs first. • Serum chemistry and PT/INR assessments at Month 17, Month 21, and Month 25 (+/- 2 weeks) or at disease progression, whichever occurs first.

	The site will contact all subjects every 3 months after radiological progression to document vital status until the subject expires or withdraws consent from the study. The follow-up contacts across all clinical sites may be coordinated using the same 3-month interval.
Inclusion Criteria	Subjects must meet <u>all</u> of the following criteria to be eligible for the study: 1. Male or female ≥ 18 years of age. 2. Diagnosed with a single HCC lesion ≥ 3.0 cm but ≤ 7.0 cm in maximum diameter based on diagnosis at screening. • Subjects meeting the American Association for the Study of Liver Disease (AASLD) criteria may be randomized without a biopsy, but will undergo a biopsy during the RFA procedure unless contraindicated or unattainable. • Subjects <u>not</u> meeting the AASLD criteria for HCC will need a biopsy to confirm HCC prior to randomization. 3. Be an appropriate candidate for receiving RFA as a medically indicated treatment as evaluated by the following factors: • The position and accessibility of the target lesion allows for the safe administration of multiple ablation cycles or deployments to achieve a probe dwell time of ≥ 45 minutes. • Not a candidate for surgical resection according to the local guidelines for resection and in the Investigator's judgment. 4. Child-Pugh Class A without either current encephalopathy or ascites. 5. Left Ventricular Ejection Fraction (LVEF) ≥ 50%. 6. ECOG performance status 0. 7. Willing to sign an informed consent form, indicating awareness of the investigational nature of this study that is in keeping with the policies of the institution.
Exclusion Criteria	Subjects meeting <u>any</u> of the following criteria will be excluded from the study: 1. Is scheduled for liver transplantation 2. Expected ablation volume > 30% of total liver volume or removal of 3 hepatic segments 3. More than 1 lesion identified during baseline. 4. Have previously received therapeutic treatment for HCC outside the study protocol or is expected to receive concomitant HCC treatment prior to PFS event. 5. Have serious medical illnesses including, but not limited to, congestive heart failure, myocardial infarction or cerebral vascular accident within the last six months, or life threatening cardiac arrhythmias. 6. Have previously received any anthracycline outside the protocol 7. Have extrahepatic metastasis. 8. Have portal or hepatic vein tumor invasion/thrombosis. 9. Have body temperature >101ºF (38.3ºC) immediately prior to study treatment. 10. Baseline laboratories (repeat lab tests are permitted to evaluate eligibility during the Screening Period. Lab results must be within protocol range prior to study treatment.) • Absolute neutrophil count < 1500/mm^3

	• Platelet count < 75,000/mm^3 • Hgb < 10.0 g/dL (unless the hemoglobin value has been stable, the subject is cardiovascularly stable, asymptomatic and judged able to withstand the RFA procedure) Note: If clinically indicated, subjects may receive platelets or packed RBC transfusions and be re-evaluated after condition is treated. 11. Baseline Chemistry • Serum creatinine ≥ 2.5 mg/dL or calculated creatinine clearance (CrCl) ≤25.0 mL/min. • Serum bilirubin > 3.0 mg/dL. • Serum albumin < 2.8 g/dL. 12. Have any known allergic reactions to any of the drugs or liposomal components or intravenous imaging agents that prohibit the ability to complete the imaging requirements. 13. Are pregnant or breast-feeding. In women of childbearing potential, a negative serum pregnancy test is required prior to study treatment. 14. Women of childbearing potential and men who are not practicing an acceptable form of birth control (i.e. diaphragm, cervical cap, condom, surgical sterility or birth control pills. Women whose partner has or men who have undergone a vasectomy must use a second form of birth control). 15. Have INR > 1.5 times the institution's upper normal limit (UNL), except in subjects who are therapeutically anticoagulated for medical conditions unrelated to HCC such as atrial fibrillation. Subjects may be re-screened after condition is treated or anticoagulant is withheld. 16. Have contraindications to receiving doxorubicin HCl. 17. Are being treated with other investigational agents. 18. Use of an investigational drug outside this study within 30 days or 5 half-lives, whichever is longer, preceding the first dose of study medication. 19. Have other concurrent malignancy (subjects with treated squamous cell carcinoma of the skin or basal cell carcinoma of the skin may be included), evidence of extrahepatic cancer from their primary malignancy, or ongoing, medically significant active infection. 20. HIV positive. 21. NYHA class III or IV functional classification for heart failure. 22. Evidence of hemachromatosis.
Trial Drug, Dose and Administration	Oral Premedication: As part of a blinded premedication regimen, subjects will be assigned to take 20 mg of oral dexamethasone (ThermoDox arm) or matching dummy capsule (Control arm) 24 hours (+/- 2 hours) prior to the scheduled RFA procedure. Intravenous Premedication: IV premedication regimen of steroids H1 and H2 antihistamines (ThermoDox arm) according to local formularies or dummy IV premedication of Sodium Chloride 0.9% or D5W (Control arm) will be administered in a blinded fashion. IV medications should be given within 30 minutes (+15 min window) prior to infusion of study drug.

	Study Drug Infusion: A 50 mg/m^2 ThermoDox infusion (ThermoDox arm) or dummy infusion of Dextrose 5% Solution or Sodium Chloride 0.9% (Control arm) will be administered IV over 30 minutes. The Sponsor will provide blinded masking materials to maintain the blind and unblinded site representative(s) (i.e. pharmacist) will prepare the materials to cover IV bags and tubing. Radiofrequency Ablation: Only FDA approved RFA devices are permitted for this trial. RFA requiring a standardized dwell time ≥ 45 minutes will be initiated 15 minutes (+5 min.) after the start of the study drug infusion with an estimated overall procedure time of less than 3 hours. A standardized Radiofrequency Ablation sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment.. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. An ablation cycle for this protocol is defined as the single activation of the probe or electrode in order to achieve a local coagulation necrosis in target tissue. The cycle may be completed by achieving a target temperature in the tissue or a target impedance. The goal is to ablate the tumor as well as a 360º 1.0 cm tumor-free margin surrounding the tumor. Tumor uptake of ThermoDox is enhanced with a standardized treatment dwell time of at least 45 minutes (30 minutes with a switchbox). The literature recommends tumors ≥ 3 cm should require at least four ablation spheres (if using simple needle electrode) or at least four deployments (if using multiple array LeVeen needle electrode). More ablations may be performed at the discretion of the operator, however the RFA probe dwell time must reach 45 minutes. Pringle maneuver or balloon occlusions are prohibited.
Sample Size:	The study is designed to detect with 80% power a hazard ratio for OS of 0.67 (33% risk reduction) in the ThermoDox (TR) arm compared with the control (PR) arm with an overall 1-sided type 1 error of 0.025. An OS hazard ratio of 0.63 was observed in an earlier trial among HCC patients with a solitary 3-7 cm lesion treated with ≥ 45 minutes of RFA treatment dwell time. A 3%/year loss to survival follow-up rate has been assumed and using a 1:1 treatment allocation (TR:PR) of 550 subjects, a target of 197 events (deaths) will be required for the primary analysis. Two interim analyses, both for efficacy and futility, are planned for the study. The first is planned after 60% of the target events is reached (118 deaths) and the second after 80% of the events has been reached (158 deaths).
Criteria for Evaluation	Efficacy Evaluations: Overall survival is defined as the time from the date of randomization to the date of death. If death is not observed during the study, survival time will be censored at the date of last study follow-up, or the cut-off date, whichever is earlier.

	Progression-free survival is defined as the time from the date of randomization until the date of Investigator-assessed radiological disease progression or death due to any cause. Subjects who are alive with no disease progression as of the analysis cut-off date will be censored at the date of the last tumor assessment. Safety All subjects will have adverse event (AE) assessments as well as other safety assessments such as physical examinations, vital signs, ECGs, echocardiograms/MUGA scans, hematology, clinical chemistry, and urinalysis beginning at time of signing of informed consent and continuing through Day 28 following study drug administration. To provide surveillance for any late hematologic, cardiac, or liver toxicity, the following additional safety assessments will be done: • Physical examination, assessment of vital signs, ECGs, CBC with differential, serum chemistry, PT/INR and urinalysis at Month 5, Month 9, and Month 13 (+/- 2 weeks) or at disease progression, whichever occurs first. • LVEF monitoring at Month 13 or at disease progression, whichever occurs first • Serum chemistry and PT/INR assessments at Month 17, Month 21, and Month 25 (+/- 2 weeks) or at disease progression, whichever occurs first. Subjects with elevated bilirubin levels > 2.0 mg/dL and ≤ 3.0 mg/dL at baseline will return to the clinic on Day 7 for additional safety assessments. Adverse event (AE) assessments will begin at time of signing informed consent through Day 28 visit after the last study treatment. AEs which are assessed as possibly, probably, or definitely related to study treatment will be recorded at any point during the trial and must be followed until resolution or until the patient is clinically stable.
Data Monitoring Committee	An independent Data Monitoring Committee (DMC) will be established by the Sponsor to review accumulating safety data at regular intervals throughout the study and to monitor trial data integrity. The DMC is responsible for unblinded monitoring of subjects with elevated (> 2.0 mg/dL and ≤ 3.0 mg/dL) serum bilirubin levels and will advise if any dose or protocol modification is needed to mitigate risk to such patients. The DMC will also evaluate the results of preplanned interim efficacy and futility analyses for OS. The DMC's specific duties as well as statistical monitoring guidelines and procedures will be fully described in the DMC Charter.
Statistical Methods	OS in the Intent to Treat population will be compared between the two treatment groups using the stratified log-rank test. The estimate of the hazard ratio and corresponding 95% CI will be provided using a Cox proportional hazards (CPH) model including treatment and the stratification factors (maximum lesion diameter [3-5 cm versus > 5-7 cm] and RFA route [laparoscopic, open surgical, percutaneous[) in the model. The survival curves will be estimated using Kaplan-Meier estimates. PFS will be analyzed using the same methodology.

Sponsor Contact	Celsion Corporation 997 Lenox Drive, Suite 100 Lawrenceville, NJ 08648, USA Tel: [redacted] Fax: [redacted]

2. LIST OF ABBREVIATIONS

Abbreviation	Definition
AASLD	American Association for the Study of Liver Disease
ACS	Almac Clinical Services
AE	Adverse Event
AFP	Alpha-Fetoprotein
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CI	Confidence Interval
CIN	Contrast-Induced Nephropathy
CR	Complete Response
CrCl	Creatinine Clearance
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CYP3A4	Cytochrome P450 3A4
D5W	5% Dextrose Solution
DHR	Distant Hepatic Recurrence
DLT	Dose-limiting toxicity
DMC	Data Monitoring Committee
DSPE-MPEG	N-(methoxypolyethyleneglycolcarbomyl) distearoylphosphatidyl-ethanolamine
EC	Ethics Committee
EF	Ejection Fraction
ECG	Electrocardiogram
EHR	Extrahepatic Recurrence
FDA	Food and Drug Administration
GCPs	Good Clinical Practices
GCSF	Granulocyte Colony-Stimulating Factor
GGT	Gamma Glutamyl Transpeptidase
GI	Gastrointestinal
HCC	Hepatocellular Carcinoma
Hct	Hematocrit

Abbreviation	Definition
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HT	Hyperthermia
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	International Normalized Ratio
IR	Incomplete Response
IRB	Institutional Review Board
IRC	Independent Radiology Charter
IRRC	Independent Radiology Review Committee
IV	Intravenous
LDH	Lactate Dehydrogenase
LR	Local Recurrence
LTLD	Lyso-Thermosensitive Liposomal Doxorubicin; ThermoDox®
LTSL	Lyso-Thermosensitive Liposome
LTFS	Liver Transplant-Free Survival
LVEF	Left Ventricular Ejection Fraction
MLC	Metastatic Liver Cancer
MR	Minor Response
MSPC	1-steroyl-2-hydroxy-SN-glycero-3-phosphoaholine
MTD	Maximum Tolerated Dose
MUGA	Multiple gated acquisition
NCR	No Carbon Required
NYHA	New York Heart Association
OS	Overall Survival
PD	Pharmacodynamics
PFS	Progression-Free Survival
PEI	Percutaneous Ethanol Injection
PO	Orally
PR	Partial Response
PRO	Patient-Reported Outcomes
PT/PTT	Prothrombin Time/Partial Thromboplastin Time
RBC	Red Blood Cells
RCW	Recurrent Chest Wall
RF	Radiofrequency
RFA	Radiofrequency Ablation
SAE	Serious Adverse Event

Abbreviation	Definition
SC	Steering Committee
SD	Stable Disease
sRFA	Standardized Radiofrequency Ablation with treatment dwell time ≥45 min.
CFDA	China Food and Drug Administration
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamate pyruvate transaminase
TACE	Transarterial Chemoembolization
TTLR	Time to Local Recurrence
TTP	Time to Progression
UA	Urinalysis
UNL	Upper Normal Limit
WBC	White Blood Cells

3. INTRODUCTION

3.1 Overview

Hepatocellular carcinoma is the sixth most common neoplasm worldwide, but its very poor prognosis makes it the third leading cause of cancer-related mortality, responsible for about 600,000 deaths annually (Parkin, et al. 2005). In the US, 18,000 new cases of HCC are diagnosed each year and the incidence is steadily increasing, almost doubling since 1998. HCC is commonly diagnosed in patients with longstanding hepatic disease and cirrhosis (primarily due to hepatitis C in US and Europe and to hepatitis B in Asia). Mortality and hospitalization due to HCC, as well as hospital-related costs (inflation adjusted), increased approximately two-fold from 1988 to 2000 (Kim et al. 2005, Thomas and Abbruzzese 2005). The incidence of extrahepatic metastases in patients with HCC is generally around 15%, which includes such metastases both at the initial diagnosis of HCC or during follow-up regardless of treatment (Uka, et al. 2007; Yang, et al. 2007).

The Barcelona Clinic Liver Cancer (BCLC) staging and treatment system is widely accepted in clinical practice and is also being used in many clinical trials of new drugs to treat HCC. Therefore, it has become the *de facto* staging system that guides HCC treatment. The BCLC system recognizes three treatments as curative for patients with up to 3 HCC lesions, each < 3.0 cm, who have WHO performance status 0 and no more than Child-Pugh class B liver impairment. The three curative therapies are surgical resection, liver transplantation, and radiofrequency ablation (RFA). Patients with HCC lesions ≥ 3 cm are considered incurable. Transarterial chemoembolization (TACE) is recommended as a palliative treatment for patients with WHO performance status 0 and sorafenib is recommended as a palliative treatment for patients with WHO performance status 1-2 (Bruix and Sherman, 2011).

Surgical resection is the mainstay of curative treatment. However, no more than 30% of HCC patients are considered suitable for surgical treatment because of tumor size, multifocal tumors, vascular invasion, presence of extrahepatic metastases, and/or extensive liver impairment. Liver transplantation is an alternative curative treatment, but its application is limited by a severe shortage of liver graft donors (Llovet, et al. 2008). Thermal ablation modalities such as radiofrequency ablation (RFA), microwave ablation, and high-intensity focused ultrasound have emerged as important treatment options for such patients in recent years.

To sum up, the worldwide HCC population is large and growing. The vast majority, including all those with tumors ≥ 3 cm, are incurable. These patients have an unmet need for more effective therapies.

3.2 Radiofrequency Ablation (RFA)

RFA can be applied repeatedly to the same patient to treat multiple tumors at time of diagnosis or upon tumor recurrence. There are presently three RFA systems approved by the FDA and currently marketed in the U.S for ablation of soft tissue: Angiodynamics, Boston Scientific, and Covidien. Please refer to Appendix IV for equipment specifications.

In general, RFA systems are comprised of a generator and a family of application-specific electrodes containing deployable arrays, saline infusion, or water-cooled needles, all of which enable predictable volumes of tissue to be ablated. Ablation is usually guided by computerized tomography (CT), magnetic resonance (MR), or ultrasound. Temperature, impedance (the ratio of voltage to electric current), and time may be monitored during the treatment to allow for accurate control of the energy delivered to the lesion and thus the amount of tissue subjected to thermal injury. Both complete and partial responses have been observed utilizing RFA and results have been comparable to those obtained with cryotherapy (Siperstein, et al. 1997; Rossi, et al. 1995; Buscarini, et al. 1995). A recent study in China (Chen, et al. 2006) compared RFA and surgery and found no difference in mortality between the two treatments. This study suggests that there are clinicians who consider RFA to be a viable alternative to surgery. Randomized trials have found that RFA has overall survival similar to surgical resection for solitary tumors < 5 cm (Chen, et al. 2006) and better overall survival than percutaneous ethanol injection (PEI) for 1-3 tumors < 3 cm (Lin, et al. 2005; Shiina, et al. 2005) and for 1-3 tumors < 4 cm (Lin, et al. 2004). The better performance of RFA versus PEI in these HCC studies may relate to RFA's greater certainty of achieving a 0.5 to 1.0 cm tumor-free margin around the tumor boundary.

Approximately 2% of patients undergoing RFA of liver tumors experience major complications due to the procedure including intraperitoneal hemorrhage, intrahepatic abscesses, gastrointestinal wall perforations, and hemothorax (Berber, et al. 2005a and 2005b). More common minor complications, typically reported in 5% or less of patients, can include altered liver function tests, self-limited bleeding, effusions, pain, fever, infection, and skin burn (Berber, et al. 2007).

RFA has been used safely in tumors up to 7 cm (Chen, et al. 2004; Curley, et al. 2004; Dodd, et al. 2001; Tateishi, et al. 2005). In the United States, palliative treatments such as transcatheter arterial chemoembolization (TACE) and sorafenib are the standard treatment options for HCC tumors > 5 cm. However, in this study the combination of RFA and ThermoDox is being investigated as a curative treatment for patients with tumors > 5 cm to $\leq$ 7 cm rather than for their palliation.

For HCC tumors $\geq$ 3 cm, overlapping ablations are required, and this overlapping can miss some micrometastases (Chen, et al. 2004; Curley, 2001; Dodd, et al. 2001; Chen, et al. 2006). If the efficacy of RFA for HCC tumors $\geq$ 3.0 cm could be increased, as by an adjuvant, it would be a formidable curative modality.

