INVESTIGATIONAL PLAN

"MULTI-CENTER, OPEN-LABELED, NON-RANDOMIZED STUDY TO EVALUATE THE TECHNICAL PERFORMANCE AND SAFETY PROFILE OF THE VORTX Rx® FOR ABLATION OF PRIMARY AND METASTATIC LIVER TUMORS (Theresa Study)"

Protocol Number: 03.CP.0.3

Protocol version: 3.0, 26 September 2018

Sponsor: HistoSonics, Inc.

Coordinating investigator:

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1 PROTOCOL SUMMARY

1.1 STUDY TITLE

Multi-center, open-labeled, non-randomized study to evaluate the acute technical performance and safety profile of the VORTX Rx® for ablation of primary and metastatic liver tumors (Theresa Study).

1.2 PROTOCOL CODE

03.CP.0.3

1.3 PROTOCOL VERSION

3.0, 26 September 2018

1.4 SPONSOR

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1.5 INVESTIGATORS AND SITES

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See signature page for sponsor and Principal Investigator agreement on the protocol, including compliance with ISO 14155, data protection laws, and Declaration of Helsinki.

Participating investigators and sites:

This is a multi-center study comprised of three (3) independent centers located in the greater metropolitan Barcelona area. The coordinating investigator, Dr. Joan Vidal-Jove, will perform all ablation procedures during the study.

The coordinating investigator, Dr. Vidal-Jove, is an experienced surgical oncologist who leads the first High Intensity Focused Ultrasound (HIFU) Unit in Surgical Oncology in Spain, where he has performed more than 200 HIFU procedures in the last years for liver, pancreas and other solid tumors.

1.6 Monitor Local Contact

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1.7 Clinical Research Ethics Committee

The study will be evaluated by the Ethics Committee for investigation with medicinal products (hereinafter CEIm for its initials in Spanish, *Comité Ético de la Investigación con medicamentos*) of Mútua Terrassa University Hospital.

1.8 Disease under study

The disease under study is liver cancer. Patients with primary hepatocellular carcinoma (HCC) and patients with liver metastases from colorectal, breast, lung or pancreatic cancers will be included.

1.9 Primary Objective

The primary objective of the study is to evaluate the acute technical performance of the VORTX Rx® medical device for the ablation of primary and metastatic liver tumors.

1.10 Primary Endpoint

The primary endpoint of the study is acute technical success, consisting of success in treating the planned ablation volume (PAV), defined as the ability of the investigational device to create an ablation zone per the PAV (a three-dimensional volume defined by the investigator using the VORTX Rx® in the planning phase of the software workflow), as measured by multi-dimensional imaging *a*nd characterized qualitatively in the radiological report 1-day post ablation. The acute technical success of up to 3 separate tumor ablations in a single patient will be assessed.

1.11 Secondary Objectives

- Assessment of the safety profile of the VORTX Rx®.
- Assessment of local tumor progression by MRI at 1 week, 1 month and 2 months, post-procedure.
- Assessment of the involution of the ablation zone at 1 week, 1 month and 2 months, post-procedure.

- Assessment of liver panel.
- Immunologic assessment.
- Evaluation of quality of life.
- Assessment of pain and analgesic requirements after the ablation procedure.

1.12 STUDY DESIGN

This is an open-label (no masking), non-randomized, multi-center study comprised of three (3) independent centers located in the greater metropolitan Barcelona area.

The coordinating investigator, Dr. Joan Vidal-Jove, will perform all ablation procedures during the study.

The primary objective of this study is to evaluate the acute technical performance of the VORTX Rx® medical device for the ablation of primary and metastatic liver tumors.

Ablation of the targeted tissue will be evaluated using MRI analysis following a predefined imaging protocol to assess acute technical success defined as the ability of the investigational device to create an ablation zone per the planned ablation volume (PAV) (a three-dimensional volume defined by the investigator using the VORTX Rx in the planning phase of the software workflow). CT analysis and ultrasound with or without contrast may be also utilized per investigator discretion.

The pre-specified technical success criteria and imaging protocol greatly reduces the possibility of unplanned changes being made based on interim trial findings, reducing operational bias and supporting findings of scientific integrity of this study.

This technical performance study will use the VORTX Rx® device to deliver acoustic energy for cavitation-based cellular destruction. The planned ablation duration of the target tumor will be 60 minutes or less and adjusted intra-procedurally as necessary per investigator discretion. Subjects in this study must have an adequate acoustic window in the abdominal space in order to be eligible for enrollment. All patients who undergo ablation with the investigational device will be treated in a hospital environment under general anesthesia not to exceed four (4) hours.

Up to three separate ablation sessions will be allowed to be performed in a single patient during the study with a minimum of 1 month between sessions. It will be allowed to treat more than one tumor in the same session whenever the total volume of the tumors ablated is determined to be less than or equal to the volume of a single 3 cm diameter tumor. Each tumor is required to meet the criteria defined for a targeted tumor in the study protocol: diameter of less than or equal to 3 cm (≤3 cm) and clearly separated from other tumors or other critical areas (i.e. located in different segments of the liver).

Targeted tumors can be retreated upon investigator discretion (i.e. in case that residual tumor is detected). Retreatment of a tumor will be performed 1 month after the prior ablation of the tumor.

In all cases, at least a one-month period must have elapsed between the last ablation performed and the next ablation session in any individual patient.

Retreatment of a tumor and ablation of a new tumor are not allowed to be performed in the same session. Any required re-treatment will be performed prior to any new treatment in any individual patient. Treatment of a new tumor in a new patient will be allowed prior to retreatment of a tumor in a previous patient.

For safety purposes, this study will take a two (2) step approach:

Step 1:

a. The first subject enrolled will be observed and assessed over a 24-hour period and shall not have experienced any unexpected serious adverse event attributable to the study device.

b. After completion of Step 1a, the second subject enrolled will be observed and assessed over a 24-hour period and shall not have experienced any unexpected serious adverse event attributable to the study device.

Step 2:

- Once Step 1a and 1b are complete, the remaining subjects, after meeting all inclusion (and no exclusion) criteria may be enrolled and treated on a first-come, first-served basis.

Should the first or second subject experience an unexpected serious adverse event attributable to the investigational device, the principal investigator will consult with the Data and Safety Monitoring Board (DSMB), his co-investigator(s) and the sponsor and together determine the appropriate next course of action regarding study. If after such consultation and review, it is determined that the study resume, a third patient will be enrolled and monitored in a similar fashion before commencing with successive enrollment.

An independent DSMB will be used for review of safety data to provide advice to the Sponsor to ensure safety, protection and well-being of subjects enrolled in the study. Additional details regarding the structure of the DSMB is provided in Section 9.5.

The study enrollment period is expected to require up to twelve (12) months and the study follow-up period per patient will be a minimum of two (2) months with a maximum follow up period to be determined by the number of tumors ablated and what is medically appropriate. However, it is anticipated that no more than 9 months should be required to complete patient follow up, or until the withdrawal of the patient due to any reason, whichever is first. Therefore, the total study duration may extend to approximately 21 months.

The study will consist of:

- a screening period (within the 2-month period prior to the ablation procedure) that includes a pre-planning visit in which the procedure simulation will be performed using the VORTX Rx® medical device to confirm visualization of target tumor and expected ablation volume using B-Mode Ultrasound,
- the ablation procedure,
- four post-ablation study visits: one (1) day, one (1) week and one (1) and two (2) months post-ablation.

This schedule will apply for each tumor ablated (or combinations of tumors ablated in the same session) (up to a total of 3 separate ablation sessions) and for retreated tumors.

1.13 STUDY MEDICAL DEVICE

Investigational medical device: VORTX Rx® (HistoSonics, Inc., Ann Arbor, MI, USA)

The HistoSonics VORTX Rx design and proposed intended purpose is for the ablation of soft tissue, including liver tumors. This device is for *Investigational Purposes Only* and will NOT be CE Marked. The proposed conformity assessment and rational of the VORTX RX® System is a Class IIb, rule 9 device. It is an active, non-implantable medical device. The primary mode of action is to ablate soft tissue through mechanical focused ultrasound cavitation and is software controlling. (*Global Medical Device Nomenclature (GMDN* - 57873*)* - therapeutic ultrasound systems and associated devices). The system is a portable ultrasound medical device designed to ablate soft tissues, including liver tissue, using very low duty cycle ultrasound pulses applied

from an ultrasound transducer outside the patient's body (extracorporeal). Ultrasound imaging is used for procedure planning, image-guided targeting, and real-time ablation feed-back while under direct physician control.

The fundamental part of the ultrasonic transducer is the piezoelectric element, which vibrates when electricity is applied to it. This produces ultrasonic waves that propagate through tissue. By arranging the array of elements into a concave formation, the transducer is capable of focusing ultrasonic waves that increase in strength at the focal point. When these waves are applied in pulses they create an effect known as "histotripsy," focused non-thermal ultrasound ablation.

The transducer is aimed at a target, in this case liver tissue. Alternating pressure waves pass through the tissue with the negative pressures being low enough to pull gas out of surrounding tissues and form bubbles. The collapse of these bubbles and repeated pressure changes ablate the targeted tissue. Using this method, the ablation can be precisely controlled.

1.14 STUDY POPULATION

Study population: consenting adult patients (aged ≥18 years) with inoperable HCC or patients with unresectable liver metastases from colorectal, breast, lung or pancreas cancer, with a maximum of 3 targeted tumors of less than or equal to 3 cm (≤3 cm) are candidates for this study. The target population for this study will be patients with liver tumors that are not candidates for surgical resection and not suitable for other locoregional treatments for any reason or have not responded to conventional therapies. Inclusion/exclusion criteria are fully described in section 5.

Number of patients: up to 10 patients will be included in the study.

1.15 STUDY TIMELINES

The study is expected to follow the timelines below (subject to changes due to administrative processes and study start-up):

- Study submission to the CEIm and Spanish Agency of Medicines and Medical Devices (AEMPS for its initials in Spanish, Agencia Española de Medicamentos y Productos Sanitarios): Q4 2017.
- Study approval (CEIm and AEMPS]): Q4 2017.
- Inclusion of patients in the study: Q1 2018 – Q1 2019 (12 months)
- Follow-up period end: Q2-Q3 2019.
- Closure of database: Q4 2019.
- Data analysis: Q4 2019.
- Final study report: Q1 2020.

2 BACKGROUND AND STUDY RATIONALE

2.1 LIVER TUMORS

The liver is a common site for both primary malignancy and metastatic disease. Hepatocellular carcinoma (HCC), accounting for 70-90% of cases of primary liver cancer, is the most common primary malignancy of the liver. HCC is a major health problem worldwide associated with high morbidity and mortality. It is the fifth most common cancer and the second most common cause of cancer-related death in the world (1). Its age-adjusted worldwide incidence has been estimated at 16 cases per 100,000 people (2) and accounts for >600,000 new cases annually worldwide (3-5). In Spain over 3,000 cases are diagnosed every year (14 for every 100,000 men and 4 for every 100,000 women). Most cases of HCC are secondary to either viral hepatitis infection or cirrhosis. Indeed, HCC is the leading cause of death among patients with liver cirrhosis (6).