In this study, RFA treatments will be administered according to the RFA Treatment Procedure and device instructions (Appendix IV). Investigators at each site that meet the experience qualifications to perform RFA in this study will complete formal training with an accompanying RFA training manual developed by the Sponsor. All subjects will undergo an RFA treatment dwell time lasting $\geq$ 45 minutes.

3.3 ThermoDox

Lyso-Thermosensitive Liposomal Doxorubicin (ThermoDox) is designed for use with RFA and consists of the heat-enhanced cytotoxic anthracycline antibiotic doxorubicin within a heat-activated liposome. At normal body temperatures (i.e., 37°C), the product is designed to retain doxorubicin within the liposome as it travels around the body. However, with mild heating, (i.e., $\geq$ 40.0°C), the liposome component of ThermoDox very quickly releases its active doxorubicin ingredient, which rapidly diffuses into the local tissue.

ThermoDox is administered intravenously and, because it is a liposome, exhibits prolonged systemic circulation relative to free (non-liposomal) doxorubicin, with a small portion being taken up by mononuclear phagocytic system (MPS) cells in the liver. During RFA/ThermoDox therapy, cytocidal heat is directed to a tumor. When heat-sensitive liposomes encounter a temperature of 40.0°C or above, their doxorubicin is released into the heated area. The released doxorubicin remains stable up to 90°C (Wood, et al. 2012). So, the antitumor activity of ThermoDox/doxorubicin requires mild ($\geq$ 40.0°C) hyperthermia and tissue perfusion to deliver a high concentration of doxorubicin to the tumor margins.

The systemic delivery of ThermoDox in conjunction with local hyperthermia creates a high concentration of doxorubicin in the immediate region of the tumor, through the release of doxorubicin from ThermoDox liposomes at the site. The temperature isotherms produced in this boundary region during RFA should be adequate to activate the release of encapsulated doxorubicin deposited locally around the ablation zone and in the tumor vasculature at the time of heating. This process extends the region of tissue that can be treated beyond that achievable by use of RFA alone.

Gasselhuber, et al. (2012) published a computational model of doxorubicin concentration in liver tumor tissue following combination therapy with ThermoDox and mild hyperthermia, (target temperature 43°C). Their model found a direct correlation between duration of hyperthermia and doxorubicin tumor tissue concentration. This finding suggests the hypothesis that, when used with ThermoDox, length of heating time can be used to ensure delivery of a doxorubicin dose needed to provide a therapeutic effect in tumor tissue. Although this study utilized mild hyperthermia, the principle of heat time translating into doxorubicin dose is readily applicable to RFA procedures.

A 2013 study in healthy pigs receiving intravenous ThermoDox and RFA of the liver is consistent with the hypothesis that doxorubicin target tissue concentration directly covaries with duration of hyperthermia (Swenson, 2014). Furthermore, fluorescence imaging found high doxorubicin concentrations in the margin of the ablation zone and in areas where ablations overlapped, supporting the hypothesis that ThermoDox addresses any microscopic tumors left behind in the clefts of overlapping ablations.

Haemmerich and Rossmann (2013) conducted a ThermoDox + RFA modeling study. They found that the amount of bioavailable doxorubicin in the tumor margin increases as the actual heating time increases. They also found that beginning RFA 15 minutes after starting a 30-minute ThermoDox infusion maximizes the amount of bioavailable doxorubicin in the tumor margin compared to beginning RFA later.

In Celsion's initial phase III trial, the HEAT Study (Protocol 104-06-301), the criterion for RFA adequacy was that it ablate each target lesion plus a 360° 1-cm tumor margin; there was no attempt to manage RFA so as to also increase doxorubicin tumor tissue concentration. The results of the HEAT Study became available in 2013. The trial achieved the expected level of safety. The overall safety profile was consistent with literature reports for liposomal doxorubicin and free doxorubicin commercial labels. Other than reversible neutropenia and leukopenia, both treatment-related adverse events ≥ grade 3 and serious adverse events were rare, affecting < 5% of patients in either arm. However, the primary endpoint of progression free survival (PFS) was not met. Patients are still being followed for overall survival (OS).

Post hoc analysis of the PFS and OS data from the HEAT Study suggests that ThermoDox efficacy for the treatment of single lesions is greatly improved when RFA dwell time is ≥ 45 minutes. An sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. Dwell time is relevant for ThermoDox activity because the target tissue and surrounding tissue remain at ≥ 40.0°C, even between ablation cycles. This minimum effective dose (MED) of RFA dwell time is achieved by administering 4 ablation cycles. In this trial, all patients will receive the RFA MED of ≥ 45 minutes dwell time; in addition, half will be randomized to the ThermoDox MTD of 50 mg/m^2 and half will be randomized to a dummy infusion of D5W or saline. Randomization and analysis will be stratified by lesion diameter (3-5 cm versus > 5-7 cm) and RFA route (laparoscopic, open surgical, percutaneous) Lesion diameter was found to be a statistically significant prognostic factor in the prior study. Although nearly all subjects in the prior trial (90.7%; 636/701) received percutaneous RFA, this study will also stratify by RFA route for the sake of consistency with the earlier trial's design. Unlike the prior trial, Child Pugh B patients will not be enrolled. They are much more likely than Child Pugh A patients to die from their underlying liver disease, confounding the effect of anticancer treatment (Llovet, et al. 2008). In the HEAT Study, deaths prior to HCC progression occurred at a rate almost five times higher in Child Pugh B patients (19.5%; 8/41) than in Child Pugh A patients (4.1%; 27/660).

4. OBJECTIVES

4.1 Primary Objective

The primary objective is to compare overall survival (OS) between patients receiving RFA plus ThermoDox versus RFA alone, using a standardized Radiofrequency Ablation (sRFA) treatment dwell time ≥ 45 minutes.

4.1.1 Measurement of the Primary Objective

Overall survival will be measured by time from randomization to death from any cause or the end of the study. OS will be compared between the treatment arms.

4.2 Secondary Objectives

The secondary objectives are to compare progression-free survival (PFS) and safety between patients receiving sRFA plus ThermoDox versus sRFA alone, using a standardized RFA treatment dwell time ≥ 45 minutes.

4.2.1 Measurement of Secondary Objectives

PFS will be measured from the date of randomization to the first date on which one of the following occurs, as determined by CT or MRI scan:

- Death of any cause
- Treatment failure (inability to achieve CR after two RFA ± ThermoDox treatment sessions)
- Progression due to local tumor recurrence after initial CR
- Progression due to distant intrahepatic tumor recurrence
- Progression due to extrahepatic tumor recurrence

Adverse events (AEs) assessment will begin at time of signing informed consent through Day 28 visit following the last study treatment. Adverse events which are assessed as possibly, probably, or definitely related to study treatment will be recorded at any point during the trial and must be followed until resolution or the patient is clinically stable.

Other safety data including physical examinations, vital signs, ECGs, echocardiograms/MUGA scans, hematology, clinical chemistry, and urinalysis will be assessed through Day 28 following study treatment..

To provide surveillance for any late hematologic, cardiac, or liver toxicity, the following additional safety assessments will be done:

- Physical examination, assessment of vital signs, ECGs, CBC with differential, serum chemistry, PT/INR and urinalysis at Month 5, Month 9, and Month 13 (+/- 2 weeks) or at disease progression, whichever occurs first.
- LVEF monitoring at Month 13 or at disease progression, whichever occurs first
- Serum chemistry and PT/INR assessments at Month 17, Month 21, and Month 25 (+/- 2 weeks) or at disease progression, whichever occurs first.

Subjects with elevated bilirubin levels > 2.0 mg/dL and ≤ 3.0 mg/dL at baseline will return to the clinic on Day 7 for additional safety assessments.

5. STUDY PLAN

5.1 Design Overview

This is a randomized, double blind, dummy controlled multicenter trial to evaluate the safety and efficacy of ThermoDox plus sRFA compared to sRFA alone using standardized treatment dwell time for solitary HCC lesions ≥ 3.0 cm to ≤ 7.0 cm. An sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient.

Eligible HCC patients will have a solitary lesion not amenable to curative resection consistent with clinical diagnosis by AASLD classification guidelines and will be candidates for RFA. Subjects will be randomly assigned to receive sRFA plus 50 mg/m^2 of ThermoDox or sRFA plus a dummy infusion using a standardized RFA dwell time ≥ 45 minutes. Randomization and analysis will be stratified by maximum lesion diameter (≥ 3.0 to 5.0 cm versus > 5.0 to 7.0 cm) and RFA route (laparoscopic, open surgical, percutaneous).

Screening Period
Subjects will be evaluated up to 21 days prior to the RFA procedure date to establish eligibility for the study treatment. Subjects must meet all of the study inclusion/exclusion criteria prior to randomization to a treatment arm.

Treatment Period
Subjects will be randomly assigned to either sRFA plus ThermoDox or sRFA plus dummy infusion using a central web-based randomization system. The RFA procedure day will be Day 0 and subjects will return to the clinic Day 14 and Day 28 (+/- 3 days). Subjects with a complete ablation by imaging will continue in the follow up period described below.

As part of a blinded premedication regimen, subjects will be assigned to take 20 mg of oral dexamethasone (ThermoDox arm) or matching dummy capsule (control arm) 24 hours (+/- 2 hours) prior to the scheduled sRFA procedure.

The IV premedication should be administered within 30 minutes (+15 min.) prior to the start of the ThermoDox infusion. The following may serve as a guide:

- Dexamethasone 20 mg IV or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)
- Diphenhydramine 50 mg IV (chlorpheniramine 10 mg IV is permitted) or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)
- Ranitidine 50 mg IV or famotidine 20 mg IV or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)

Study drug will be administered IV over 30 minutes in a blinded fashion.

- Treatment arm subjects will receive a 50 mg/m^2 ThermoDox infusion.

- Control arm subjects will receive a dummy infusion of D5W (250 cc of 5% Dextrose solution) or Saline (250 cc of 0.9% Sodium Chloride).

The Sponsor will provide blinded masking materials to maintain the blind and unblinded site representative(s) (i.e., pharmacist) will prepare the materials to cover IV bags and tubing.

Only US FDA approved RFA devices are permitted for this trial. RFA requiring a standardized dwell time ≥ 45 minutes will be initiated at minute 15 (+5 minutes) following the start of the study drug infusion with an estimated overall procedure time of less than 3 hours. An sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. An ablation cycle for this protocol is defined as the single activation of the probe or electrode in order to achieve a local coagulation necrosis in target tissue. The cycle may be completed by achieving a target temperature in the tissue or a target impedance.

The goal is to reach a > 45 minute dwell time which can be achieved by employing at least four ablation cycles or deployments in order to ablate the tumor as well as a 360º 1.0 cm tumor-free margin surrounding the tumor. Tumor uptake of ThermoDox is enhanced with standardized treatment times of at least 45 minutes (30 minutes with a switchbox). The RFA literature recommends a minimum of 4 ablation cycles to ablate tumors of ≥ 3.0 cm together with a 360° 1.0-cm margin (Chen, et al. 2004).

According to literature, tumors ≥ 3 cm should require at least four ablation spheres (if using simple needle electrode) or at least four deployments (if using multiple array LeVeen needle electrode). More or fewer ablations may be performed at the discretion of the operator, however the RFA probe dwell time must reach 45 minutes.

The sRFA procedure day will be recorded as Day 0 and subjects will return to the clinic Day 14 and Day 28 (+/- 3 days). Subjects with baseline bilirubin levels > 2.0 mg/dL and ≤ 3.0 mg/dL will return to the clinic on Day 7 for additional safety assessments. Subjects with a complete ablation by imaging will continue in the follow-Up period described below.

Safety data including physical examinations, vital signs, ECGs, echocardiograms/MUGA scans, hematology, clinical chemistry, and urinalysis will be assessed through Day 28 following study treatment. Adverse events (AEs) will be reported and exclusion concomitant medications are restricted through Day 28 following the last study treatment. Adverse events which are assessed as possibly, probably, or definitely related to study treatment will be recorded at any point during the trial and must be followed until resolution or the patient is clinically stable.

Subjects with a complete ablation by imaging at Day 28 will continue in the follow-up period and be followed for PFS and OS.

A subject who has an incomplete ablation is eligible for 1 retreatment procedure within 21 days after the radiological imaging exam showing residual disease at Day 28. Subjects will be retreated only once with the same RFA equipment and treatment assigned at randomization. Baseline safety evaluations must meet the eligibility parameters prior to a retreatment. Repeat screening data will be recorded into the Case Report Form. The second ablation must occur no later than 21 days after the first post-ablation scan assessment at Day 28. Subjects with a complete ablation after retreatment will be followed both for PFS and for OS. If after 2 ablations the subject has local, distant intrahepatic, or extrahepatic HCC, then the subject will be considered a treatment failure and will have met the PFS endpoint. The subject will be followed for OS every 3 months.

Among subjects who are not treatment failures, five repeat treatments are permitted to treat a recurrent lesion or to treat newly-identified local or distant intrahepatic lesions at the Investigator's discretion after the PFS endpoint is reported and with agreement from the Sponsor. The subject must be eligible for retreatment consistent with the safety eligibility criteria and will be retreated with the same randomized treatment.

Subjects who develop extrahepatic lesions will have met the PFS endpoint and are no longer eligible for further protocol treatment; they will be followed for OS.

Follow-Up Period

Following study treatment subjects will undergo CT or MRI imaging scans (chest, abdomen and pelvis) at months 1, 5, 9, 13, 17, 21, 25, then every 6 months (+/- 2 weeks) until radiological progression is seen. The same imaging equipment, technique, and measurement of assessment should be used to characterize disease at baseline and during follow up for an individual patient. Investigator determined radiological progression must be observed and recorded prior to beginning alternate treatments for HCC. Post-progression treatments will be reported and the subject will continue to be followed for OS.

To provide surveillance for any late hematologic, cardiac, or liver toxicity, the following additional safety assessments will be done:

- Physical examination, assessment of vital signs, ECGs, CBC with differential, serum chemistry, PT/INR and urinalysis at Month 5, Month 9, and Month 13 (+/- 2 weeks) or at disease progression, whichever occurs first.
- LVEF monitoring at Month 13 or at disease progression, whichever occurs first
- Serum chemistry and PT/INR assessments at Month 17, Month 21, and Month 25 (+/- 2 weeks) or at disease progression, whichever occurs first.

The site will contact all subjects every 3 months after radiological progression to document vital status until the subject expires or withdraws consent from the study. These follow-up contacts across all clinical sites may be coordinated using the same 3-month interval.

5.2 Trial Termination

For reasonable cause, either the Investigator or the Sponsor may terminate this study prematurely. Written notification of the termination is required. Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study.
- Failure of the Investigator to enter subjects at an acceptable rate.
- Insufficient adherence to protocol requirements (non-compliance).
- Lack of evaluable and/or complete data.
- Decision to modify the developmental plan of the drug.
- A decision on the part of the Sponsor to suspend or discontinue development of the drug.
- Regulatory authority decision.

6. STUDY POPULATION

6.1 Source and Number of Subjects

Approximately 550 subjects will be randomized at approximately 100 centers worldwide.

6.2 Subject Selection Criteria

6.2.1 Overview

This clinical trial can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom study participation is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Eligibility criteria may not be waived by the Investigator and are subject to review in the case of a GCP or a regulatory authority audit. Any questions regarding a subject's eligibility should be discussed with the Celsion medical monitor (see List of Study Representatives at the beginning of the protocol) prior to study treatment.

6.2.2 Inclusion Criteria

Subjects must fulfill all of the following inclusion criteria to be eligible to participate in the study.

Subjects may be randomized without a biopsy if they meet American Association for the Study of Liver Disease (AASLD) criteria for the diagnosis of HCC (see Appendix VIII). Subjects not meeting AASLD criteria for HCC will need a biopsy for confirming HCC prior to randomization.

1. Male or female ≥ 18 years of age
2. Diagnosed with a single HCC lesion ≥ 3.0 cm but ≤ 7.0 cm in maximum diameter based on diagnosis at screening.
   - Subjects meeting the AASLD criteria may be randomized without a biopsy, but will undergo a biopsy during the RFA procedure unless contraindicated or unattainable.
   - Subjects not meeting the AASLD criteria for HCC will need a biopsy to confirm HCC prior to randomization.
3. Be an appropriate candidate for receiving RFA as a medically indicated treatment as evaluated by the following factors:
   - The position and accessibility of the target lesion allows for the safe administration of multiple ablation cycles or deployments to achieve a probe dwell time of ≥ 45 minutes.
   -
   - Not a candidate for surgical resection according to the local guidelines for resection and in the Investigator's judgment.
4. Child-Pugh Class A without either current encephalopathy or ascites
5. Left ventricular ejection fraction (LVEF) ≥ 50%
6. ECOG performance status 0
7. Willing to sign an informed consent form, indicating awareness of the investigational nature of this study that is in keeping with the policies of the institution.

6.2.3 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. Is scheduled for liver transplantation.
2. Expected ablation volume > 30% of total liver volume or removal of 3 hepatic segments
3. More than 1 lesion identified during baseline.
4. Have previously received therapeutic treatment for HCC outside the study protocol or is expected to receive concomitant HCC treatment prior to PFS event.
5. Have serious medical illnesses including, but not limited to, congestive heart failure, myocardial infarction or cerebral vascular accident within the last six months, or life threatening cardiac arrhythmias.
6. Have previously received any anthracycline outside the protocol.
7. Have extrahepatic metastasis.
8. Have portal or hepatic vein tumor invasion/thrombosis.
9. Have body temperature > 101ºF (38.3ºC) immediately prior to study treatment.
10. Baseline laboratories (repeat lab tests are permitted to evaluate eligibility during the Screening Period. Lab results must be within protocol range prior to study treatment.)
    - Absolute neutrophil count < 1500/mm^3
    - Platelet count < 75,000/mm^3
    - Hgb < 10.0 g/dL (unless the hemoglobin value has been stable, the subject is cardiovascularly stable, asymptomatic and judged able to withstand the RFA procedure)

    Note: If clinically indicated, subjects may receive platelets or packed RBC transfusions and be re-evaluated after condition is treated.

11. Baseline Chemistry
    - Serum creatinine ≥ 2.5 mg/dL or calculated creatinine clearance (CrCl) ≤ 25.0 mL/min.
    - Serum bilirubin > 3.0 mg/dL.
    - Serum albumin < 2.8 g/dL.
12. Have any known allergic reactions to any of the drugs or liposomal components or intravenous imaging agents that prohibit the ability to complete the imaging requirements.
13. Are pregnant or breast-feeding. In women of childbearing potential, a negative serum pregnancy test is required prior to study treatment.
14. Women of childbearing potential and men who are not practicing an acceptable form of birth control (i.e. diaphragm, cervical cap, condom, surgical sterility or birth control pills. Women whose partner has or men who have undergone a vasectomy must use a second form of birth control).
15. Have INR > 1.5 times the institution's upper normal limit (UNL), except in subjects who are therapeutically anticoagulated for medical conditions unrelated to HCC such as atrial fibrillation. Subjects may be re-screened after condition is treated or anticoagulant is withheld.
16. Have contraindications to receiving doxorubicin HCl.
17. Are being treated with other investigational agents.
18. Use of an investigational drug outside this study within 30 days or 5 half-lives, whichever is longer, preceding the first dose of study medication.
19. Have other concurrent malignancy (subjects with treated squamous cell carcinoma of the skin or basal cell carcinoma of the skin may be included), evidence of extrahepatic cancer from their primary malignancy, or ongoing, medically significant active infection.
20. HIV positive.
21. NYHA class III or IV functional classification for heart failure.
22. Evidence of hemachromatosis.

6.2.4 Potential Risks and Benefits

Potential Risks

There are risks to subjects participating in this study. As reviewed in Sections 3.2 and 8.4, there are risks associated with the RFA procedure. There are also risks associated with the use of doxorubicin HCl. Based on the clinical experience to date, there does not appear to be a substantial unique risk for ThermoDox, but unexpected AEs could occur. The RFA procedure itself has a relatively low major complication rate. The safety profile of RFA is provided in Sections 3.2 and 8.4. Doxorubicin HCl, the active agent in ThermoDox, is routinely given in chemotherapy regimens for multiple cycles after appropriate recovery intervals, usually at least 3 weeks. In contrast, the vast majority (84.8%; 291/343) of subjects in the RFA plus ThermoDox arm in the prior trial received only a single dose of 50 mg/m^2 of ThermoDox.

The primary toxicities of doxorubicin are myelosuppression and cumulative-dose cardio-toxicity. The risk of developing impaired ventricular ejection fraction (LVEF) is generally proportional to the cumulative exposure. The probability of developing cardiomyopathy isestimated to be 1 to 2% at total cumulative dose of 300 mg/m^2 of doxorubicin HCL. (Pfizer Doxorubicin HCL Prescribing Information). The clinical safety profile of ThermoDox as observed to date is consistent with the known safety profile of doxorubicin HCl. Subjects in this study must have reasonable marrow reserve and adequate cardiac function. Subjects with prior doxorubicin exposure are excluded from this study (except in the case of completion of treatment or re-treatment). Expected non-hematological toxicities include cardio-toxicity, neuropathy, volume retention, hypersensitivity, mucositis, GI disturbances, nausea, and vomiting. Local toxicity in the liver will be carefully evaluated. Renal function will be closely monitored with urinalysis. BUN/Urea and creatinine will be monitored as nephrotoxicity has been seen in previous animal studies.

The protocol does not require dose reduction in patients with elevated bilirubin. Dose adjustment for doxorubicin-containing chemotherapy was based on data from Benjamin, et al. (1974). However, an indiscriminate dose reduction based solely on serum bilirubin concentrations may not only reduce doxorubicin myelosupression but also the therapeutic outcome (Donelli, et al. 1998; Johnson, et al. 1986). In the prior study, subjects with baseline serum bilirubin > 2.0 mg/dL had a higher incidence subsequent grade 3+ hyperbilirubinemia and of grade 3+ hepatic failure. However, the greater incidence of hepatic failure consisted of a single patient with grade 3 liver failure. Among patients with an elevated bilirubin level at baseline, there were no subsequent serious hepatobiliary adverse events except for 2 cases of hyperbilirubinemia/blood bilirubin increased. The DMC is responsible for unblinded monitoring of subjects with elevated (> 2.0 mg/dL and $\leq$ 3.0 mg/dL) serum bilirubin levels and will advise if any dose or protocol modification is needed to mitigate risk to such patients.

Doxorubicin imparts a red coloration to the urine for 1 to 2 days after administration. Subjects should be advised that this is expected.

Dose adjustments are recommended in study patients who demonstrate significant myelosuppression from the initial dose of ThermoDox. Guidance on dose adjustment in patients receiving additional treatment of study drug is provided in section 8.5.4 of this protocol. Among patients requiring re-treatment after incomplete initial ablation who have developed a non-hematologic toxicity $\geq$ grade 3, no dose modification is required. Instead, such subjects will not be re-treated until the severity of the non-hematologic toxicity drops to $\leq$ grade 2.

It is possible that the concomitant use of ThermoDox with RFA may produce larger than usual ablation volume post-RFA. Liposomal doxorubicin has been observed to increase ablation volume, seemingly by sensitizing the tissue peripheral to the ablation margin to thermal injury and, therefore, effectively reducing the temperature threshold for ablation (Goldberg, et al. 2002). However, any relative increase in ablation volume is expected to be achieved in a controlled manner, since the ThermoDox-related enhanced release of doxorubicin will occur in the immediate region surrounding the RFA zone. There are

potential risks and benefits from this possibility; however, it has not been an apparent clinical problem in subjects treated to date with ThermoDox or in reports of combination of RFA and Doxil®, a non-heat-sensitive liposomal formulation (Goldberg, et al. 2002).

Twenty five to thirty percent of subjects are at risk of developing a "post-ablation syndrome" with flu-like symptoms that in most cases appear three to five days after the RFA procedure and usually last about five days, although some subjects may remain ill for two to three weeks. Subjects with post-ablation syndrome may present with fever, delayed pain, malaise, chills, nausea after the procedure, even if only briefly (Dodd, et al. 2005), and transient elevation of transaminases. Oral acetaminophen is commonly given to control fever, which is the most common symptom. However, treatment of post-ablation syndrome should be based on the best judgment of treating physician. All subjects are required to undergo evaluation of their vital signs at specified time points after the ablation procedure.

AASLD treatment guidelines permit diagnosis of HCC with the use of imaging only and without a biopsy for histological or cytological confirmation of disease. Completion of a biopsy sample increases risk to subjects with liver disease and therefore is not always indicated. However patients who undergo an RFA treatment without a biopsy may be at increased risk for undergoing an unnecessary ablation or be at risk for seeding for subcapsular or poorly differentiated disease. This risk is minimal and accepted within clinical practice guidelines.

RFA has been used safely in tumors up to 7 cm (Chen, et al. 2004; Curley, et al. 2004; Dodd, et al. 2001; Tateishi, et al. 2005). In the United States and other local regions, palliative treatments such as transcatheter arterial chemoembolization (TACE) and sorafenib are the standard of care for HCC tumors > 5 cm. However, in this study the combination of RFA and ThermoDox is being investigated as a curative treatment for patients with tumors > 5 cm to $\leq$ 7 cm rather than for their palliation. There is risk that RFA is not as effective in the larger tumors, however this has not been established. Subjects are eligible to receive palliative therapy as medically indicated after progression is observed.

There is a low but definite risk of hemorrhage in the liver or the peritoneal cavity following RFA procedure (Berber, et al. 2004 and 2007). After treatment, subjects will be monitored closely for at least one day and immediate interventional back-up, i.e., transcatheter arterial embolization or surgery, will be available at each study site in accordance with study site's standard of care. Any adverse event must be monitored and recorded.

There is a risk of infusion reactions with ThermoDox since such reactions to the IV administration of liposomal drugs have been previously reported. Severe infusion reactions in animals have been reported with ThermoDox in unprotected and less than optimally protected animals. A summary of this experience is provided in the non-clinical pharmacology and toxicology section of the Investigators brochure (IB). Celsion's approach to this has been to provide protection through broad-spectrum prophylaxis using steroids and antihistamines.

IV contrast should not be administered if medically contraindicated. Subjects that meet the protocol's eligibility criteria may be at risk for contrast-induced nephropathy (CIN) when

undergoing CT with contrast procedures. Investigators must be mindful of the risk factors associated with CIN and employ strategies to reduce the risk of CIN. In subjects with diabetes or borderline renal function (creatinine > 1.5 mg/dL), special precautions guided by the locally accepted medical standards (e.g., hydration, contrast dose reduction, follow up creatinine determination) should be employed. An accepted procedure may include adequate intravenous volume expansion with isotonic saline (1.0 – 1.5 mL/kg per hour) for 3-12 hours before the procedure and continued for 6-24 hours if clinically indicated and based on the treating physician's medical judgment.

Potential Benefits

It is expected that thermal ablation of a subject's liver lesion may result in local disease control and improved survival duration. The addition of ThermoDox to this ablation procedure has the potential to improve local control by the creation of high local concentrations of doxorubicin in the boundary of the ablation margin resulting in increased ablation volume and better tumor cell kill in and around the tumor margin. Thus, ThermoDox has the potential to enhance the local effects of RFA procedures. However, as an investigational product, it is not known at this time if ThermoDox will have a clinical benefit.

7. ENROLLMENT AND WITHDRAWAL PROCEDURES

7.1 Enrollment Procedures

The Investigator must inform each prospective subject of the nature of the study, explain the potential risks, answer questions, and obtain written informed consent from the subject prior to performing any study-related screening procedures or assessments. The subject must also give consent, in verbal and/or written form, as directed by the ethics committee and/or local law. After written informed consent has been obtained, screening procedures for determination of eligibility can be initiated.

Once screening procedures have been completed, the site will use a centralized randomization procedure in order to assign subjects to treatment with RFA plus ThermoDox or RFA plus dummy infusion.

7.2 Subject Discontinuation

Subjects will be discontinued from the study if they withdraw consent.

Following progression, sites are instructed to contact subjects at 3-months intervals. At each follow-up interval, if contact cannot be made on the first attempt, then the site MUST document 3 attempts to contact the subject using phone calls or visits to the physician's offices. If the site is unable to contact the subject, then a "certified or tracking letter" MUST be sent to the subject's last known residence. This method of contact should be attempted at 3 consecutive follow-up intervals prior to the subject being considered lost to follow up.

If a subject discontinues from retreatments or from the PFS imaging interval, the patient should still be contacted for overall survival until withdraw consent for study participation.

7.3 Study Noncompliance

All instances of noncompliance and all significant protocol violations will be reported to the appropriate ethics committee per local regulations. All instances of eligibility violations or deviations will be recorded on the case report forms and reported to Sponsor.

If a subject is discontinued from the study due to noncompliance of follow up visits, the patient should still be contacted for overall survival until end of study or withdraw consent from study participation.

7.4 End of the Study

The primary analysis will be based on the first 197 events (deaths) observed. Survival follow-up will be continued for 5 years after the last patient is randomized, to enhance OS evidence for regional or other subgroup assessments.

8. STUDY TREATMENT ADMINISTRATION

8.1 Allocation to Treatment

Eligible subjects will be randomized through an Interactive Response Technology (IRT) web-based system. Subjects will be randomized in a 1:1 ratio to either the active treatment of ThermoDox (50 mg/m^2) or to the non-active dummy infusion. Both treatment arms will be administered in conjunction with standardized RFA using a ≥ 45 minute treatment dwell time. The IVR system will advise the unblinded site representative (i.e. pharmacist) the treatment arm the subject has been randomized to and the kit number to dispense the pre-medication (oral capsule) to the subject. Therefore, randomization must occur at least 24 hours prior to the scheduled RFA procedure.

A centralized computer-generated randomization scheme has been prepared before the study and controlled by the unblinded Sponsor representative. Subjects will be stratified by maximum lesion diameter (≥ 3.0 to 5.0 versus > 5.0 to ≤ 7.0) and RFA route (laparoscopic, open surgical, percutaneous).

The Sponsor will provide all masking materials to maintain the blind. Each center will have identified unblinded representatives (i.e., pharmacist) to prepare the materials and to cover IV bags and tubing. Only designated Sponsor representatives may be unblinded as well.

8.2 Pre-Medication and ThermoDox Administration

Please refer to Pharmacy Manual for preparation and administration details. ThermoDox is calculated using Boyd's formula as indicated in the Manual. Accurate records must be kept regarding calculation and dispensing of study medication for each individual subject in the study.

Subjects assigned to the ThermoDox arm will be assigned a steroid, H1, and H2 antihistamine pre-treatment regimen and subjects assigned to dummy infusion arm will receive non-active pre-treatment regimen in a blinded fashion.

Oral premedication is supplied to the site as over encapsulated dexamethasone (20 mg/m^2) capsules OR identical dummy capsules. Oral pre-medication is a single dose taken by the subject 24 hours (+/- 2 hours) prior to the scheduled study drug infusion.

The day of the RFA procedure, subjects are required to be administered an IV premedication regimen according to local medical practice or as noted in the pharmacy manual. The IV premedication should be administered within 30 minutes (+15 min.) prior to the start of the ThermoDox infusion. The following may serve as a guide:

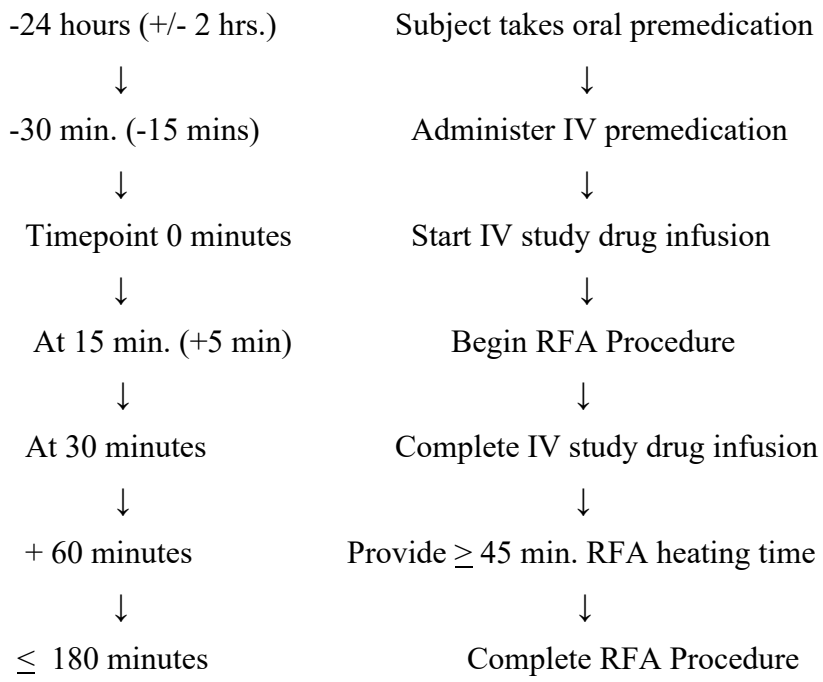
- Dexamethasone 20 mg IV or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)
- Diphenhydramine 50 mg IV (chlorpheniramine 10 mg IV is permitted) or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)
- Ranitidine 50 mg IV or famotidine 20 mg IV or dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W)

These drugs are to be administered intravenously in the listed order. Intravenous premedications are secured using local formulary or matching dummy infusion of Saline or D5W. The premedications should not be mixed in the same bag. The unblinded study representative will prepare the pre-medications consistent with the randomized treatment arm so that other study personnel remain blinded.

The total 250 cc volume of ThermoDox (50 mg/m^2) or masked dummy infusion is delivered in a single 30-minute period through a free flowing IV line. The entire content of the infusion bag must be administered.

RFA will be initiated at minute 15 (+5 minutes) following the start of study drug infusion and should be completed within 3 hours after study drug infusion initiation. The RFA procedure should achieve a dwell time of ≥ 45 minutes, which can be done by employing at multiple ablation cycles or deployments.

Figure 1: Flowchart of Study Drug Administration in Relation to RFA



8.3 Use of Radiofrequency Ablation Devices

Only FDA approved RFA devices including the Covidien switchbox and all manufacturer probes for these devices are permitted for this trial. The choice of RFA devices (Angiodynamics, Boston Scientific, or Covidien) will be made at each institution. Physicians (Principal Investigator or sub-Investigator) performing RFA may use more than one RFA device at their local institution. However, the same device must be used for all treatments of an individual patient.

8.4 Radiofrequency Ablation Procedure

The goal is to reach a ≥ 45 minute dwell time which can be achieved by employing multiple ablation cycles or deployments in order to ablate the tumor as well as a 360º 1.0 cm tumor-free margin surrounding the tumor. Tumor uptake of ThermoDox is enhanced with a standardized RFA treatment dwell time of ≥ 45 minutes (≥ 30 minutes with a switchbox). An sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. An ablation cycle for this protocol is defined as the single activation of the probe or electrode in order to achieve a local coagulation necrosis in target tissue. The cycle may be completed by achieving a target temperature in the tissue or a target impedance.

The RFA operator must have experience with performing at least 15 procedures. A minimum of 5 prior cases with the device should be documented.

The standard RFA procedures to achieve adequate dwell time of ≥ 45 minutes must be implemented by the sites.

a. The tumor should be treated with the goal of reaching a > 45 minute dwell time which can be achieved by employing multiple overlapping ablation cycles (according to literature recommend 4 cycles) thus ablating the tumor as well as a 360° 1.0 cm tumor-free margin around the tumor.
b. Devices should be operated within manufacturer's instructions.
c. A percutaneous, laparoscopic, or open surgical approach may be utilized.
d. The RFA device operator will activate the RFA generator to initiate tumor tissue ablation temperatures at 15 minutes (+5 min.) after the start of the study drug infusion.
e. Enhanced local concentration of doxorubicin and tumor uptake from ThermoDox requires at least 45 minutes RFA dwell time. This dwell time is achieved by administering multiple ablation cycles, so 4 ablation spheres (if using a simple needle electrode) or 4 deployments (if using multiple array LeVeen needle electrode) are recommended, but fewer or more may be performed at discretion of the investigator provided the dwell time reaches 45 minutes. The RFA literature recommends a minimum of 4 ablation cycles to ablate tumors of ≥ 3.0 cm together with a 360º 1.0–cm margin (Chen, et al. 2004). More ablations may be performed at the discretion of the operator (Curley, 2001; Dodd, et al. 2001; Chen, et al. 2006).
f. Investigators are to follow their chosen RFA device's particular guidelines for overlapping ablation procedures (See Appendix IV).
g. Operators should employ strategies for maximal patient safety based on tumor size and tumor location (Rhim, et al. 2001).
h. The procedure must be completed within 3 hours from the start of the infusion.
i. The adequacy of ablation will be monitored by ultrasound, MRI, or CT scan throughout the procedure in accordance to the site's standard of practice.
j. Pringle maneuver or balloon occlusions are prohibited.