Hepatic metastases are present in approximately 40% to 50% of adult patients with extra-hepatic primary tumors (7). The liver is the most common site of distant metastasis from colorectal cancer (CRC) or other gastrointestinal tract cancers (colorectal liver metastases [CLM]). CRC is a major cause of death due to cancer worldwide and the second leading cause of cancer mortality in Europe (1). Approximately 25% of patients with CRC present with liver metastases at diagnosis and up to 50% develop liver metastases over the course of the disease (6). Liver metastases are found in 6-25% of patients with metastatic breast cancer (8) and in up to 50% of patients with pancreatic cancer (9).

The first-line treatment for hepatic tumors is surgical resection or transplantation. Liver transplantation is the optimal choice for patients with a single HCC or early multifocal disease and decompensated cirrhosis. However, only a small patient population will receive this treatment (about 5%) since eligibility for liver transplantation requires a unique tumor lesion <5 cm in diameter or up to 3 tumor lesions <3 cm (3) confined to regions without major hepatic vessels.

Surgical resection is the optimal first-line treatment for primary and metastatic liver cancer. However, few patients are suitable for surgery due to poor baseline function, overall tumor burden, or hepatic vessel invasion. Liver resection is also considered the only potentially curative treatment in patients with CLM providing significant improvement of overall survival (10). However, surgical resection with curative intent is only feasible for a minority of patients with liver metastases (10-25%) since only a small proportion of patients have resectable disease at disease presentation (11). Despite the survival advantage of hepatic resection on CLM, relapse is common following curative resection (12). In addition, surgery is an invasive procedure associated with high rates of morbidity and mortality (13).

The use of systemic chemotherapy has also been attempted in patients with liver cancer. Unfortunately, HCC is a relatively chemotherapy-resistant tumor, therefore, outcomes using this treatment are unsatisfactory (14, 15). Also, systemic therapy is often ineffective with underlying cirrhosis, and it is only used in patient's ineligible for surgical resection, liver transplant and local ablation. In addition, the use of chemotherapy is outweighed by frequent toxicity and lack of significant survival benefit. Radiotherapy is also an ineffective strategy for primary liver cancer. Radiation therapy is limited by dose-related radiation hepatitis, which precludes the administration of external beam radiation in doses effective for tumor eradication (16).

Transarterial chemoembolization (TACE), based on the delivery of chemotherapeutic agents directly to the tumor lesions via the hepatic artery, has become one of the most widely accepted palliative therapy for unresectable HCC (17). However, complete necrosis rate of tumors after TACE only reaches 10-20%, and the prognosis of these patients remains poor (18-20).

Ablation techniques have emerged as promising alternatives for those patients who are not eligible for surgical resection or who have failed medical and/or radiotherapy therapies. Current ablation methods include non-thermal ablation methods such as percutaneous ethanol injection (PEI) and irreversible electroporation (IRE) and thermal modalities such as radiofrequency ablation (RFA), microwave ablation (MWA), and high-intensity focused ultrasound (HIFU) (3).

The limitations of the non-thermal ablation methods: For PEI, high rates of tumor recurrence and multiple treatment sessions required have limited the use of PEI in routine clinical practice. For IRE, most devices require simulation planning with the use of multiple probes placed in parallel to achieve the desired ablation zone. The requirement of multiple treatment sessions and the use of multiple parallel probes results in a significant increase in procedural cost and complexity. Also, IRE requires general anaesthesia with paralytic agents as the electrical current generated during the procedure can cause muscle spasms and arrhythmias (16).

The limitations of thermal ablation methods: Despite the efficacy of some of these local thermal ablation modalities, significant limitations exist due to their mode of action (thermal tissue destruction). Thermal ablation is inconsistent in tissue with non-uniform heat dissipation patterns, which is common in hypervascular liver tumors (21). Thus, thermal ablation often results in incomplete tumor necrosis in tissue near major vessels (22, 23). Consequently, the shape and the size of the ablation zone may be unpredictable and the efficacy of thermal ablation may be restricted as multiple sessions are necessary to complete tumor ablation (24). In addition, thermal ablation methods are often unsuitable for treating tumors larger than 3 cm due to excessive treatment time and practical probe sizes (25-27). Besides, all ablation modalities available share a high rate of local recurrence in HCC. Most complications associated with use of RFA and MWA are consequences relating to thermal injury (28). Another limitation of these methods is the lack of imaging feed-back during treatment. Thus, the effect of ablation treatment is evaluated after the application of thermal treatment by CT or MRI while no real-time imaging provides monitoring during treatment (29).

HIFU is a non-invasive, image-guided, thermal ablation method. Unlike percutaneous thermal modalities, HIFU is completely extracorporeal and lacks the risks of bleeding and tumor seeding with the direct puncture of tumors. HIFU may improve upon other thermal ablation modalities due to its non-invasiveness, real-time feed-back and the ability to scan the focal zone over a large volume (29). As the other thermal-based methods, HIFU is also limited by the heat-sink effect, resulting in reduced effectiveness in ablating tissue near major vessels and extended treatment time for larger hypervascular liver volumes (29). Also, a major challenge in the non-invasive treatment of liver tumors using HIFU ultrasound is rib obstruction, which may result in secondary hot-spots near the treatment main focal zone, inducing loss of therapeutic precision and collateral damage (30). Moreover, because of the high ultrasound absorption coefficient of HIFU at the bone-tissue interface, overheating of ribs and surrounding tissue often results in unwanted tissue damage. Skin burns and subcostal edema have been reported with HIFU liver ablation cases (31, 32).