RFA will be performed in accordance with RFA Treatment Procedure described in Appendix IV and manufacturer's instructions provided separately.

In order to avoid potential heating or burns to the skin area in contact with the grounding pads, handling of the skin area around the pads as well as the number of pads used will be determined per the RFA device manual's instructions. Should the temperature of the ground pads rise above 39°C, the RFA treatment will be discontinued until the temperature returns to baseline; additional grounding pads can be employed to avoid skin burns and rising temperature.

8.5 Dose Modifications

8.5.1 Hepatobiliary Surveillance

Subjects with an elevated serum bilirubin level (> 2.0 and ≤ 3.0 mg/dL) at baseline will have additional safety assesments at Day 7 post-ablation and at any other time the physician deems it necessary. These assessments should include a physical exam, vital sign, AE and concomitant medications assessment, and a CBC, UA and chemistry laboratory draw. Grade 3 – 4 hepatic toxicities are to be followed until resolution.

No dose modification is required for patients with elevated serum bilirubin.

8.5.2 Suggested Management for Hypersensitivity Reactions:

Severity of Symptoms:	**Treatment Guidelines:**
Mild Symptoms: localized cutaneous reactions such as mild pruritis, mild flushing, and mild rash. (Grades 0-1)	• Consider decreasing the rate of infusion until recovery from symptoms, stay at bedside and monitor subject. • Then, complete study drug infusion at the initial planned rate.
Moderate to Severe Symptoms: any symptom that is not mild (see above) such as generalized pruritis, generalized flushing, generalized rash, dyspnea, hypotension with systolic BP < 80 mm Hg, bronchospasm, angioedema and generalized angioedema. (Grades 2-4)	• Stop infusion. • WITHDRAW FROM STUDY TREATMENT
Anaphylaxis (Any degree of anaphylaxis): (Grade 4)	• Stop infusion. • WITHDRAW FROM STUDY TREATMENT

8.5.3 Extravasations

Infusion of ThermoDox should be closely monitored for local site reaction. If there are initial signs of extravasation, then the infusion must be immediately stopped and continued in another vein. Local protocol should be used to treat the affected area. Application of ice over the site of extravasation for approximately 30 minutes may be helpful in alleviating the local reaction.

8.5.4 Dose Modification for Re-Treatment ONLY

Among patients requiring re-treatment after incomplete initial ablation who have developed Grade 4 hematologic toxicities, the absolute neutrophil and platelet counts must return to less than or equal to grade 1 before re-treatment.

The investigator should follow local/institutional cytokine support or in the absence of local guidelines for cytokine support, the investigator may use the "American Society of Clinical Oncology 2006 Update of recommendations for the use of white blood cell growth factors: An Evidence-Based Clinical Practice Guideline" (Smith et al. 2006). The second ablation must occur no later than 21 days after the first post-ablation radiographical imaging assessment at Day 28. If the patient does not meet the dose modification criteria within the timeframe, then patient should be taken off study and treated as clinically necessary.

HEMATOLOGICAL TOXICITY*			
GRADE	**ANC**	**PLATELETS**	**MODIFICATION**
1	1500-1900	75,000-150,000	Resume treatment with no dose reduction
2	1000-<1500	50,000-<75,000	Wait until ANC ≥ 1,500 and platelets ≥ 75,000; redose with no dose reduction
3	500-<999	25,000-<50,000	Wait until ANC ≥ 1,500 and platelets ≥75,000; redose with no dose reduction
4	<500	<25,000	Wait until ANC ≥ 1,500 and platelets ≥ 75.000; redose at 25% dose reduction or continue full dose with cytokine support

*Dose reduction guidelines to investigators for subjects who experience Grade 4 absolute neutrophil counts (ANC) or Grade 4 platelet count and require additional ablation due to incomplete ablation

Among patients requiring re-treatment after incomplete initial ablation who have developed a non-hematologic toxicity ≥ grade 3, no dose modification is required. Instead, such subjects will not be re-treated until the severity of the non-hematologic toxicity drops to ≤ grade 2.

9. STUDY MEDICATION

The Sponsor has prepared a Pharmacy Manual for further description of premedication and ThermoDox treatment dose preparation. The active pharmaceutical ingredient (API) in ThermoDox is doxorubicin hydrochloride for injection, USP. Doxorubicin is a cytotoxic anthracycline antibiotic. The investigational product ThermoDox combines doxorubicin HCl with a lyso-thermosensitive liposomal carrier. Staff must be properly delegated and trained to handle, prepare, instruct the subject, and administer the investigational product.

9.1 Clinical Supplies

ThermoDox will be supplied as 15 mL clear vials containing 2 mg/mL doxorubicin. Multiple vial packaging will be supplied to the sites. The appearance of ThermoDox requires masking to maintain the blind of the study, therefore matching dummy vials will not be supplied for the trial. All treatment medication and premedication will be masked by the pharmacy in order to maintain blinding.

The pre-treatment kits will contain over encapsulated Swedish orange opaque size DB AA capsules containing Dexamethasone 20 mg (5) 4 mg tablets or dummy tablets.

Intravenous premedications are secured using local formulary or matching dummy infusion of Sodium Chloride 0.9% or 5% Dextrose (D5W). The Sponsor may procure and ship IV premedications to site if there are local regional formulary restrictions.

Only subjects that sign informed consent and are randomized in the study may receive the investigational study medication (ThermoDox or dummy infusion), in accordance with all applicable regulatory requirements.

9.2 Packaging and Labeling

The vial of ThermoDox for Injection will be labeled Caution: New Drug Limited by Federal Law to Investigational Use Only, as presented below (or as required by local regulatory authority).

Randomization identifiers will be placed on active and dummy pre-medication kits to guide the randomization process.

Sample Label:

Protocol # 104-13-302

ThermoDox® 15 mL x 4 vials

Store at ≤ - 60 °C
This box contains 4 vials
Each vial contains 2 mg/mL Doxorubicin Hydrochloride, DPPC, MSPC, DSPE-MPEG 2000, sodium citrate, citric acid, lactose, sodium carbonate and Water for Injection.
Direction: See preparation procedure provided
Caution: New Drug – Limited by Federal law to Investigational Use Only
To be used only by Qualified Investigators
Celsion Corporation. Lawrenceville, NJ 08648 USA

(i) Lot #: (ii) Retest date: (iii) Mfg Date:

9.3 Storage of Study Medication

ThermoDox must be stored frozen at colder than -60°C as per the manufacturer label. All Sponsor supplied investigational product must be kept in a secure area with access limited to the Investigator and authorized site staff. Storage conditions must be monitored for temperature deviations using a freezer with an appropriate monitoring device.

9.4 Thawing, Stability, and Reconstitution of ThermoDox

ThermoDox vials must be thawed at room temperature for a minimum of 75 minutes prior to reconstitution with 250 mLs of 0.9% Sodium Chloride Injection USP or 250 mLs 5% Dextrose Injection USP (D5W). The site will remove the number of ThermoDox vials necessary for the dose based on the subject's body surface area (BSA) from Boyd's formula.

Do NOT heat ThermoDox vials to speed up the thawing process. Accelerated thawing techniques are not permitted. After thawing, the vials are stable for 4 hours at room temperature (15°C to 30°C).

ThermoDox vials must be thawed and diluted to a final volume of 250 mLs using sterile 5% Dextrose Injection, USP (D5W) or 250 mLs of sterile 0.9% of Sodium Chloride Injection, USP (Saline). The total volume of 250 mLs is infused over a 30-minute period through a free flowing IV infusion pump.

ThermoDox should be infused at room temperature. Do not heat ThermoDox solutions above room temperature during administration.

ThermoDox is supplied sterile and pyrogen free; aseptic procedures should be employed during preparation for administration. For detailed storage and preparation instructions, refer to the Pharmacy Manual that is provided to the pharmacist by Celsion.

9.5 Drug Accountability/Disposition of Clinical Trial Supplies

Drug accountability records are required to be maintained by the site for all clinical trial supplies. Used clinical supplies will be destroyed at the site in accordance with the site's local processes.

Unused clinical supplies will be destroyed in accordance with the site's regulations only after completion of the study supplies reconciliation by the Sponsor's monitor.

If it is not possible to destroy unused clinical supplies, Celsion Corporation should be contacted in order to make alternative arrangements.

10. SCHEDULE OF STUDY PROCEDURES

Table 1: Schedule of Study Evaluations

Protocol Section	Procedure/Test	Screening / Baseline	Treatment Period[1]							Follow-Up Period			
			Procedure Day Day 0				Post-Procedure Visits (+/- 3 days)			Safety and Efficacy Assessments			
		-21 Days	Pre-Dose	Time 0	RFA + 15 min.	Post RFA	Day 7[2]	Day 14	Day 28	M5, M9, M13 or Progression[3]	M17, M21, M25	6 Month Interval after M25	3 Month Interval after Progression
Evaluation and Observations													
17.1	Informed Consent	X[4]											
10.1.3	Demographics and Medical History	X											
10.1.5	Physical exam	X				X	X	X	X	X			
10.1.6	Vital Signs	X[5]	X[5]			X[5]	X	X	X	X			
10.1.7	ECOG Performance	X											
14	Adverse Event Eval.	X	→	→	→	→	→	→	X[6]	X[6]			
11	Con. Med. Update	X	→	→	→	→	→	→	X				
Treatment Procedures													
8.1	IRT System/ Randomization	X[7]											
8.2	Pre-Medication Regimen		X[8]										
8.2	IV Study Drug Infusion			X[9]									
8.3-8.4	RFA Procedure				X[10]								

Protocol Section	Procedure/Test	Screening / Baseline	Treatment Period[1]							Follow-Up Period			
			Procedure Day Day 0				Post-Procedure Visits (+/- 3 days)			Safety and Efficacy Assessments			
		-21 Days	Pre-Dose	Time 0	RFA + 15 min.	Post RFA	Day 7[2]	Day 14	Day 28	M5, M9, M13 or Progression[3]	M17, M21, M25	6 Month Interval after M25	3 Month Interval after Progression
Safety Procedures/Lab Tests													
10.1.10	LVEF Monitoring	X[11]							X[11]	X[11]			
10.1.9	12-Lead ECG	X				X			X	X			
10.1.11	Serum pregnancy test	X											
10.1.12	CBC with differential	X					X	X	X	X			
10.1.12	Serum chemistry[12]	X					X	X	X	X	X		
10.1.12	PT/INR	X					X	X	X	X	X		
10.1.12	Urinalysis	X					X	X	X	X			
Disease Assessment													
10.1.4	Biopsy		X[13]										
10.1.8	Imaging[14]	X							X	X	X	X	
15.2.1	Overall Survival[15]												X[15]

1. **Retreatment –** Subjects are eligible for one retreatment no later than 21 days after the Day 28 imaging scan. The retreatment must be the same (sRFA + dummy or sRFA + ThermoDox) as initially administered.
2. **Day 7 Visit –** Only subjects with a total bilirubin > 2.0 but ≤ 3.0 mg/dL must undergo the additional safety visit on Day 7 post-RFA.
3. **Time of Progression** – Month 13 visit assessments are required at time of disease progression, whichever occurs first within the first 13 months. No further assessments required after progression is recorded
4. **Informed Consent –** Is required within 28 days prior to study drug infusion. Informed Consent should be obtained prior to any study-related procedure performed outside standard medical care and required only to evaluate for participation in this trial.

5. **Vital Signs –** Assess screening vital signs prior to randomization and perform a ***pre-dose assessment the morning of the RFA procedure***. Post-RFA vital signs should be assessed within 15 minutes after completion of study drug infusion, within 30 minutes and 1 hour (+/- 15 min.) after RFA procedure, then every 2 hours (+/- 15 minutes) until the subject is stable or discharged.
6. **Adverse Event Evaluation –** Assess adverse events (AEs) through Day 28 following study drug treatment. AEs after Day 28 evaluated as possibly, probably or definitely related to the study drug or the RFA procedure will be recorded at any point during the trial and are to be followed until resolution or the patient is clinically stable.
7. **Randomization –** Eligibility criteria must be assessed prior to randomization. ***Randomization must occur at least 24 hours prior to scheduled RFA treatment time*** to ensure that the oral pre-medication regimen can be started.
8. **Pre-Medication Regimen -** A blinded oral pre-medication capsule is dispensed after randomization with instructions to take the pre-medication 24 hours (+/- 2 hours) prior to the scheduled RFA procedure time. Recommend a reminder phone call be made to the subject the morning prior to RFA treatment. IV premedication (active or dummy infusion) is administered within 30 mins. (+15 min.) prior to study drug infusion.
9. **IV Study Drug Infusion –** Infusion is intended to be 30 minutes in duration; however, the complete contents of the infusion bag must be infused.
10. **RFA Procedure –** 15 minutes (+5 min) after the start of study drug infusion, RFA is to be initiated and is to be completed within 3 hours of the start of study drug infusion. **RFA procedure should achieve an RFA dwell time of ≥ 45 minutes.**
11. **LVEF Monitoring –** LVEF monitoring is required at baseline, at Day 28, and at Month 13 or radiologic progression, whichever occurs first. Echocardiogram is preferred. MUGA scans are permitted if an echo cannot be completed; however, the same method of measurement must be used to evaluate LVEF in an individual subject.
12. **Liver Function Tests –** Serum Chemistry and PT/INR will be assessed at baseline, day 14, day 28, month 5, 9, 13, 17, 21, and 25 or time of progression, whichever comes first. Patients with elevated bilirubin at baseline will also be assessed at day 7.
13. **Biopsy –**Subjects not meeting AASLD diagnosis should have histological confirmation of HCC prior to randomization.
14. **Imaging –** CT or MRI imaging (abdomen, chest, and pelvis) is required; however, the same modality used at baseline must be used for all further imaging in an individual subject. Scans must be done within 21 days prior to initial treatment to confirm the presence of an evaluable lesion and at Day 28 to evaluate whether a complete initial ablation was achieved. Follow-up imaging will be done at months 5, 9, 13, 17, 21, and 25 (+/- 2 weeks), then at 6-month intervals (+/- 2 weeks) until radiologic progression is seen. For trial purposes, an imaging scan demonstrating progression must be recorded before beginning alternative treatment.
15. **Overall Survival –** All subjects will be contacted every 3 months after radiological progression for vital status reporting.

10.1 Study Procedures

10.1.1 Screening Overview

Written informed consent must be obtained ≤ 28 days prior to initiation of treatment and before any procedures specifically for inclusion in the protocol are performed.

Screening assessments will be performed after obtaining informed consent and within 21 days prior to the initiation of treatment. Screening procedures will include a medical history, physical examination, vital signs, ECOG performance status, adverse event evaluation, concomitant medicine update, ECG, ECHO or MUGA for LVEF measurement, laboratory tests, and radiological imaging scans. Screening laboratory procedures may be repeated to assess eligibility parameters during the screening period.

Screening assessment will be collected, reviewed and determined to be acceptable by the Principal Investigator prior to randomization and must provide sufficient time for the subject to begin the pre-medication regimen. Any change in health evaluated by physical exam or vital signs should be acceptable to the Investigator before study treatment is started.

10.1.2 Subject Numbering

Each subject for whom written informed consent has been obtained will be assigned an eight-digit subject number. The first four digits of the subject number will be the investigational site numbers. The remaining digits will be a number starting with 7001 for the first subject enrolled at the site and incrementing by "1" for each sequential subject.

Never reassign patient numbers. In the event that a subject withdraws from the study, the number assigned to that subject is retired and the next subject receives the next sequential number.

10.1.3 Demographics and Medical History

Demographic data (date of birth, race, and sex) will be recorded up to 21 days prior to study drug treatment. Medical history including details of cancer and non-cancer history will be collected.

10.1.4 Disease History and Biopsy

Data on the current malignancy will include: date of diagnosis and stage of cancer,

The following information must be obtained to confirm HCC:

1. The date and results of the HCC biopsy.
2. If a biopsy was not performed or not confirmatory of HCC then
   - Patient must meet AASLD criteria of
     - Hypervascular with washout in the portal/venous phase (on CT) OR
     - AFP > 200 ng/ml (for detailed description see Appendix VIII)
   - Record the reason why biopsy not performed.

Subjects may be randomized without a biopsy if they meet American Association for the Study of Liver Disease (AASLD) criteria for the diagnosis of HCC (see Appendix VIII). Subjects <u>not</u> meeting AASLD criteria for HCC will need a biopsy for confirming HCC prior to randomization.

10.1.5 Physical Exam

Physical examinations will be conducted within 21 days for eligibility assessment prior to randomization. An important component of the physical exam is the accurate measurement of height (in cm) and weight (in kg). All pertinent findings will be recorded on the appropriate pages of the CRF. All changes relative to baseline considered to be clinically significant by the Investigator will be reported as AE's and recorded in the CRF.

The physical exam must be conducted at the following times:

- Once within 21 days of RFA treatment (initial and any re-treatment).
- Post RFA prior to discharge.
- Day 14 (+/- 3 days)
- Day 28 (+/- 3 days)
- Month 5, Month 9, Month 13 (+/- 2 weeks) or disease progression, whichever occurs first
- Day 7 (+/- 3 days) for subjects with elevated bilirubin

10.1.6 Vital Signs

Vital signs, which include temperature (in Celsius), blood pressure, pulse rate, and respiration must be obtained. Vital signs must be collected in a position that is consistent for all time points for each subject and will be collected at the following times:

- Once within 21 days of RFA treatment (initial and any retreatment)
- Before administration of the investigational product. Note: subjects with a temperature or blood pressure outside the eligibility criteria at the pre-dose evaluation should NOT receive treatment with investigational product.
- Within 15 minutes after completing study drug infusion and after RFA completion at 30 minutes (+/- 15 minutes), 1 hour (+/- 15 minutes), every 2 hours (+/- 15 minutes) until the subject is stable or discharged.
- Day 14 (+/- 3 days)
- Day 28 (+/- 3 days)
- Month 5, Month 9, Month 13 (+/- 2 weeks) or disease progression, whichever occurs first
- Day 7 (+/- 3 days) for subjects with elevated bilirubin

10.1.7 ECOG Performance Status

The Investigator will evaluate the patient's ECOG performance status within 21 days prior to study treatment.

10.1.8 Imaging

CT or MRI scans (Chest, Abdomen and Pelvis) will be done within 21 days prior to study treatment to confirm the presence of an evaluable lesion and at Day 28 to assess whether a complete initial ablation was achieved. Additional imaging will be done at months 5, 9, 13, 17, 21, 25, then every 6 months (+/- 2 weeks) until disease progression is seen. The following requirements apply to imaging for this study:

- The same modality and technique must be used for all scans of an individual patient.
- Investigator-determined radiological progression must be documented prior to any non-study HCC treatment. Imaging follow-up should continue for subjects with clinical evidence of progression until radiological disease progression is confirmed before beginning any non-study cancer treatment.
- The date of progression will be reported to the CRF.

Once the PFS endpoint is met, no further CT and MRI scans are required for this study.

For subjects with incomplete ablations who are re-treated a second time and complete ablation is achieved, the imaging studies will continue per protocol with Day 0 set as the date of the second ablation procedure. If the lesion cannot be completely ablated after the second treatment, then the subject will be considered a treatment failure. Such subjects will continue to be followed for adverse events through Day 28 following the re-treatment; they will also continue to be followed for OS every 3 months.

10.1.9 ECG

Acquire an ECG (12 lead with paper printout) at the following time points in relation to study drug treatment:

<u>Pre-Treatment</u>

- Within 21 days prior to study drug infusion

<u>Post-Treatment</u>

- Once subject is stable after RFA procedure
- Day 28 (+/- 3 days)
- Month 5, Month 9, Month 13 (+/- 2 weeks) or disease progression, whichever occurs first

The Investigator will comment on any changes considered to be clinically significant and report as an AE. All changes considered clinically significant after Day 28 should be reported as AEs and recorded in the CRF if considered possibly, probably or definitely related.

10.1.10 ECHO/MUGA Scan for LVEF Monitoring

A baseline echocardiogram (ECHO) must be carried out within 21 days prior to study treatment. Measurements with a MUGA scan are allowed if an echocardiogram cannot be performed; however, the same modality used at baseline must be used for follow-up. Follow-up scans must be completed on Day 28 (+/- 3 days) and at Month 13 or at time progression of disease, whichever occurs first.

A decrease of >10% in resting LVEF that becomes abnormal (lower than the institutional lower limit of normal) should be further evaluated by repeat examination for subjects with a total cumulative doxorubicin of ≥300 mg/m^2.

10.1.11 Serum Pregnancy Test

Women of child bearing potential must undergo a serum pregnancy test within 21 days prior to each administration of study drug.

10.1.12 Laboratory Analytes

Table 2: Laboratory Analytes

Hematology	**Clinical Chemistry**		**Urinalysis**
Hematocrit	Sodium	AST/SGOT	pH
Hemoglobin	Potassium	ALT/SGPT	specific gravity
RBC count	Chloride	Alkaline Phosphatase	Protein
WBC count	Glucose	Total protein	Glucose
Differential WBC count	Urea nitrogen	Albumin	Ketones
Platelets	Creatinine	Calcium	Blood
PT	Total bilirubin	AFP	WBC/HPF
INR			RBC/HPF
			Crystals

The laboratory will perform evaluations of the analytes listed in Table 2 above. Evaluations should take place as follows:

- Within 21 days prior to study drug infusion- Repeat laboratory analysis is permitted to evaluate eligibility prior to treatment. The repeat analysis results must meet the eligibility criteria prior to study drug infusion.
- Day 14 (+/- 3 days)
- Day 28 (+/- 3 days)
- Day 7 (+/- 3 days) for subjects with elevated bilirubin

Additional hematologic tests (CBC with differential) will be collected at months 5, 9, and 13 or disease progression, whichever occurs first.

Additional post-treatment liver function tests (Chemistry and PT/INR), will be collected at months 5, 9, 13, 17, 21, and 25 (+/- 2 weeks) or disease progression, whichever occurs first.

10.2 Re-Treatment

A subject who has an incomplete initial ablation is eligible for 1 retreatment procedure within 21 days after the scan showing residual disease at Day 28. Subjects will be retreated only once with the same treatment assigned at randomization. Baseline safety evaluations must meet the eligibility parameters prior to a retreatment. The second ablation must occur no later than 21 days after the first post-ablation scan assessment at Day 28. Such subjects will continue to be followed for AEs/SAEs through Day 28 after the second study drug administration. Subjects with a complete ablation after retreatment will be followed for all primary and secondary endpoints. If after 2 ablations the subject has local, distant intrahepatic, or extrahepatic HCC, then the subject will be considered a treatment failure and will have met the PFS endpoint. The subject will still be followed for OS.

Among subjects who are not treatment failures, five repeat treatments may be permitted to treat a recurrent lesion or to treat newly-identified local or distant intrahepatic lesions at the Investigator's discretion after the PFS endpoint is reported and with agreement from the Sponsor. The subject must be eligible for retreatment consistent with the safety eligibility criteria and will be retreated with the same randomized treatment.

Subjects who develop extrahepatic lesions will have met the PFS endpoint and are no longer eligible for further protocol treatment; they will be followed for OS.

10.3 Unscheduled Assessments and Early Discontinuation

The Investigator may, at his or her discretion, arrange for a subject to have an unscheduled assessment(s), especially in the case of adverse events that require follow-up. If the subject is experiencing an adverse event considered by the Investigator to be possibly related to the use of study treatment, then an unscheduled visit should be performed.

If the subject is discontinued from participation within the first year for any reason, the Investigator must make every effort to perform Month 13 evaluations as described in Table 1.

11. CONCOMITANT MEDICATIONS

Subjects may take any medication that is not restricted by the protocol and would not be expected to interfere with the intent and conduct of the study. Chronic medications should be dosed on a stable regimen, if possible. In the case of medications restricted by the protocol, adequate washout times must be observed. Please reference Appendix III for an explanation of washout times. All medications (including herbal preparations) at the time of screening and within 30 days prior to study drug treatment and other treatments taken by the subject during the study and up to 24 hours following treatment, including those treatments initiated prior to the start of the study (ICF signing), must be recorded on the Case Report Form.

Subjects should receive full supportive care during the study, including transfusion of blood and blood products, and treatment with antibiotics, antiemetics, antidiarrheals and analgesics, as appropriate.

Supportive medications may be administered during the course of study for toxicity at the discretion of the Investigator. Subjects with myelosuppression may require blood or platelet transfusions, filgrastim (or other colony stimulating factors), or other supportive care medications, all of which may be administered at the Investigator's discretion. Antiemetics may be administered to control nausea and vomiting, and antidiarrheals may be prescribed for diarrhea.

Liposomal drugs (Abelect™, Ambisome™, Nyotran™, etc.), or lipid-complexed drugs (fentanyl, etc.) or intravenous fat emulsions could change the pharmacokinetic profile of ThermoDox and should not be administered to study subjects while on the trial. Intravenous fat emulsions, except propofol, should be prohibited as instructed in Appendix III.

Doxorubicin is a substrate of CYP3A4. The following medications are prohibited from the specified time-point before the first dose of study drug treatment:

- Other anti-cancer therapy should not be given until Investigator determined radiological disease progression, at minimum 30 days after the last study treatment, or withdrawal from the study. Subjects who receive concurrent anti-cancer therapy will be discontinued from the study.
- Any other investigational drugs, from 30 days or 5 half-lives, whichever is longer, prior to the first dose of study treatment until Investigator determined radiological progression. Subjects should not start another investigational drug without discussion with the medical monitor. If an investigational drug is initiated, it must be a minimum of 30 days after the last study drug treatment (except in re-treatment)

- Inducers and inhibitors of CYP3A4 – these may alter the metabolism of ThermoDox, as the drug is a substrate for CYP3A4. CYP3A4 inducers and inhibitors that are prohibited from screening through 30 days after the last study treatment can be found in Appendix III Prohibited Medications;
- Certain herbal supplements are prohibited as listed in Appendix III.

12. ASSESSMENT OF EFFICACY

12.1 Primary Endpoint: Survival

All patients will be monitored for survival by recording their visits during routine follow up for response to treatment. The visits are scheduled to occur every four months from the first imaging study confirming complete ablation until month 25 or radiological progression, whichever comes first. If patients have not demonstrated radiological progression at month 25 then the imaging visit schedule is reduced to every six months until progression. Survival is confirmed at every imaging visit.

Once radiological progression is confirmed then follow up for overall survival is required. Sites are required to confirm contact with the subject during either a clinic visit or a telephone contact every three months. It is expected that subject follow up will be about five years.

12.2 Secondary Endpoint: Progression Free Survival

The protocol incorporates modified RECIST (mRECIST) developed for HCC clinical research as a basis to evaluate tumor response. The mRECIST enables assessment of overall response by taking into account target lesion response, and presence or absence of new lesions. A separate imaging manual and imaging training program has been developed for this study in order to ensure uniformity in imaging technique and imaging review in accordance with mRECIST.

12.3 Baseline Assessment

The baseline assessment must include a comprehensive evaluation of the chest, abdomen and pelvis. The HCC tumor identified on the eligibility scan should be defined as target lesion and its longest diameter should be measured on the contrast-enhanced CT or contrast-enhanced MRI scan. This measurement should reflect the overall longest tumor diameter, regardless of the presence of internal areas of spontaneous necrosis. In the study protocol, intermediate size is defined as a tumor lesion larger than 3.0 cm but not exceeding 7.0 cm in longest diameter. Since the study will recruit patients with solitary tumors and no evidence of vascular invasion or extrahepatic spread, the eligible size HCC lesion detected on the eligibility scan is expected to be the only target lesion. No additional target or non-target lesions should be detectable on the eligibility scan to allow enrollment in the study

12.4 Target Lesion Response and Local Recurrence

The image acquisition protocols used for any post-baseline assessments should be consistent with the protocol used for the baseline eligibility scan. Baseline images for treatment eligibility will be confirmed centrally before RFA treatment. Investigator determined radiological confirmation of complete ablation will occur at one month post RFA. If initial RFA is incomplete then a second, and final, attempt may be made for a complete ablation for subject to stay on protocol. Once a complete ablation is confirmed then patients are monitored radiologically until progression. The imaging interval will be every four months until month 25 then every six months thereafter. No imaging studies are required once progression is confirmed.

The disappearance of any intratumoral enhancement in the target tumor lesion after treatment with study treatment will be classified as complete response (CR), while the persistence of any areas of intratumoral enhancement consistent with residual viable tumor will be considered as incomplete response (IR). Failure to achieve a CR after two RFA ± ThermoDox treatment sessions will be classified as treatment failure. Tumor recurrence detected within or around the target lesion after having achieved initial CR will be classified as local recurrence. Tumor recurrences will be defined as local if they are located 1 cm or less from the ablation margin

12.4.1 Response Criteria for Assessing Index Lesion

PFS will be measured from the date of randomization to the first date on which one of the following occurs:

- Death of any cause
- Treatment failure (inability to achieve CR after two RFA ± ThermoDox treatment sessions)
- Progression due to local tumor recurrence after initial CR
- Progression due to distant intrahepatic tumor recurrence
- Progression due to extrahepatic tumor recurrence

Disease progression will be assessed locally at each study site. CT or MRI scans (chest, abdomen, and pelvis) will be done at baseline and post-treatment at months 1, 5, 9, 13, 17, 21, 25, then every 6 months (+/- 2 weeks) until radiological progression is seen. The definitions below will be used to assess disease progression. All imaging scans must be submitted centrally. Progression must be documented prior to any non-study HCC treatment.

Table 3: Radiological Progression Definitions

Complete Response (CR):	Complete disappearance of viable tumor on follow-up scan (Day 28) when compared to the pretreatment images. The ablation zone is defined radiographically as a homogeneous, avascular, non-enhancing, sharply marginated ablation site following RFA evaluated on both arterial and portal venous phases and complete disappearance of the lesion. Ablation zones that are larger than the tumor (including a rim of surrounding liver parenchyma) are intentionally created to reduce the risk of local recurrence and are a much better predictor of actual complete ablation than is an ablation zone that is only equal in size to the native tumor.
Incomplete Response (IR):	Incomplete Response (IR): Any image-detected residual viable tumor at or within 1 cm of the ablation zone on Day 28 scan following initial ablation therapy that is smaller in size compared to pre-treatment scans, irrespective of the size of the zone of ablation. Incomplete responses will be allowed to be re-treated once.
Local Recurrence (LR):	**Local recurrence (LR):** Recurrence of viable tumor at or contiguous (within 1 cm) to the site of a prior ablation in the liver based on meeting one of any of the specific imaging criteria and features listed below when comparing the recent imaging (latest) to the reference imaging (Day 28 post-RFA, assuming complete treatment on Day 28 scan): • New soft tissue attenuation nodules that abnormally enhance and washout following intravenous contrast administration. • On subsequent scans at time points longer than 4-6 weeks after RFA: Presence of a measurable increase (at least 10 mm) in size of the abnormally enhancing nodular soft tissue component within or adjacent to the treated tissue, as compared with baseline scan at approximately Day 28 post-RFA. • A new HCC lesion or nodule signifying local recurrence is defined as a hypervascular lesion or nodule with abnormal enhancement and washout as defined above, and measuring greater than 10 mm in any axial dimension. • A lesion that does not show the prior typical features of HCC, but demonstrates serial enlargement of at least 10mm.
Distant Hepatic Recurrence (DHR):	Development of a new lesion (meeting the criteria described above for a new lesion) in the liver at a site greater than 1 cm from a prior ablation site, compared with nadir.

Extrahepatic Recurrence (EHR):	Appearance of a new lesion outside of the liver. Note: extrahepatic HCC lesions may not demonstrate the same enhancement criteria as indicated for intrahepatic lesions; however, they should be unequivocally malignant and new since nadir. There are no size criteria.
Unable to Evaluate (UE):	A lesion present at baseline which was not measured or which was unable to be evaluated leading to an inability to determine the status of that particular tumor for the time point in question.
Not Done (ND):	Scans were not performed at this time point to evaluate the lesions.
Equivocal (EQ)	A new liver lesion that is smaller than 1 cm in longest diameter OR fails to show a typical enhancement profile should be considered as equivocal and not conclusive for disease progression. Such lesions can be diagnosed as HCC by evidence of either a change in enhancement pattern OR at least 1-cm interval growth in subsequent scans.

Table 4: Overall responses for possible combinations of tumor responses in the target lesion with or without the appearance of new intrahepatic or extrahepatic lesions.

Target Lesion	Distant Intrahepatic Recurrence	Extrahepatic Disease	Overall Response
CR	No	No	CR
IR*	No	No	Treatment Failure
IR*	Yes	No	Progression
IR*	No	Yes	Progression
Local Recurrence	Yes or No	Yes or No	Progression
Any	Yes	Yes or No	Progression
Any	Yes or No	Yes	Progression

*After 2 RFA +/- ThermoDox treatment sessions within 21 days after Day 28 radiological examination.

13. ASSESSMENT OF SAFETY

Safety data will be tabulated for all subjects who were treated under the study protocol in either treatment group and will include vital signs, laboratory parameters, ECG, echocardiograms/MUGA, and adverse events. The Principal Investigator is responsible for recognizing and reporting adverse events during the conduct of the trial.

A Data Monitoring Committee (DMC) will evaluate safety data on an ongoing basis at regular intervals during subject accrual and follow-up. The DMC is responsible for unblinded monitoring of subjects with elevated (> 2.0 mg/dL and ≤ 3.0 mg/dL) serum bilirubin levels and will advise if any dose or protocol modification is needed to mitigate risk to such patients. The Sponsor is responsible to report relevant SAEs to applicable local, national, and/or international regulatory body.

14. ADVERSE EVENTS

An adverse event is defined as any unfavorable and unintended sign, including an abnormal laboratory finding, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product. This includes any occurrence that was new in onset or aggravated in severity or frequency from the baseline condition. Adverse events (AEs) assessments will begin at time of signing of informed consent and will continue through Day 28 following study drug administration. AEs which are assessed as possibly, probably, or definitely related to study drug must be followed until resolution or until a stable clinical endpoint is reached.

Other safety data including physical examinations, vital signs, ECGs, echocardiograms/MUGA scans, hematology, clinical chemistry, and urinalysis will be assessed through Day 28 following study treatment. Subjects with an elevated serum bilirubin level (> 2.0 and ≤ 3.0 mg/dL) at baseline will have additional safety assessments at Day 7 post-ablation and at any other time the physician deems it necessary.

To provide surveillance for any late hematologic, cardiac, or liver toxicity, the following additional safety assessments will be done:

- Physical examination, assessment of vital signs, ECGs, CBC with differential, serum chemistry, PT/INR and urinalysis at Month 5, Month 9, and Month 13 (+/- 2 weeks) or at disease progression, whichever occurs first.
- LVEF monitoring at Month 13 or at disease progression, whichever occurs first
- Serum chemistry and PT/INR assessments at Month 17, Month 21, and Month 25 (+/- 2 weeks) or at disease progression, whichever occurs first.

The condition which is detected by the diagnostic procedure conducted to test the efficacy of the investigational agent is not considered an AE.

Symptoms or clinically significant laboratory or instrumental (e.g., electrocardiographic) abnormalities of a pre-existing disease, such as cancer or other disease, should not be considered an AE. However, occurrence of new symptoms or laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, are considered AEs.

Abnormal results of diagnostic procedures, including laboratory test abnormalities, are considered adverse events if they result in:

- Treatment or any other therapeutic intervention.
- Further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality).
- Associated clinical signs or symptoms that would have a significant clinical impact, as determined by the Investigator.

Any untoward medical event that occurs from the time of signed informed consent to the time immediately prior to the first study drug administration will be reported as a "predose event" in the Medical History CRF.

All adverse events that occur following study drug treatment will be documented on the AE CRF with indications of onset, duration, severity (NCI Common Terminology Criteria for Adverse Events), presumed relationship to study medication/RFA (not related, unlikely, possibly, probably, definitely), remedial actions taken, and outcome.

An adverse event will be considered associated with the use of the study drug/RFA if the attribution was possibly, probably, or definitely.

All subjects will be encouraged to report adverse events spontaneously or in response to general, open-ended questioning. All adverse events which are assessed as possibly, probably, or definitely related to study drug are to be followed up until resolution or the patient is clinically stable.

Adverse events will be graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 (See Appendix IX).