Therefore, developing new strategies in which a liver tumor can be ablated non-invasively and avoiding thermal-related collateral damage and ineffectiveness would be a major clinical advancement in the treatment of soft tissues, particularly liver tumors.

2.2 HISTOTRIPSY - NON-THERMAL; FOCUSED ULTRASOUND

To address these unmet clinical needs, cavitation-based focused ultrasound (histotripsy) is a promising option to ablate liver tumors and overcome the limitations of currently available ablation modalities.

Histotripsy is an ablative technology that mechanically destroys targeted tissue through the precise targeting of acoustic cavitation (33-35). The VORTX Rx® is an image-guided device designed to deliver non-invasive, non-thermal histotripsy for local ablation that has the potential to overcome many limitations of other ablation devices.

The Histotripsy Group in the University of Michigan´s Department of Biomedical Engineering invented and pioneered the development of focused ultrasound histotripsy for more than twelve years. Starting with their earliest work of in the use of microbubbles to cause tissue damage, this group developed histotripsy into a highly controlled and predictable tool to remove unwanted tissue with microscopic precision. In 2010, HistoSonics, Inc. entered into a worldwide exclusive license with the University of Michigan for exclusive rights to the entire portfolio of histotripsy patents and patent applications. HistoSonics has advanced histotripsy toward clinical application with the development of the VORTX Rx® medical device to deliver histotripsy.

Histotripsy is based on the delivery of acoustic energy in the form of short (<50 microseconds) very high intensity pulses which induces controlled cavitation to mechanically homogenize targeted tissue (33). Cavitation is a process that occurs when a sufficiently negative pressure is applied to a fluid or tissue to cause microbubble formation from fluid vaporization and release of dissolved gas (36). Microsecond, high-pressure pulses applied by an ultrasound transducer outside the body and focused on the target tissue are used to generate a cluster of microbubbles. Once formed, the microbubbles exhibit highly dynamic patterns of oscillation and inertial collapse which impart severe stresses on surrounding cells and tissues to produce cellular and tissue destruction at the target (37). With sufficiently high pressure and adequate number of pulses, the target tissue can be completely destroyed, as demonstrated pre-clinically, creating a fluid homogenate with no recognizable cellular structures.

Favorable characteristics of histotripsy ablation method:

- No insertion of probes or needle electrodes required
- Ultrasound imaging feed-back for pre-operative planning and real-time visualization of target tumor and image-guided tissue destruction
- Prevents damage to adjacent structures
- Overcomes rib obstruction
- Precise targeting
- Complete resorption of ablation zone
- Minimal scarring
- Immediate treatment imaging feed-back

2.3 HISTOTRIPSY FOR LIVER TUMORS

The characteristics of histotripsy provides for potentially significant benefit in the application for the ablation of liver tumors. Several preclinical studies were conducted that demonstrated the performance and safety profile of histotripsy to safely ablate liver tissue. Refer to the Investigator's Brochure (IB) for a detailed description of those studies.

As an overview, the following summarizes those key pre-clinical studies, conducted to provide foundational evidence of the feasibility of histotripsy to safely and effectively target and ablate the liver. Various preclinical and bench models were employed in the following studies:

Study 1 demonstrates in a porcine model that histotripsy can ablate liver tissue while preserving surrounding critical structures (29). Eight pigs were treated in this study. Twelve lesions of ~1

cm^3 were created in the livers of six pigs and two larger volume lesions of 18 cm^3 and 60 cm^3 were generated in the livers of 2 pigs. Histological evaluation of the lesions showed complete ablation of hepatic parenchyma inside the treated volume with sharp boundaries and only a small area of partially ablated liver tissue <500 µm likely caused by respiratory motion. There were no signs of damage to overlying skin, muscle, or ribs found. Histological evaluation showed no damage observed in any of the large vessels inside the treated region; no visible damage or perforation to the gallbladder was observed in harvested samples.

Study 2 demonstrates in a porcine model that histotripsy transmitted through the ribs can safely and effectively ablate the liver without the need for aberration correction (38). Non-invasive liver histotripsy ablations were generated in 8 pigs, with 4 lesions through acoustic windows with full ribcage obstruction and 4 lesions without rib coverage. The treatments were applied by a 750-kHz focused transducer using 5 cycle pulses at 200 Hz PRF. Temperatures on overlying tissues including ribs were measured with needle thermocouples. Statistically comparable ablation volumes were generated for transcostal and transabdominal treatments. The average temperature increase on the ribs in all transcostal treatments was 3.9±2.1 ºC, while in transabdominal treatments an increase of 1.7±1.3 ºC was observed. No damage was observed on ribs and other overlying tissues.

Study 3 demonstrates the chronic response and healing effect post-histotripsy in a rat model, with complete healing within 28 days (39). Histotripsy was applied to the livers of 20 rats through the intact abdomen transcutaneously at a pulse repetition frequency of 100 Hz. Lesions were generated by mechanically scanning the ablation focus in a 4 mm x 4 mm square grid inside the rat liver. Lesion progression of the rats was monitored with MRI on days 0, 3, 7, 14 and 28 (n=4 each). Histologic evaluation of ablated liver lesions 1 hour after the procedure showed complete ablation, with no recognizable cellular structures within the ablated region. No signs of damage to overlying skin, muscle or ribs. The chronic response was assessed over 28 days with the tissue homogenate inside the lesions being gradually replaced by regenerated liver parenchyma and scar. By day 28, the lesion was mainly replaced by normal parenchyma.