14.1 Laboratory Test Results

Clinically significant laboratory or instrumental (e.g., electrocardiographic) abnormalities of a pre-existing disease, such as cancer or other disease, should not be considered an AE. Any laboratory test result considered by the Investigator to be unusual, associated with clinical signs and/or symptoms, and clinically significant should be reported as an adverse event (clinically significant adverse events include those which require an intervention). Grade 3 laboratory abnormalities meeting the above description should be recorded as AEs. All grade 4 abnormal laboratory results must be recorded as AEs.

Significant abnormal values occurring during the trial will be followed until repeat test results return to normal, stabilize, or are no longer clinically significant. Any positive

pregnancy test result must be repeated, study treatment withdrawn, and any pregnancy must be followed to term.

14.2 Overdose

Any instance of overdose (suspected or confirmed) must be communicated to the medical monitor within 24 hours of knowledge and be fully documented as a serious adverse event. An overdose should be evaluated by the Principal Investigator and potentially reported as an unanticipated problem to the site's IRB according to local requirements.

An overdose in which signs, symptoms, or abnormalities meet the definition of an adverse event or serious adverse event must be fully documented and reported consistent with AE and SAE reporting guidelines. Details of any signs or symptoms and their management must be recorded in the CRF.

14.3 Non-Serious Clinical Adverse Events

A **non-serious adverse event** is any AE that does not meet the criteria listed in section 14.4 for a serious AE or the outcome (i.e., subject treatment, life-threatening condition, hospitalization, recovery) cannot be determined with the information provided.

For detailed description of relationship of AE to study treatment refer to Appendix V.

14.4 Serious Adverse Events (SAE)

Serious adverse events are defined as any untoward medical occurrences that at any dosage results in one or more of the following:

- Death.
- Is immediately life-threatening, (i.e., presented an immediate risk of death at the time of the adverse event, not an adverse event that hypothetically might have caused death if it were more severe).
- Requires or prolongs inpatient hospitalization.
- Causes persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Significant medical event that requires intervention to prevent one of the above outcomes listed in the above definition

Any SAE, which occurs after a subject has signed consent and until Day 28 following last study drug administration, whether or not related to study treatment, must be reported to Pharmacovigilance immediately (within 24 hours of knowledge of the event) via telephone, facsimile or email. If initially reported via telephone or email, this must be followed up by a facsimile of the written SAE report (within 24 hours of the initial telephone report).

Country specific reporting requirements apply. For example, all China local SAEs during the study must be reported within 24 hours to the CFDA.

14.4.1 Definition of Life-Threatening Adverse Experiences

An adverse experience is life-threatening if the subject is at immediate risk of death from the event as it occurred; i.e., it does not include a reaction that, if it had occurred in a more severe form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening, even though drug-induced hepatitis can be fatal.

14.4.2 Definition of Disabling/Incapacitating Experiences

An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions.

14.4.3 Reporting of SAEs

Any serious event, including death from any cause that occurs up to one month following last study treatment administration, whether or not related to the investigational drug or RFA, must be reported to Pharmacovigilance immediately (within 24 hours) via telephone, fax, or e-mail.

If initially reported via telephone or e-mail, this must be followed-up by a written faxed report to be submitted within 24 hours of the initial report.

Pharmacovigilance (SAE) contact phone numbers:

InVentiv Pharmacovigilance

Region	North America	Mainland China	Europe & Rest of World
Fax (SAE reporting)	[redacted]	[redacted]	[redacted]
Email	[redacted] (NA) [redacted] (ROW/Asia Pac)		

<u>Initial Reports</u>

Within 24 hours of the site's knowledge of a serious adverse event:

- Complete a Serious Adverse Event Report Form (SAER), sign it, and fax it to the Pharmacovigilance using the designated fax transmittal form.
- Place the initial version of SAER in the subject's file.

Follow-Up Reports

New information received spontaneously or by request of the Medical Monitor or Safety Surveillance:

Within 48 hours of the receipt of new information:

- Complete a new SAER with the new information. Sign and fax the form to Pharmacovigilance.
- Fax copies of supporting documents (e.g., hospital discharge summaries, lab test results with normal ranges, autopsy or biopsy reports) to Pharmacovigilance.
- Place the follow-up version of the SAE and all supporting documentation in the subject's file.

Final Report

Within 48 hours of the receipt of final information:

- Determine that there is no further information available and this update may be considered final.
- Complete a new SAER form with the new and final information. Sign and fax the form to Pharmacovigilance. As above, send copies of any additional supporting information.
- Place this version of the final SAER into the subject's file.

It is imperative that Pharmacovigilance be informed within 24 hours of a serious adverse experience (including overdose with clinical effect, which is considered an SAE) so that reporting to the FDA and other regulatory agencies can be met within the required time frame (7 or 15 calendar days).

Because of the need to report to health authorities all serious adverse experiences in a timely manner, it is vitally important that an Investigator report immediately any adverse experiences, which would be considered serious, even if the Investigator does not consider the adverse experience to be clinically significant or drug/RFA-related.

Should the Investigator become aware of an SAE (regardless of relationship to study drug) that occurs while the subject is on the study, the SAE must be reported in accordance with the procedures specified in this protocol.

If the subject is withdrawn less than 28 days after study drug treatment, any SAEs which occur within 28 days after study drug treatment must be reported in accordance with the procedures specified in this protocol.

All serious adverse events that are assessed as possibly, probably, or definitely related to study drug are to be recorded at any point during the trial and followed until either: the adverse event resolves, the adverse event stabilizes, the adverse event returns to baseline

values (if a baseline value is available), or it is shown that the adverse event is not attributable to the study drug or study conduct.

14.5 Other Events

If a female subject becomes pregnant during the study, follow-up of the outcome of the pregnancy and any postnatal sequelae in the infant is required. Pregnancies are not considered SAEs but are considered immediately reportable (within 24 hours) and are to be documented on the Pregnancy Report form.

Medical and scientific judgment is to be exercised in deciding whether expedited reporting is appropriate in other situations, such as for important medical events that were not immediately life-threatening or did not result in death or hospitalization but are jeopardizing the subject or require intervention to prevent one of the outcomes listed above.

15. STATISTICS

A Statistical Analysis Plan (SAP) that includes a more technical and detailed description (including templates for Tables, Listings, and Figures) of the planned statistical summaries and analyses will be prepared.

15.1 Study Populations

The safety and efficacy study populations will be defined as follows:

- Intent-to-Treat (ITT) population: This population includes all randomized subjects and is the primary population for subject characteristics and all efficacy parameters. All analyses using this population will be based on the treatment to which each subject was randomized.
- As-Treated (AT) population: This population includes all subjects who actually received at least one application of RFA plus ThermoDox (TR) or RFA + dummy infusion (PR). This population will be used for the safety analyses. All analyses using this population will be based on the treatment actually received.

15.2 Study Endpoints

15.2.1 Primary Endpoint – Overall Survival (OS)

Overall Survival is the primary endpoint of this study and is defined as the time (in months) from the date of randomization to the death date. In the absence of death confirmation or for subjects alive as of the OS cut-off date, survival time will be censored at the date of last study follow-up, or the cut-off date, whichever is earlier.

The OS cut-off date used for the primary analysis will be based on the observations of the 197th death in the study. With the OS cut-off date being event driven, for operational

efficiency, the cut-off date for all other study endpoints (e.g., PFS) will be fixed at close proximity of the OS cut-off date, when the milestone is nearing completion.

15.2.2 Secondary Endpoints

15.2.2.1 Progression-Free Survival (PFS)

Progression-free survival is defined as the time (in months) from the date of randomization until the date of the Investigator-assessed radiological disease progression or death due to any cause. Subjects who are alive with no disease progression as of the analysis cut-off date will be censored at the date of the last tumor assessment. Subjects who receive non-study cancer treatment before disease progression, or subjects with clinical but not radiologic evidence of progression will be censored at the date of the last evaluable tumor assessment before the non-study cancer treatment is initiated. If a subject undergoes a liver transplant or liver resection before progression is observed, then the PFS for this subject will be censored on the date of last assessment without documented progression prior to the procedure. Treatment failures (subjects with incomplete ablations after two RFA procedures) will be considered to have met the PFS endpoint and the date of the Day 28 assessment following the 2nd ablation procedure will be used as the progression date. Additional censoring rules details will be outlined in the SAP.

15.3 Statistical Analysis

15.3.1 Subject Disposition, Baseline and Treatment Characteristics

15.3.1.1 Subject Disposition

The number of subjects in each study population and the reasons for exclusion, along with any randomization and/or stratification errors will be summarized. In addition, subjects that discontinue study treatment or study follow-up will also be summarized, along with reasons for study discontinuation.

15.3.1.2 Subject Baseline Characteristics

Subject characteristics at Baseline will be summarized in frequency tables or with summary statistics for continuous variables.

15.3.1.3 Study Treatment

The study treatment administration and compliance profile will be summarized descriptively.

15.3.1.4 Non-Study Treatment in the Study Follow-up Period

The number, type, and extent of use of non-study cancer treatment after study treatment discontinuation will be summarized.

15.3.2 Efficacy Analyses

15.3.2.1 Primary Efficacy Analysis

OS in the ITT population will be compared between the 2 treatment groups using the stratified log-rank test. The estimate of the hazard ratio and corresponding 95% CI will be provided using a Cox proportional hazards (CPH) model including treatment and the

stratification factors (maximum lesion diameter [3-5 cm versus >5–7 cm] and RFA route [laparoscopic, open surgical, percutaneous]) in the model. The survival curves will be estimated using Kaplan-Meier estimates. The stratification factors will be populated as per the randomization assignment.

15.3.2.2 OS - Supportive Analyses

Supportive analyses for OS, conducted in the ITT population (unless otherwise noted), will include:

a. The unstratified log-rank test and a CPH model (only treatment effect t in the model).

b. Multivariate analysis using the CPH model, including the stratification factors and the following set of potential prognostic/predictive factors: age (< 65 v. ≥ 65), race (Caucasian, Asian, Other), region (EU and US, China, Korea, Other), baseline platelets, RFA start time, RFA dwell time (≤ 45 min – for any outliers, ≥ 45 min and ≤ 90 min, ≥ 90 min), device, disease etiology (Hep B, Other).

   Factors included in the model will be assessed for co-linearity and a stepwise selection process will be applied to identify a final subset of prognostic/predictive factors in the model. Once the subset has been established, treatment will be added to the final model to assess its effect.

   An exploratory analysis of treatment by factor interactions using the CPH model will be conducted, using the factors identified in the final model above.

c. Subgroup analyses will also be conducted for the stratification factors and the potential prognostic/predictive factors identified in Section 15.3.2.2.b above. The HR and associated 95% CI will be presented for each subgroup.

d. The primary efficacy analysis, as outlined in Section 15.3.2.1, will also be run excluding any subjects that did not meet key eligibility criteria: a) single HCC lesion between ≥ 3.0 and ≤ 7.0 cm, b) ECOG performance status, c) RFA start time within 20 minutes of starting the infusion, d) RFA dwell time ≥ 45 minutes, and e) baseline platelets ≥ 75,000/mm^3 and f) Child-Pugh A classification. Additional sensitivity analyses will be defined in the SAP.

15.3.2.3 Secondary Endpoint Analyses

All secondary endpoints comparisons will be made at the 2-sided 0.05 significance level. Since PFS is the only secondary endpoint for regulatory registration purposes, no further multiplicity adjustments will be made. Assuming that OS demonstrates significance at the 1-sided 0.025 level, PFS can subsequently be tested at the 1-sided 0.025 level.

PFS will be analyzed with methodology applied to the OS endpoint. Specifically, PFS will be analyzed with the methodology specified in Section 15.3.2.1 and 15.3.2.2. Additional sensitivity analyses, as detailed in the SAP, will account for clinical progression as a PFS event.

15.3.2.4 Safety Analyses

The safety evaluations will focus primarily on AEs and laboratory assessments, but will also include physical examinations, vital signs, ECGs, echocardiograms/MUGA scans hematology, clinical chemistry, and urinalysis beginning at time of signing of informed consent and continuing through Day 28 following study drug administration.. AEs after day 28 visit which are assessed as possibly, probably or definitely related to study treatment will be recorded at any point during the trial. All subjects included in the As-Treated Population will be evaluated by treatment arm in the safety analysis.

Adverse events will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) terminology and the severity of the toxicities will be graded according to the NCI CTCAE criteria, v4.03, where applicable. Concomitant medications will be coded according to the World Health Organization (WHO) Medication Dictionary for Concomitant Medication.

All AEs will be summarized (incidence) and listed by the System Organ Class (SOC), preferred term, toxicity/severity grade, and causal relationship to study medication. In addition, separate summaries of SAEs and Grade 3 and 4 AEs will be presented.

Hematological and chemistry laboratory parameters will be graded according to the NCI CTCAE v.4.03 criteria, where applicable. Absolute values and changes from baseline will be summarized. In addition, worst severity grade, time to event, and time to resolution will also be summarized.

15.4 Determination of Sample Size

15.4.1 Study Population

The study is designed to detect with 80% power a hazard ratio for OS of 0.67 (33% risk reduction) in the ThermoDox (TR) arm compared with the control (PR) arm with an overall 1-sided type 1 error of 0.025. An OS hazard ratio of 0.63 was observed among HEAT Study patients with a solitary 3-7 cm lesion treated with ≥ 45 minutes of RFA. A 3%/year loss to survival follow-up rate has been assumed and using a 1:1 treatment allocation (TR:PR) of 550 subjects, a target of 197 events (deaths) will be required for the primary analysis.

Based on these design operating characteristics and assuming a median survival time of approximately 4.5 years in the control arm, the primary analysis target events milestone will be reached approximately 30 months after the last subject is randomized in the study. The median OS in the control arm was estimated based on the interim OS results in the HEAT study.

15.5 Interim Analyses

An independent Data Monitoring Committee (DMC) will periodically assess the safety data. A description of the roles and responsibilities and details of the review processes are provided in a separate DMC charter.

Two interim analyses, both for efficacy and futility, are planned for the study. The first is planned after 60% of the target events is reached (118 deaths) and the second after 80% of the events has been reached (158 deaths). The Lan-DeMets alpha-spending approach will be used with O'Brien-Fleming stopping boundaries to evaluate efficacy. Fixed HR boundaries will be used to assess futility. This approach will account for multiple testing and preserve the overall 1-sided study significance level of 0.025. Additional details will be provided in the DMC charter.

No futility analyses are planned during the accrual period. Considering the relative short time of the accrual period compared to the slower accumulation rate of the events, there will not be sufficient events to make such assessment in the accrual study stage.

16. DATA HANDLING

16.1 Direct Access to Source Data/Documents

The Investigator and institution agree that Celsion, its representatives (includes CRO, auditors, and monitors), the IRB/REB/IEC, and representatives from worldwide Regulatory Agencies will have the right, both during and after the clinical trial, to review and inspect pertinent medical records related to the clinical trial.

The Investigator must maintain the primary records (i.e., source documents) of each subject's data at all times. Examples of source documents are subject progress notes, clinic notes, hospital records, laboratory reports, drug inventory, study drug label records, and those CRFs that are used as source documentation.

The Investigator will maintain a confidential subject identification list that allows the unambiguous identification of each subject. All study-related documents must be kept until notification by Celsion instructs otherwise.

16.2 Quality Control and Quality Assurance

16.2.1 Monitoring of the Study and Regulatory Compliance

The project manager, or their monitor designee, will make an initiation site visit to each institution to review the protocol and its requirements with the Investigator(s), inspect the drug storage area, and fully inform the Investigator of his/her responsibilities and the procedures for assuring adequate and correct documentation. During the initiation site visit the case report forms (CRFs) and other pertinent study materials will be reviewed with the Investigator's research staff. During the course of the study, the monitor will make regular site visits in order to review protocol compliance, examine CRFs and individual subjects' medical records, and assure that the study is being conducted according to pertinent regulatory requirements. All CRF entries will be 100% verified with source documentation. The review of medical records will be done in a manner to assure that subject confidentiality

is maintained. Sponsor or Regulatory body inspections may occur as well to assure the accuracy of study conduct.

16.2.2 Curricula Vitae and Financial Disclosure of Investigators

All Principal Investigators will be required to provide a current signed and dated curriculum vitae, a completed FDA Form 1572, and a financial disclosure statement to Celsion or their authorized representative. In addition, all Sub-Investigators will be required to provide a current curriculum vitae and a financial disclosure statement.

16.2.3 Protocol Modifications

No modification of the protocol should be implemented without the prior written approval of the Sponsor or the Sponsor's representative. Any such changes, which may affect a subject's treatment or informed consent, especially those increasing potential risks, must receive prior approval by the IRB/REB/IEC. The exception to this is where modifications are necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial (e.g., change in monitor, change in telephone number). Other administrative revisions, which may affect the clinical portion of a study, will be duly reported to the IRB/REB/IEC by the Principal Investigator.

16.3 Data Handling and Record Keeping

The trial developed Case Report Form (CRF) is the primary data collection instrument for the trial. All subject data requested on the CRF must be supported by and be consistent with the patient's source documentation. All missing data must be explained. When a laboratory test, assessment, or evaluation has not been performed or is "unknown", a note should be created verifying that the field was "Not Done" or "Unknown". For any entry error made, the errors must be corrected, and a note explaining the reason for change should be provided.

Any electronic Case Report Forms (eCRF) system would be developed, maintained and utilized consistent with 21 CFR part 11 requirements. Authorized site personnel would require proper training on the system and would have an individual username and password to complete data entry and to address data clarifications.

Any paper Case Report Forms will consist of two-part no-carbon-required paper (NCR) and should be completed in black ink (hard point pen). Incorrect data should not be obliterated. Use of white-out is prohibited. Any correction or deletion should be made by drawing a single line through the entry so that the original entry is still legible. This change must be initialed and dated by the site study coordinator or Investigator. The top sheet will be collected by the monitor. The bottom sheet is for the Investigator files and must be made available for review by Sponsor, IRB/REB/IEC, or appropriate regulatory agencies.

Any changes made to data after collection will be made through the use of Data Clarification Forms (DCF). Data reported on the case report forms, which is derived from source documents, should be consistent with the source documents or the discrepancies should be explained. Case report forms will be considered complete when all missing and/or incorrect

data have been resolved. Please refer to the separate Case Report Form Completion Guideline for further instructions.