Study 4 demonstrates histotripsy's ability to ablate cancer cells in a mouse model (40). Liver cancer patient-derived xenografts (Hep3B HCC cell line) were subcutaneously implanted in 8 NSG mice. When the tumors had grown to approximately ~1 cm in diameter, histotripsy ablation was applied non-invasively using a custom-built 1 MHz histotripsy therapy system designed for small animal experiments. Gross analysis and histological evaluation demonstrated that histotripsy ablated the targeted tumor into an acellular debris with no remaining cellular structures with sharp boundaries between treated and untreated tissue. Histology of the lung and brain was assessed in these animals 4 weeks after a partial tumor ablation, showing normal lung parenchyma and brain parenchyma with no HCC metastasis. MRI showed nearly complete destruction of the tumor with only small nodules of residual tumor remaining after ablation, likely due to limitations in the murine model (i.e. unable to treat a margin around the tumor due to the subcutaneous location).

Study 5 demonstrates, using imaging data and modeling, that there is ample acoustic window in the human for histotripsy access in greater than 90% of liver targets analyzed (internal Scientific Report (SR) No. 0203). 21 de-identified human CT data sets were collected with data from BMI categories ranging from <18.5 to >40 and were analyzed to assess the available acoustic window for target locations throughout the liver. These data sets were segmented using Mimic so that various tissue types could be considered and analyzed in the acoustic aperture calculation. The collection of all possible acoustic apertures and focal pressures for these subjects was used to define a theoretical histotripsy transducer. The analysis determined that 93% of target liver points were accessible with the theoretical transducer when considering the assumptions of patient preparation (for gas removal) and pressure thresholds.

Study 6 confirms a strong safety profile in a porcine model wherein a supratherapeutic histotripsy dose was applied directly to major hepatic veins (SR 0204). Histotripsy was applied non-invasively to 22 pigs (11 heparinized and 11 non-heparinized) using 5 cycle pulses applied at a pulse repetition frequency (PRF) of 50 Hz. Four histotripsy treatments were applied: 1 and 15-minute treatments applied to a region of the liver adjacent to a large hepatic vein, followed by 1 and 15-minute treatments applied within the hepatic vein (diameter ≥ 5 mm). For all subjects (heparinized and non-heparinized), bubble clouds were successfully initiated in the liver and hepatic vein without inducing any significant changes in blood pressure, ECG, or SpO2. Small but significant decreases in heart rate and core body temperature were observed in both groups during the experiment, with these decreases likely being a response to the general animal procedures rather than histotripsy, consistent with intra-procedural observations noted before and between histotripsy treatments. All animals survived the treatment. Acute hypotension during treatment was observed in one subject which returned to baseline levels by the 1-hour post-treatment time point.

With these foundational studies providing a strong proof of concept, the VORTX Rx® device was further developed for use in the ablation of soft tissues, including liver tumors. As part of the development process, histotripsy was applied to livers using the VORTX Rx® in 48 porcine subjects (39 acute; 9 chronic) in a series of experiments, with the goal of safe, effective, and time efficient histotripsy ablation of the liver.

- In the first series, ablations were created in 30 minutes or less of energy delivery with results from contrast-enhanced MRI imaging and histology consistently showing complete ablations within the targeted tissue volume. Chronic animals were each survived 28 days (+/- 2 days) with the involution of the ablation zone resulting in an average reduction of 65% by the 28-day time point. The ablations in these 24 subjects met the efficacy and time efficiency requirements, however, body wall effects were apparent in 13 of the 24 subjects (54%).
- The next 12 subjects were treated with thermocouples placed to study temperature rise as a function of various operating parameters and the research team conducted a rigorous study with theoretical analysis and bench testing to optimize device parameters to derive parameters that would deliver safe, effective, and time efficient ablations.
- The next series of experiments was an in-vivo acute porcine model with 6-animal livers ablated with the same procedural protocol as the previous subjects using the parameters derived from the thermocouple studies. The ablations in all 6 subjects were excellent as confirmed with both imaging and histology. None of the 6 subjects showed any signs of body wall effects.
- The next step was a Good Laboratory Practice (GLP) confirmatory study to demonstrate that the system developed could safely and effectively ablate a clinically relevant volume of liver tissue using the system and workflow in the same manner as it would be used clinically. Clinically relevant ablation volumes were generated in six additional porcine subjects using these optimized parameters in a study which confirmed the device, the VORTX Rx®, is safe and effective. Please see IB for additional details.

2.4 Clinical experience with the VORTX Rx®

2.4.1 Benign Prostatic Hyperplasia

The VORTX Rx® is a platform technology. This means some aspects of the platform hardware and software can be leveraged for different indications using different device configurations. As

an example, in 2013, a previous configuration of the VORTX Rx® was approved by the United States Food and Drug Administration (US FDA) under an Investigational Device Exemption (IDE) for use in a human pilot trial indicated for benign prostatic hyperplasia (BPH) treatment. The VORTX Rx® BPH-indicated configuration had hardware and software similar to the current investigational device adapted for soft tissue ablation. This design was a portable ultrasound device to apply histotripsy ablation used in this human trial for non-invasive image-guided ablation in male subjects with benign prostate enlargement.

Under the FDA-approved IDE, a prospective, non-randomized pilot study was conducted in two centers in the United States. The primary objective of this first-in-human (FIH) study was to evaluate safety of the device for treatment of BPH and secondarily to perform an initial efficacy assessment using histotripsy ablation. Twenty-five adult men in a first cohort and three additional men in a second cohort with moderate to severe symptomatic BPH received an ablation. Using the VORTX Rx®, 60 minutes of non-invasive acoustic energy (histotripsy) was delivered through the perineum to the prostate with real-time transrectal ultrasound monitoring. Subjects were either under general anesthesia or IV-sedation (based on investigator and patient preference). All patients who received an ablation were followed up at post-operative day 1, and 1, 3 and 6 months after treatment.