16.3.1 Data Collection

Intraoperative data for device type, the number of ablation cycles, the RFA dwell time, probe type, tip length, and probe length will be documented.

Note: A standard sRFA treatment is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to ablate tissue through turning off the RFA generator after the final ablation cycle. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient.

16.3.2 Recording of Data

The Investigator will be responsible for the recording of all data on the CRFs provided, as certified by the Investigator's signature and date on the designated pages. Should any value be significantly different from normal, the Investigator or designee will comment in the appropriate sections provided in the CRFs.

The Investigator will provide access to his/her original records to permit a representative from the Sponsor to verify the proper transcription of data. To facilitate photocopying, any paper CRF entries must be recorded legibly in black ink only. Erroneous entries will be crossed out with a single line, so as to remain legible. The correct value will be entered above the error and then initialed and dated by the person authorized to make the correction.

16.3.3 Study Records

Investigators in the United States, Canada or EU must maintain all study records for the indication under investigation for two years following the date a Product Licensing Application is approved or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and the FDA is notified.

Investigators in Asia must maintain all study records for the indication under investigation for five years following the date a Product Licensing Application is approved.

Subject's medical files should be retained in accordance with applicable legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice. Investigator must contact the Sponsor before moving or removing the study files.

16.4 Financing and Insurance

Financing and insurance information is provided for this study under separate cover.

16.5 Publication Policy

Since the Phase III study is a blinded, randomized, international study for registration purposes, no individual Investigator may publish or disclose clinical trial results until the study is completed. Study completion is defined as FDA review and response of the NDA submission. In addition, Institution acknowledges that, due to the limited patient population in its treatment group, the data generated from its individual participation in the Study and evaluation of its individual results may not be sufficient from which to draw any meaningful scientific conclusions.

Upon study completion, Institution may, upon written notice to Sponsor, participate in a joint, multi-center publication of the study results with other third party Principal Investigators and/or institutions, provided that the proposed publication is first reviewed, commented, and approved by expressed written consent by Sponsor.

Sponsor will provide authorship rights (and lead authorship rights) to Phase III site Investigators in order of greatest contribution of evaluable patients to the Phase III registration study. The term “evaluable” is distinguished from “enrollment contribution” and defined as patients that the FDA accepts as patients qualified for NDA approval. In addition, Sponsor may form a publication committee to evaluate and give final approval of publication submission.

At least sixty (60) days prior to submitting a manuscript to a publisher or other outside persons (i.e., reviewer[s] or prior to any public presentation), a copy of the manuscript or presentation will be provided to Sponsor by the Investigator for review and comment. The Investigator understands and agrees that participation in the Study may involve a commitment to publish the results of the Study in a cooperative publication with other Investigators prior to publication or oral presentation on an individual basis. No publication of confidential information shall be made without Sponsor’s prior written consent, which shall not be unreasonably withheld or delayed. The Investigator agrees, following a request from Sponsor, to delete any confidential information which would prejudice the securing of adequate intellectual property protection from the proposed publication.

17. ETHICAL CONSIDERATIONS

The study will be conducted in the US under Investigational New Drug (IND) Application 66,827, in compliance with Title 21 of the Code of Federal Regulations (CFR), Part 50 (Protection of Human Subjects), and Part 56 (Institutional Review Board) as well as the principles of the Declaration of Helsinki and its amendments and local country-specific requirements. Institutional Review Boards (IRBs) reviewed and approved the protocol and informed consent. All subjects will be required to give written informed consent prior to participation in the study. This study will be performed in accordance with Good Clinical Practices (GCP) by qualified Investigators.

The study specifically incorporates the following features:

- Randomized study design
- Blinding of subjects and Investigators
- Parallel active control group
- Prospectively stated objectives and analytical plan
- Accepted, pre-specified outcome measures for safety and efficacy
- Investigator meeting (and/or a site initiation visit) prior to study start and a detailed protocol to promote consistency across sites
- Compliance with Good Clinical Practices (GCP), with assessment via regular monitoring.

Quality assurance procedures will be performed at study sites and during data management to assure that safety and efficacy data were adequate and well documented.

17.1 Informed Consent

The Investigator will obtain written informed consent from each subject (or their authorized representative) participating in the study. The form must be signed, witnessed and dated according to local acceptable standards. The informed consent form will contain all the Essential Elements of Informed Consent set forth in 21 CFR Part 50, and the ICH Guideline for Good Clinical Practice, Section 4.8 (see Appendix VIII), and the terms of the Declaration of Helsinki. Copies of the signed document should be given to the subject and filed in the Investigator's study file, as well as the subject's medical record if in conformance with the institution's Standard Operating Procedures.

17.2 Institutional Review Board/Research Ethics Board/Independent Ethics Committee

The study will not be initiated without approval of the appropriate Institutional Review Board/Research Ethics Board/Independent Ethics Committee (IRB/REB/IEC) and will comply with all administrative requirements of the governing body of the institution. This protocol, consent procedures, and any amendments must be approved by the IRB/REB/IEC in compliance with current regulations of the FDA and in accordance with ICH/GCPs. A letter of approval will be sent to the Sponsor prior to initiation of the study and when any subsequent modifications are made. The Sponsor or their authorized representative will keep the IRB/REB/IEC informed, as required by regulations, of the progress of the study as well as of any serious and unexpected adverse events.

17.3 Subject Protection

17.3.1 Subject Privacy

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify subjects by initials and assigned subject numbers; subjects should not be identified by name. In accordance with local, national or federal regulations, the Investigator will allow the Sponsor or their designee personnel access

to all pertinent medical records in order to verify the data gathered on the case report forms and to audit the data collection process. Regulatory agencies such as the US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection. No material bearing a patient's name will be kept on file by the Sponsor and patients will be informed of their rights within the ICF.

17.3.2 Rationale for Subject Selection

The selection of subjects for this protocol will not be based on sex, race, or ethnic background.

17.3.3 Participation of Children

Children will not be enrolled in this study.

18. TRIAL ADMINISTRATIVE INFORMATION

18.1 Protocol Amendments

As the trial progresses, any amendments to the study protocol deemed necessary will be communicated to the Investigator by Celsion or its designee. All protocol amendments will undergo the same review and approval process as the original protocol. A protocol amendment may be implemented after it has been approved by the IRB/REB/IEC, unless immediate implementation of the change is necessary for subject safety. In that case, the situation must be documented and reported to the IRB/REB/IEC within 5 working days.

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20. APPENDICES

20.1 Appendix I: Child-Pugh Classification of Hepatic Impairment

	Encephalopathy*	Ascites	Bilirubin (mg/dL)	Albumin (g/dL)	INR
Score					
1	Absent	Absent	< 2	> 3.5	< 1.3
2	I / II	Mild / moderate	2 - 3	2.8 - 3.5	1.3 - 1.5
3	III / IV	Severe	> 3	< 2.8	> 1.5

*** Encephalopathy grades:**

Grade 0: normal consciousness personality, neurological exam and electroencephalogram

Grade 1: restless, sleep disturbed, irritable/agitated, impaired handwriting, 5 cps waves

Grade 2: lethargic, time-disoriented, inappropriate, asterixis, ataxia, slow triphasic waves

Grade 3: somnolent, stuporous, place-disoriented, hyperactive reflexes, rigidity, slower waves

Grade 4: unrousable coma, no personality/behavior, decerebrate, slow 2-3 cps activity

Interpretation:

Child Class A: 5 to 6 points

Child Class B: 7 to 9 points

Child Class C: 10 to 15 points

Source:

Child CG, Turcotte JG. Surgery and portal hypertension. In: The liver and portal hypertension. Edited by CG Child. Philadelphia: Saunders. 1964:50-64.

Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the esophagus in bleeding oesophageal varices. Br J Surg. 1973;60:648-52.

20.2 Appendix II: New York Heart Association (NYHA) Functional Classification for Heart Failure

A functional and therapeutic classification for prescription of physical activity for cardiac patients.

Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities.

Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.

Class III: patients with marked limitation of activity; they are comfortable only at rest.

Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

20.3 Appendix III: Prohibited Medications

The active ingredient compound of ThermoDox (Doxorubicin) is a substrate (major) of CYP2D6, 3A4. Inducers and inhibitors of CYP3A4 may alter the metabolism of ThermoDox. The following CYP3A4 inducers and inhibitors are prohibited from screening through after study treatment. Additionally, medications that could change the pharmacokinetic profile of ThermoDox or modify gastric pH are prohibited. Future updates to the list will be provided to the site as new information becomes available.

Drug Class	Agent	Wash-out[1]
CYP3A4 Inducers		
Antibiotics	all rifamycin class agents (e.g., rifampicin, rifabutin, rifapentine)	14 days
Anticonvulsants	phenytoin, carbamazepine, barbiturates (e.g., phenobarbital)	
Antiretrovirals	efavirenz, nevirapine	
Glucocorticoids (oral)	cortisone (> 50 mg), hydrocortisone (> 40 mg), prednisone (> 10 mg), methylprednisolone (> 8 mg), dexamethasone (> 1.5 mg, except the oral premedication which is part of the protocol)[2]	
Other	St. John's Wort, modafinil	
CYP3A4 Inhibitors		
Antibiotics	clarithromycin, erythromycin, troleandomycin	7 days
Antifungals	itraconazole, ketoconazole, fluconazole (> 150 mg daily), voriconazole	
Antiretrovirals, Protease Inhibitors	delaviridine, nelfinavir, amprenavir, ritonavir, indinavir, saquinavir, lopinivir	
Calcium channel blockers	verapamil, diltiazem	
Antidepressants	nefazodone, fluvoxamine	
GI Agents	cimetidine, aprepitant	
Other	grapefruit, grapefruit juice	
	amiodarone	6 months
Miscellaneous		
Antacids[3]	Mylanta, Maalox, Tums, Rennies	1 hour before and after dosing
Herbal or dietary supplements	Ginkgo biloba, kava, grape seed, valerian, ginseng, echinacea, evening primrose oil	14 days
Intravenous fat emulsions[4]		1 day before and after dosing
Lipid complexed drugs	Fentanyl,	1 day before and after dosing
Liposomal drugs	Abelect™, Ambisome™, Nyotran™	1 day before and after dosing

1. At the time of screening, if a subject is receiving any of the above listed medications/substances, the medication or substance must be discontinued (if clinically appropriate) for the period of time specified prior to administration of the first dose of study drug treatment and throughout the period discussed above in order for the subject to be eligible to participate in the study.
2. Glucocorticoid daily doses (oral) ≤ 1.5 mg dexamethasone (or equivalent) are allowed. Glucocorticoid conversions are provided in parentheses.
3. Antacids may have an impact on absorption of oral premedication dexamethasone due to a change in stomach pH.
4. Intravenous fat emulsions, with the exception of Propofol, are prohibited.

20.4 Appendix IV: RFA Treatment Procedure

Treating Physician Qualifications

- Must have performed radiofrequency ablation therapy on hepatic tumors in at least 15 patients.
- Must have used RFA device that will be used in protocol to treat hepatic tumors in at least 5 patients.

Ablation Equipment

Only FDA-approved RFA equipment marketed by Covidien, Boston Scientific Corp., and Angiodynamics (RITA) will be used in the protocol; however, each doctor (Principal Investigator or sub-Investigator) performing RFA will be limited to the use of a single manufacturer's device for all treatments for an individual patient.

- Covidien RFA Equipment
  Monopolar RF ablation generator with automated ablation algorithm (using the current model, which may be replaced with more current models during the course of the study.)
  Cool-Tip RF ablation needles.
  Covidien generator Switch Box.
  Dispersive Electrodes.
- Boston Scientific Corp. RFA Equipment
  Radiofrequency Generator (using the current model, which may be replaced with more current models during the course of the study.)
  LeVeen Needle Electrodes.
  Dispersive Electrodes.
- Angiodynamics (RITA) RFA Equipment
  Radiofrequency Generator (using the current model, which may be replaced with more current models during the course of the study).
  StarBurst Electrodes.
  Dispersive Electrodes.

Ablation Strategy for Individual Tumors

The following are the minimum requirements for ablating tumors of a given size. At least 4 ablation cycles lasting a total of ≥ 45 minutes should be performed. Since all tumors are not perfect spheres, the operator should use the maximum diameter to choose the appropriate ablation strategy. All tumors should be treated with the goal of ablating the tumor as well as a 360^{o} 1.0 cm tumor free margin around each tumor. Updated operational manuals for each RFA device, detailing the specifics of electrode vs. tumor size, should be followed accordingly.

20.5 Appendix V: Adverse Events (AEs) and Criteria for Determining Relationship of AE to Treatment

Criteria for Determining Category of Relationship of Clinical Adverse Events to Treatment

1	Not related	This category applies to those adverse events, which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
2	Unlikely (must have two)	In general, this category can be considered applicable to those adverse events, which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug or/and procedure. An adverse event may be considered unlikely if or when: 1. It does not follow a reasonable temporal sequence from administration of the test drug or/and procedure. 2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It does not follow a known pattern of response to the drug/procedure. 4. It does not reappear or worsen when the drug/procedure is re-administered.
3	Possibly (must have two)	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug or/and procedure administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when: 1. It follows a reasonable temporal sequence from administration of the test drug/procedure. 2. It could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. 3. It follows a known pattern of response to the test drug/procedure.
4	Probably (must have three)	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug or/and procedure. An adverse event may be considered probably related if or when:

1. It follows a reasonable temporal sequence from administration of the test drug/procedure.
2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.

   It disappears or decreases on cessation or reduction in dose.
3. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia).
4. It follows a known pattern of response to the test drug/procedure.

5 Definitely (must have all) This category applies to those adverse events that the Investigator feels are incontrovertibly related to test drug or/and procedure. An adverse event may be assigned an attribution of definitely related if or when:

1. It follows a reasonable temporal sequence from administration of the test drug/procedure.
2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose with re-exposure to drug. (Note: This is not to be construed as requiring re-exposure of the subject; however, a category of definitely related can only be used when a recurrence is observed.)
4. It follows a known pattern of response to the test drug/procedure.

The Investigators are to record the Outcome to Date using the following four categories:

Outcome		
Code	Descriptor	Definition
1	Recovered	The patient fully recovered from the adverse event with no residual effect observable.
2	Adverse Event Still Present	The adverse event itself is still present and observable.
3	Adverse Event Resolved	The adverse event resolved, but residual effect(s) are still present and observable.
4	Death	Death
5	Unknown	The outcome of the adverse event could not be determined.

20.6 Appendix VI: ECOG Performance Status

Grade	Functional Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care. Confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

ECOG = Eastern Cooperative Oncology Group, Robert Comis MD, Group Chair.

Source: Oken MM, Creech R, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

20.7 Appendix VII: Requirements of Written Informed Consent Document (21 CFR 50.20 and 50.25 and ICH GCP, Topic E6, Section 4.8)

Basic Elements of Informed Consent:

In seeking informed consent, the following information shall be provided to each subject:

- A statement that the study involves research, an explanation of the purpose of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures that are experimental.
- A description of any reasonably foreseeable risks or discomforts to the subject.
- A description of any benefits to the subject or to others that may reasonably be expected from the research.
- A disclosure of appropriate alternative procedures or courses of treatment, if any, which might be advantageous to the subject.
- A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration or other regulatory agency may inspect the records.

For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.

An explanation of whom to contact for answers to pertinent questions about the research and research subject's rights, and whom to contact in the event of a research-related injury to the subject.

A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Additional Elements of Informed Consent:

When appropriate, one or more of the following elements of information shall also be provided to the subject:

- A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant), which are currently unforeseeable.
- Anticipated circumstances under which the subject's participation may be terminated by the Investigator without regard to the subject's consent.
- Any additional costs to the subject that may result from participation in the research.

- The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.
- A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.
- The approximate number of subjects involved in the study.

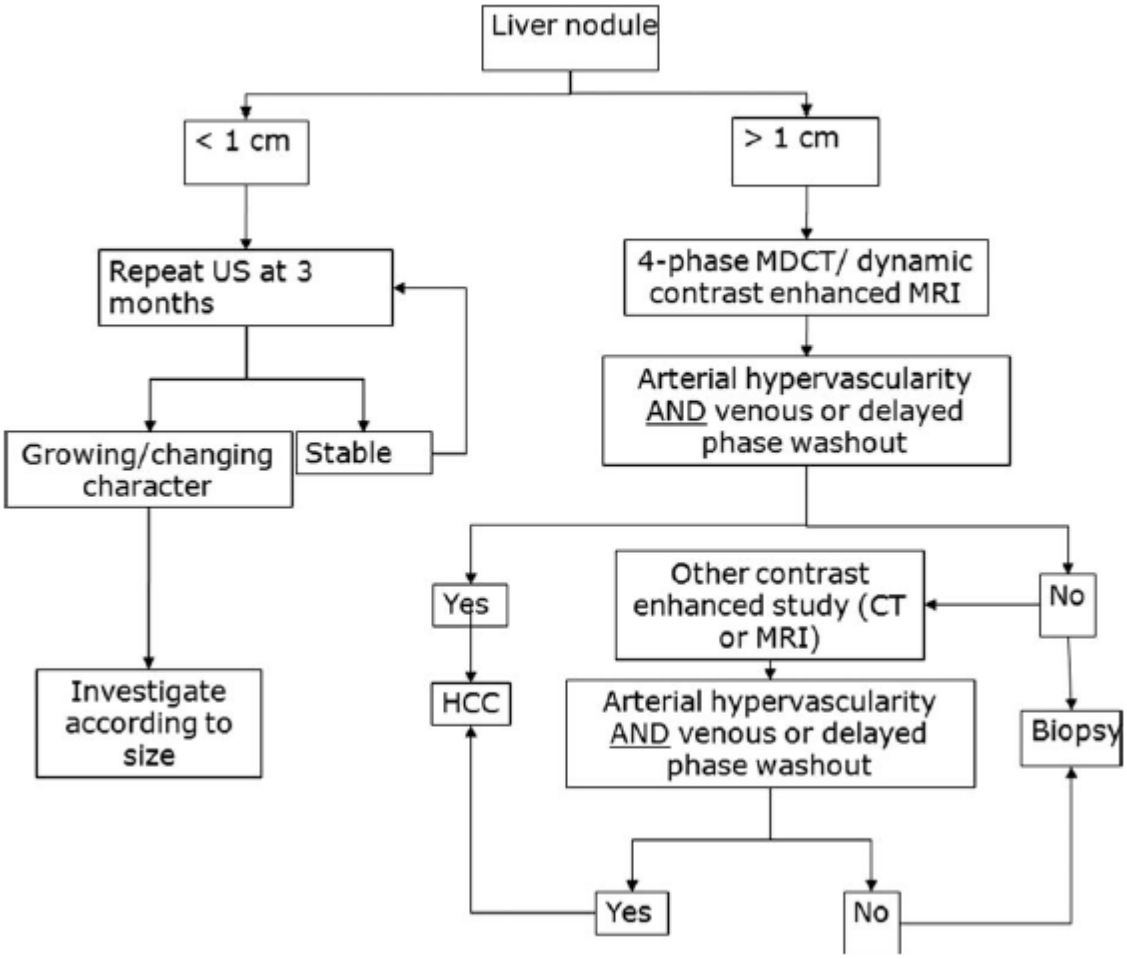
20.8 Appendix VIII: AASLD Diagnostic Criteria

This section summarizes diagnostic procedures from the American Association for the Study of Liver Diseases' 2010 update to its practice guidelines (Bruix and Sherman 2011).

HCC can be diagnosed radiologically, without the need for biopsy if the typical imaging features are present. This requires a contrast-enhanced study (dynamic CT-scan or MR). In the arterial phase, HCC enhances more intensely than the surrounding liver. In the venous phase, the HCC enhances less than the surrounding liver.

Thus, to properly document the existence of HCC, a 4-phase study is required: unenhanced, arterial, venous and delayed phases.

For lesions above 1 cm in diameter, either dynamic MRI or multidetector CT scanner should be used. If the appearances are typical for HCC on either MRI or CT scan, as described above, then no further investigation is required and the diagnosis of HCC is confirmed. If the appearances are not typical for HCC (and do not suggest hemangioma), then one of two strategies is possible. A second study (the other of CT scan or MRI) could be performed. If the appearances are typical, the diagnosis is confirmed. Alternatively, an atypical study could trigger a biopsy.


Liver nodule
< 1 cm
> 1 cm
Repeat US at 3 months
4-phase MDCT/ dynamic contrast enhanced MRI
Growing/changing character
Stable
Arterial hypervascularity AND venous or delayed phase washout
Investigate according to size
Yes
Other contrast enhanced study (CT or MRI)
No
HCC
Arterial hypervascularity AND venous or delayed phase washout
Biopsy
Yes
No

20.9 Appendix IX: Link to the most recent version of NCI Common Toxicity Criteria for Adverse Events (CTCAE), Version 4

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

20.10 Appendix X: Description of Amendment Changes

Protocol Version 1.0 13-January-2014 to Version 2.0, 14-March-2014

Sect	Description/Rationale	Changed to	Changed From
Cover	Protocol Version Date	Version 2.0, 14-Mar-2014	Version 1.0, 13-Jan-2014
Cover	The EudraCT Number was issued for this trial	2014-000934-53	2008-000963-41
Synopsis, Section (S) 3.3 ThermoDox, S8.1, S15.3.2.2	FDA requested stratification by RFA route.	Randomization and analysis will be stratified by maximum lesion diameter (3-5 cm versus > 5-7 cm) and RFA route (laparoscopic, open surgical, percutaneous).	Randomization and analysis will be stratified by maximum lesion diameter (3-5 cm versus > 5-7 cm)
Synopsis, 5.1	Standardized monitoring interval for PFS to ensure consistency across sites	Following study treatment, subjects will undergo CT or MRI imaging scans at months 1, 5, 9, 13, 17, 21, and 25 (+/- 2 weeks), then at 6-month intervals (+/- 2 weeks) until radiological progression is seen..	Following study treatment, subjects will undergo CT or MRI imaging scans consistent with local standard of care at each institution at an interval not > 6 months from the prior scan until radiological progression is seen.

Sect	Description/Rationale	Changed to	Changed From
Synopsis, 6.2.3	Inclusion criteria update to characterize RFA eligibility and removed term. In additional all investigators that are RFA operators will complete formal training with an accompanying RFA training manual developed by the Sponsor. Overall health of livers since this is repetitive to the Child-Pugh Score inclusion criteria.	• The position and accessibility of the target lesion allows for the safe administration of multiple ablation cycles or deployments to achieve a probe dwell time of > 45 minutes. • DELETED "Overall health of the liver	• Procedure is expected to require multiple burn cycles or deployments to achieve an RFA probe dwell time ≥ 45 minutes. • Overall health of the liver.
Synopsis: Exclusion Criteria	Amended to be consistent with the clinical program development to date	Platelet count < 75,000/mm^3	Platelet count < 60,000/mm^3
Synopsis; 5.1: 8.3	Added term "Only" for clarification	Only FDA approved RFA devices are permitted for this trial.	FDA approved RFA devices are permitted for this trial

Sect	Description/Rationale	Changed to	Changed From
Synopsis	Updated this section of the synopsis to be consistent with section 8.4	A standardized Radiofrequency Ablation sRFA treatment for this protocol is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to produce coagulative necrosis of target tissue through removal of the RFA probe after the final ablation cycle or deployment. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient. An ablation cycle for this protocol is defined as the single activation of the probe or electrode in order to achieve a local coagulation necrosis in target tissue. The cycle may be completed by achieving a target temperature in the tissue or a target impedance.	A standardized Radiofrequency Ablation (s)RFA treatment is defined as the dwell time of ≥ 45 minutes measured from the first activation of the RFA probe to ablate tissue through turning off the RFA generator after the final ablation cycle. This includes the multiple ablation cycles and repositioning time between cycles for an individual patient.
PROTOCOL SYNOPSISSynopsis, S4.2.1, S15.3.2.4	AE reporting requirement update to confirm events possibly, probably or definitely related to study treatment will be recorded at any point during the trial	AEs which are assessed as possibly, probably, or definitely related to study treatment will be *recorded at any point during the trial and must be* followed until resolution or until the patient is clinically stable.	AEs which are assessed as possibly, probably, or definitely related to study treatment will be followed until resolution or until the patient is clinically stable.

Sect	Description/Rationale	Changed to	Changed From
3.2	Clarification to note RFA for lesion >5 cm and < 7 cm may be outside standard treatment in some regions. The sRFA/ThermoDox combination is being investigated as a curative treatment rather than as a palliative treatment. Some centers may not enroll this population. The risks included in the consent form.	In the United States, palliative treatments such as transcatheter arterial chemoembolization (TACE) and sorafenib maybe standard treatment options for HCC tumors > 5 cm. However, in this study the combination of RFA and ThermoDox is being investigated as a curative treatment for patients with tumors > 5 cm to ≤ 7 cm rather than for their palliation.	New text.
S4.2.1, S12.4.1	PFS definitions clarified for consistency	PFS will be measured from the date of randomization to the first date on which one of the following occurs: • Death of any cause • Treatment failure (inability to achieve CR after two RFA ± ThermoDox treatment sessions) • Progression due to local tumor recurrence after initial CR • Progression due to distant intrahepatic tumor recurrence • Progression due to extrahepatic tumor recurrence	PFS will be measured from the date of randomization to the first date on which one of the following occurs: As determined by CT or MRI Scan An incomplete ablation following two RFA procedures Local recurrence of HCC tumor after complete initial ablation Any new distant intrahepatic HCC tumor Any new extrahepatic tumor Death from any cause

Sect	Description/Rationale	Changed to	Changed From
S6.2.4	Added text for explaining potential risks.	Doxorubicin HCl, the active agent in ThermoDox, is routinely given in chemotherapy regimens for multiple cycles after appropriate recovery intervals, usually at least 3 weeks. In contrast, the vast majority (84.8%; 291/343) of subjects in the RFA plus ThermoDox arm in the prior trial received only a single dose of ThermoDox.	Doxorubicin HCl, the active agent in ThermoDox, is routinely given in chemotherapy regimens for multiple cycles after appropriate recovery intervals, usually at least 3 weeks.
S6.2.4	Added text for explaining potential risks for patients with elevated bilirubin levels at baseline and to describe plan to mitigating risk during the trial	In the prior study, subjects with baseline serum bilirubin > 2.0 mg/dL had a higher incidence subsequent grade 3+ hyperbilirubinemia and of grade 3+ hepatic failure. However, the greater incidence of hepatic failure consisted of a single patient with grade 3 liver failure. Among patients with an elevated bilirubin level at baseline, there were no subsequent serious hepatobiliary adverse events except for 2 cases of hyperbilirubinemia/blood bilirubin increased. The DMC is responsible for unblinded monitoring of subjects with elevated (> 2.0 mg/dL and $\leq$ 3.0 mg/dL) serum bilirubin levels and will advise if any dose or protocol modification is needed to mitigate risk to such patients.	New text.

Sect	Description/Rationale	Changed to	Changed From
S6.2.4	Include potential for increased risk to patients if a biopsy has not confirmed HCC.	AASLD treatment guidelines permit the diagnosis with the use of imaging modality only without a biopsy histological or cytological confirmation of the specimen. Procurement of a biopsy increases risk to subjects with liver disease and therefore is not always indicated. However patients who undergo an RFA treatment without a biopsy may be at increased risk for undergoing an unnecessary ablation or be at risk for seeding for subcapsular or poorly differentiated disease. This risk is minimal and accepted within clinical practice guidelines.	New Text

Sect	Description/Rationale	Changed to	Changed From
S6.2.4	Include potential increased risk for subjects whose lesions are > 5 cm.	RFA has been used safely in tumors up to 7 cm (Chen, et al. 2004; Curley, et al. 2004; Dodd, et al. 2001; Tateishi, et al. 2005). In the United States and other local regions, palliative treatments such as transcatheter arterial chemoembolization (TACE) and sorafenib are the standard of care for HCC tumors > 5 cm. However, in this study the combination of RFA and ThermoDox is being investigated as a curative treatment for patients with tumors > 5 cm to ≤ 7 cm rather than for their palliation. There is risk that RFA is not as effective in the larger tumors, however this has not been established. Subjects would be eligible to receive palliative therapy as medically indicated after progression is observed	New Text
S 7.2	Study Discontinuation- New text to clarify subjects may select to continue in OS. Site personnel should discusswith subject.	If a subject discontinues from retreatments or from the PFS imaging interval, the patient should still be contacted for overall survival until withdraw consent for study participation.	New text

Sect	Description/Rationale	Changed to	Changed From
S7.3	Study non-compliance- New text to clarify subjects may continue in OS. Site personnel should discuss with subject.	If a subject is discontinued from the study due to noncompliance of follow up visits, the patient should still be contacted for overall survival until end of study or withdraw consent from study participation.	New text
S8.5.4	Guidance to investigator for non-hematologic toxicities >grade 3 prior to a retreatment	Among patients requiring re-treatment after incomplete initial ablation who have developed a non-hematologic toxicity ≥ grade 3, no dose modification is required. Instead, such subjects will not be re-treated until the severity of the non-hematologic toxicity drops to ≤ grade 2.	New text.
S10.1.4, S10.1.5, S10.1.8	Standardized 4 month safety interval surveillance to include PE, Vitals, ECGs.	• Month 5, Month 9, Month 13 (+/- 2 weeks) or disease progression, whichever occurs first	Patients who receive study retreatment will have an additional physical exam at Month 3 following each retreatment.

Sect	Description/Rationale	Changed to	Changed From
S10.1.7	Standardized 4 month PFS secondary endpoint monitoring	CT or MRI scans (Chest, Abdomen, and Pelvis) will be done within 21 days prior to study treatment to confirm the presence of an evaluable lesion and at Day 28 to assess whether a complete initial ablation was achieved. Additional imaging will be done at months, 5, 9, 13, 17, 21, 25, then every 6 months (+/- 2 weeks) until disease progression is seen. The following requirements apply to imaging for this study: • The same modality and technique must be used for all scans of an individual patient.	CT or MRI scans will be done within 21 days prior to study treatment to confirm the presence of an evaluable lesion and at Day 28 to assess whether a complete initial ablation was achieved. Imaging procedures, follow-up intervals, and the determination of progression will be done according to the local standard of care with the following exceptions: • The same modality and technique must be used for all scans of an individual patient. • The follow up imaging interval cannot exceed 6 months.
S10.1.9	1 month adjustment for LVEF exam at 1 year and then language to advise when repeat scan is necessary.	Follow-up scans must be completed on Day 28 (+/- 3 days) and at Month 13 or at time progression of disease, whichever occurs first. A decrease of >10% in resting LVEF that becomes abnormal (lower than the institutional lower limit of normal) should be further evaluated by repeat examination for subjects with a total cumulative doxorubicin of ≥ 300 mg/m^2.	Follow-up scans must be completed on Day 28 (+/- 3 days) and at Month 12 or at time progression of disease, whichever occurs first.

Sect	Description/Rationale	Changed to	Changed From
S10.1.11	Standardized long term safety surveillance for Liver Function Testing	Additional post-treatment liver function tests (Chemistry and PT/INR), will be collected at months 5, 9, 13, 17, 21 and 25 (+/- 2 weeks) or disease progression, whichever occurs first.	Post-treatment liver function tests (Chemistry and PT/INR), will be collected at intervals not to exceed 6 months following study drug administration through Month 12.
S10.2	Clarification to simplify understanding.	The second ablation must occur no later than 21 days after the first post-ablation scan assessment at Day 28.	The second ablation must occur no earlier than 21 days after the initial ablation and no later than 21 days after the first post-ablation scan assessment at Day 28.
S10.3	Imaging intervals and liver function test intervals are standardized elsewhere in the protocol	No replacement language	Radiological Imaging interval and Liver Function Test can be scheduled according to local standard treatment guidelines at intervals not to exceed 6 months. The radiological imaging will continue until Investigator determined radiological progression. The Liver Function Tests are to be monitored through Month 12 following last study drug infusion.

Sect	Description/Rationale	Changed to	Changed From
S12.1	Assessment of efficacy definitions were added to the protocol to ensure consistency across sites	All patients will be monitored for survival by recording their visits during routine follow up for response to treatment. The visits are scheduled to occur every four months from the first imaging study confirming complete ablation until month 25 or radiological progression, whichever comes first. If patients have not demonstrated radiological progression at month 25 then the imaging visit schedule is reduced to every six months until progression. Survival is confirmed at every imaging visit. Once radiological progression is confirmed then follow up for survival is required. Sites are required to confirm contact with the subject during either a clinic visit or a telephone contact every three months. It is expected that subject follow up will be about five years.	Additional language.

Sect	Description/Rationale	Changed to	Changed From
S12.2	Secondary Endpoint progression free survival includes mRESIST details to ensure consistency during standardized interval training.	The protocol incorporates modified RECIST (mRECIST) developed for HCC clinical research as a basis to evaluate tumor response. The mRECIST enables assessment of overall response by taking into account target lesion response, and presence or absence of new lesions. A separate imaging manual and imaging training program has been developed for this study in order to ensure uniformity in imaging technique and imaging review in accordance with mRECIST.	Additional language.

Sect	Description/Rationale	Changed to	Changed From
S12.3	Baseline Assessment definitions added for consistency	The baseline assessment must include a comprehensive evaluation of the chest, abdomen and pelvis. The HCC tumor identified on the eligibility scan should be defined as target lesion and its longest diameter should be measured on the contrast-enhanced CT or contrast-enhanced MRI scan. This measurement should reflect the overall longest tumor diameter, regardless of the presence of internal areas of spontaneous necrosis. In the study protocol, intermediate size is defined as a tumor lesion larger than 3.0 cm but not exceeding 7.0 cm in longest diameter. Since the study will recruit patients with solitary tumors and no evidence of vascular invasion or extrahepatic spread, the eligible size HCC lesion detected on the eligibility scan is expected to be the only target lesion. No additional target or non-target lesions should be detectable on the eligibility scan to allow enrollment in the study	Additional language.

Sect	Description/Rationale	Changed to	Changed From
S12.4	Target lesion local response and local recurrence added for guidance to investigators in reporting PFS endpoint.	The image acquisition protocols used for any post-baseline assessments should be consistent with the protocol used for the baseline eligibility scan. Baseline images for treatment eligibility will be confirmed centrally before RFA treatment. Investigator determined radiological confirmation of complete ablation will occur at one month post RFA. If initial RFA is incomplete then a second, and final, attempt may be made for a complete ablation for subject to stay on protocol. Once a complete ablation is confirmed then patients are monitored radiologically until progression. The imaging interval will be every four months until month 25 then every six months thereafter. No imaging studies are required once progression is confirmed. The disappearance of any intratumoral enhancement in the target tumor lesion after treatment with study treatment will be classified as complete response (CR), while the persistence of any areas of intratumoral enhancement consistent with residual viable tumor will be considered as incomplete response (IR). Failure to achieve a CR after two RFA ± ThermoDox treatment sessions will be classified as treatment failure. Tumor recurrence detected within or around the target lesion after having achieved initial CR will be classified as local recurrence. Tumor recurrences will be defined as local if they are located 1 cm or less from the ablation margin	Additional language.

Sect	Description/Rationale	Changed to	Changed From
S12.4.1	New definition of Equivocal response in newly created Table 3 and addition of Table 2Table 4, Overall Responses for Possible combination of Tumor Responses.	Equivocal (EQ) A new liver lesion that is smaller than 1 cm in longest diameter OR fails to show a typical enhancement profile should be considered as equivocal and not conclusive for disease progression. Such lesions can be diagnosed as HCC by evidence of either a change in enhancement pattern OR at least 1-cm interval growth in subsequent scans. *Plus Table 4 titled Overall Responses for possible combination of tumor responses in the target lesion with or without the appearance of new intrahepatic or extrahepatic lesions.	New text.
S14	Additional responsibilities for the DMC.	A Data Monitoring Committee (DMC) will evaluate safety data on an ongoing basis at regular intervals during subject accrual and follow-up. The DMC is responsible for unblinded monitoring of subjects with elevated (> 2.0 mg/dL and ≤ 3.0 mg/dL) serum bilirubin levels and will advise if any dose or protocol modification is needed to mitigate risk to such patients. The Sponsor is responsible to report relevant SAEs to applicable local, national, and/or international regulatory body.	A Data Monitoring Committee (DMC) will evaluate safety data on an ongoing basis at regular intervals during subject accrual and follow-up. The Sponsor is responsible to report relevant SAEs to applicable local, national, and/or international regulatory body.

END OF PROTOCOL