An excellent safety profile was established. No study-stopping adverse events (AEs) occurred (no deaths, major bleeding or fistulas). Device-related AEs (definitely or probably device-related per the DSMB) were limited to 3 cases of transient retention (<3 days), 1 case of intermittent retention (8 days), a minor anal abrasion and 1 case of microhematuria.

This study concluded that ablations delivered using the VORTX Rx® were well-tolerated, safe and led to significant improvements in patient-reported symptoms using the International Prostate Symptom Score validated tool (IPSS), without measurable changes in objective measures such as uroflow. See the IB for additional discussion on this clinical experience of the VORTX Rx®.

2.4.2 VORTX Rx® FOR LIVER TUMORS

HistoSonics is currently focused on developing the VORTX Rx® for the ablation of soft tissues. Within the application of soft tissues, the company is currently interested in studying ablation of liver tumors, given the significance of this unmet clinical need. The HistoSonics VORTX Rx® platform device has now been designed and configured for non-invasive, non-thermal, real-time image-guided procedures including soft tissue ablation, particularly liver tumors.

2.5 STUDY RATIONALE

As noted above, surgical resection is the optimal first-line treatment for primary and metastatic liver cancer. However, few patients are suitable for surgery due to poor baseline function, overall tumor burden, or hepatic vessel invasion. In addition, surgery is an invasive method associated with a high morbidity. Chemotherapy and radiotherapy treatments are often ineffective treatment strategies for liver cancer treatment, outweighed by frequent toxicity or collateral damage (41).

Ablation techniques have emerged as promising alternatives for those patients who are not eligible for surgical resection or who have failed medical and/or radiotherapy therapies. However, despite the efficacy of some of these local thermal ablation modalities, significant limitations exist due to their mode of action (thermal tissue destruction), including the heat sink effects from blood flow through the highly vascular liver, often resulting in incomplete tumor ablation or inability to treat near major blood vessels (25-27). In addition, uncontrolled thermal spread (ablating beyond planned volumes) increases the potential for damage to adjacent structures. Therefore, developing new strategies in which a liver tumor can be ablated non-

invasively and avoiding thermal-related collateral damage and ineffectiveness would be a major clinical advancement in the treatment of soft tissues, particularly liver tumors.

To address these unmet clinical needs, cavitation-based focused ultrasound (histotripsy) is a promising option to ablate liver tumors and overcome the limitations of currently available ablation modalities. Histotripsy is a non-invasive, non-thermal, image-guided focused ultrasound ablative technology that uses mechanical properties of focused ultrasound to ablate targeted tissues through the precise delivery of acoustic cavitation. Histotripsy has the potential to target multiple sites without the need for incisions or needle insertions (which may cause bleeding, infection, and/or tumor spread). Because histotripsy is non-thermal, it is not affected by the heat sink effect from blood vessels and does not have the limitations associated with thermal ablation such as collateral damage due to uncontrolled thermal spread. In addition, histotripsy may be able to overcome the interference challenge from overlying ribs better than other ablation methods. It has been demonstrated preclinically that histotripsy can generate liver ablations using acoustic energy transmitted through the ribcage without inducing unwanted thermal effects or damage to overlying tissues. Liver ablation using histotripsy results in complete ablation of target tissue into an acellular homogenate that is absorbed by the body and replaced by normal liver parenchyma over time.

As has been demonstrated through extensive preclinical experience described in Section 2.3 above and further described in the IB, histotripsy is a potential paradigm-shifting technology. The non-thermal and non-invasive characteristics of histotripsy offer patients the potential for a soft tissue/tumor ablation with fewer clinical complications and adverse events than currently available ablation methods and surgical procedures.

The safety of the VORTX Rx® has been demonstrated through rigorous performance testing to include bench and preclinical acute and chronic studies. This clinical study is intended to evaluate technical performance, including acute technical success, while collecting safety-related data of ablation using the VORTX Rx®.

2.6 Risk-benefit assessment

There are no guaranteed benefits to subjects from participation in this study. However, patients with primary and metastatic liver disease included in this study may benefit from the ablation of up to three tumors by where a complete or partial removal of targeted tumor tissue will be performed using the study medical device. Therefore, there is a potential for tumor debulking for reduced overall tumor burden, leading to a favorable impact in the prognosis and quality of life for these patients who are not candidates for surgical resection and not suitable candidates for other locoregional treatments or have not responded to conventional therapies. The characteristics of cavitation-based focused ultrasound may allow a successful ablation of liver tumor with fewer complications than currently available liver ablation modalities and surgical procedures.

The potential risks associated with the VORTX Rx® when used for the ablation of liver tumors are expected to include potential risks typically associated with ablation methods, ultrasound-imaging related risks, patient positioning-related complications and risks related to the skin contact with the transducer coupling device. In addition, considering that patients will undergo the procedure under general anesthesia, there is the potential for anesthesia-related complications.

Potential risks typically associated with ablation methods

Side effects according to state-of-the-art tumor ablations reports are expected, undesired complications of the procedure that although occurring commonly, rarely, if ever, result in substantial morbidity and mortality (42-46). The more serious complications described include, but not limited to, hemorrhage requiring transfusion, visceral perforation (bowel or stomach), biliary leakage or strictures, liver failure, myocardial infarction, lung damage, or embolism. Other complications may be abscess, soft tissue or skin lesions and burns, damage to vascular system, thrombosis, ascites, subcapsular hematoma, cryo-shock, post-ablation syndrome (low-grade fever, malaise, chills, myalgia delayed pain, and nausea and vomiting), pleural effusion, asymptomatic pleural effusions and minimal asymptomatic perihepatic (or renal) flood or blood collections seen at imaging (42-47).

As noted above, thrombosis is a possible adverse event resulting from tumor ablation. In the preclinical studies conducted in support of the VORTX Rx® product development, localized thrombosis was observed in 58% of preclinical subjects receiving histotripsy ablations in the confirmatory study phase.

As a possible adverse event in this clinical study, all subjects will be closely monitored for thrombosis or embolism and, if so determined to be of clinical importance, will be treated with standard local clinical practice appropriate for the individual patient.

Ultrasound energy delivery-related clinical risks

The study device delivers ultrasound energy to ablate liver tumors targeted by the clinician. Real-time ultrasound imaging will be used in combination with focused ultrasound ablation in order to provide ablation planning, image-guided targeting, and intraprocedural imaging feedback to the physician during the procedure. This introduces clinical risk to the patient that is typical for other ablation methods that are monitored with ultrasound imaging during the ablation. Moreover, the intervening tissues in the energy pathway are potentially at risk of physical effects associated with ablative ultrasound.

Patient´s position

The patient will remain under general anesthesia in a supine position on a procedure table throughout the procedure with the chest/abdomen coupled to the device transducer supported by a fixed (non-active) articulating arm that locks in place. This introduces clinical risk to the subject that is typical of being placed in this position.

Skin contacting during the procedure

The skin will be in contact with the device transducer via an Ultrasound Medium Container (UMC) to include a 3M Ioban™ drape, coupling container and approximately 1.5 liters of degassed water, which introduces a clinical risk regarding potential skin sensitivity or reaction to patients with known sensitivity to iodine (contraindication) see the User Manual for further information. It should be noted that through the device development process, biocompatibility risks have been minimized by limiting patient contact exposure, use of known state-of-the-art contacting materials and the clinical centers' water resources.

General anesthesia

Patients will be under general anesthesia during the procedures, as respiration needs to be controlled. The potential occurrence of anesthesia-related complications must therefore be considered. However, only patients who can undergo general anesthesia will undergo ablation procedure. Anesthetic risk will be assessed at screening and before each ablation procedure performed during the study (multiple tumors and/or retreatment of tumors).

Risk-Benefit Summary/Conclusion:

Several measures will be taken to ensure that the potential benefits outweigh the potential risks for patients participating in this study. Measures include:

- careful selection of patients;
- a detailed pre-procedure assessment;
- complete training and qualification of investigators;
- close monitoring of the study device and rigorous post-ablation follow-up.

There is reasonable assurance that the VORTX Rx® device is safe to take into the clinical setting based on:

- valid scientific evidence from bench and preclinical data,
- the application of risk management processes to the development of the medical device (per EN ISO 14971),
- the sponsor's plan for compliance with design, conduct, recording and reporting of this clinical investigation per Clinical investigation of medical devices for human subjects - Good Clinical Practice (EN ISO 14155),
- compliance with other applicable regulatory requirements,
- together with a study design similar to clinical evaluations of similar ablation technology (see the Manufacturing Standards section of the IB for Manufacturing Standards).

In addition, the probable benefits to health for this palliative patient population from use of the study device, for its intended uses, and conditions of use, when accompanied by adequate directions and warnings against unsafe use, outweigh the probable risks.

3 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVE

The primary objective of the study is to evaluate the acute technical performance of the VORTX Rx® medical device for the ablation of primary and metastatic liver tumors.

3.2 SECONDARY OBJECTIVES

- Assessment of the safety profile of the VORTX Rx®.
- Assessment of local tumor progression by MRI at 1 week, 1 month and 2 months, post-procedure.
- Assessment of the involution of the ablation zone 1 week, 1 month and 2 months post-procedure.
- Assessment of liver panel.

- Immunologic assessment.
- Evaluation of quality of life.
- Assessment of pain and analgesic requirements after the ablation procedure.

4 STUDY ENDPOINTS

4.1 PRIMARY ENDPOINT

The primary endpoint of the study is acute technical success, consisting of success in treating the PAV, defined as the ability of the investigational device to create an ablation zone per the PAV (a three-dimensional volume defined by the investigator using the VORTX Rx® in the planning phase of the software workflow), as measured by multi-dimensional imaging and characterized qualitatively in the radiological report 1-day post ablation. The acute technical success of up to 3 separate tumor ablations in a single patient will be assessed

4.2 SECONDARY ENDPOINTS

- The safety profile of the VORTX Rx® will be measured based on all adverse events (AEs) (serious and non-serious) that are probably or definitely device-related and experienced during the use of the medical device as well as those AEs and abnormal findings on physical examination, vital signs, laboratory, ECG and imaging tests through the study. Adverse Events will be captured in compliance with ISO 14155 (48), MEDDEV 2.7/3 rev 3 (49) and classified and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0 (Appendix 2).

  The incidence of any of the following serious adverse events (SAEs), if definitely attributable to the device under study, will be evaluated:

  1. Major bleeding that requires transfusion within 48 hours
  2. Visceral perforation (bowel or stomach)
  3. Major biliary duct injury
  4. Death

  The study will be considered to have a favorable safety profile given the following outcomes:

  - No deaths due to the ablation,and
  - No deaths from visceral perforation (bowel or stomach), and
  - 20% or lower incidence of major bleeding requiring transfusion within 48 hours, and
  - 20% or lower incidence major bile duct injury.

- The ablation zone created as a result of the ablation of each tumor (multiple tumor ablation sessions and/or retreatment) will be assessed post-procedurally to evaluate local tumor progression by contrast-enhanced MRI imaging at 1 week, 1 month and 2 months. Evaluations may be supplemented with optional CT or ultrasound with or without contrast imaging at the discretion of the investigator.

- The involution of the ablation zone of each tumor lesion (multiple tumor ablation sessions and/or retreatment) will be assessed post-procedurally by contrast-enhanced MRI imaging at 1 week, 1 month and 2 months. Evaluations may be supplemented with optional CT or ultrasound with or without contrast imaging at the discretion of the

investigator. Ablation zone dimensions will be determined at each time point for each tumor.

- Liver panel will be evaluated on the basis of the change of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, albumin and bilirubin, gamma glutamyl transpeptidase (GGT), prothrombin time (PT) and international normalized ratio (INR) from baseline to evaluation visits 1 day, 1 week and 1 and 2 months post procedure for each tumor ablated.

- Immunological response of ablation will be evaluated on the basis of immune tests conducted and tumor biomarker assessments (including, but not limited to, immune tests: CD3+, CD4+, CD8+, CD45+, CD16+, CD56+ and CD19+, C-reactive protein [CRP], complement C3, C4 and CH50, immunoglobulins [IgG, IgM, IgA], interleukin-6 [IL-6], carcinoembryonic antigen [CEA], alfa-fetoprotein [AFP], Cancer Antigens CA15-3 [Breast Cancer] (50) and CA 19-9 [Pancreatic Cancer] (51)) from baseline to evaluation visits 1 day, 1 week and 1 and 2 months post procedure for each tumor ablated.

- Measure Quality of life (QoL) on the basis of the score of the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 (58) (Appendix 4) at baseline and at 1 and 2 months post procedure for each tumor ablated.

- Perform pain assessment by a 100 mm visual analog scale (VAS) where 0 is “no pain” and 100 is “the maximum pain possible” (Appendix 5) at 1-day and 1-week post-procedure for each tumor ablated.

- Evaluate analgesic treatment prescription in the 24-hour period post-procedure and during the one week period post-procedure for each tumor ablated.

5 STUDY DESIGN

5.1 OVERALL STUDY DESIGN

This is an open-label (no-masking), non-randomized, multi-center study comprised of three (3) independent centers located in the greater metropolitan Barcelona area.

The PI and study coordinator, Dr. Joan Vidal-Jove, will performance all ablation procedures during the study.

The primary objective of this study is to evaluate acute technical performance of the VORTX Rx® medical device for the ablation of primary and metastatic liver tumors.

Ablation of the targeted tissue will be evaluated using MRI analysis following a predefined imaging protocol to assess acute technical success defined as the ability of the investigational device to create an ablation zone per the planned ablation volume (PAV) (a three-dimensional volume defined by the investigator using the VORTX Rx® in the planning phase of the software workflow). CT analysis may also be utilized per investigator discretion.

The pre-specified technical success criteria and imaging protocol greatly reduces the possibility of unplanned changes being made based on interim trial findings, reducing operational bias and supporting findings of scientific integrity of this study.

Up to three separate ablation sessions will be allowed to be performed in a single patient during the study with a minimum of 1 month between sessions. It will be allowed to treat more than one

tumor in the same session whenever the total volume of the tumors ablated is determined to be less than or equal to the volume of a single 3 cm diameter tumor. Each tumor is required to meet the criteria defined for a targeted tumor in the study protocol: diameter of less than or equal to 3 cm (≤3 cm) and clearly separated from other tumors or other critical areas (i.e. located in different segments of the liver).

Targeted tumors can be retreated upon investigator discretion (i.e. in case that residual tumor is detected). Retreatment of a tumor will be performed 1 month after the prior ablation of the tumor.

In all cases, at least a one-month period must have elapsed between the last ablation performed and the next ablation session.

Retreatment of a tumor and ablation of a new tumor are not allowed to be performed in the same session. Any required re-treatment will be performed prior to any new treatment in any individual patient. Treatment of a new tumor in a new patient will be allowed prior to retreatment of a tumor in a previous patient.

Medical history review, ECOG PS, physical examination, including vital signs, and anesthetic risk assessment will be carried out before ablation of additional tumors (up to 3 ablation sessions) or tumor retreatment is performed. Only patients who can undergo general anesthesia will undergo ablation procedure. Anesthetic risk will be assessed at screening and before each ablation procedure performed during the study (multiple tumors and/or retreatment of tumors) using the American Society of Anesthesiologist (ASA) score. The ASA score will be used to assess the physical status of patients before the procedure and estimate pre-operative risk.

The study enrollment period is expected to require up to twelve (12) months and the study follow-up period per patient will be a minimum of two (2) months with a maximum follow up period to be determined by the number of tumors ablated and what is medically appropriate. However, it is anticipated that no more than nine (9) months should be required to complete patient follow up, or until the withdrawal of the patient due to any reason, whichever is first. Therefore, the total study duration may extend to approximately 21 months.

The study will consist of a screening period (within the 2-month period prior to the ablation procedure), pre-planning visit including a procedure simulation (within a week prior to the procedure visit) and a procedure visit where the ablation will be performed using the study device, and 4 post-ablation study visits 1 day, 1 week and 1 and 2 months post-ablation. This schedule will apply for each tumor ablated (or combinations of tumors ablated in the same session) (up to a total of 3 separate ablation sessions) and for retreated tumors.

Once informed consent has been obtained, and the fulfillment of all pre-selection criteria has been confirmed, the patient will be scheduled for the ablation procedure. All selection criteria must be confirmed the day of the ablation procedure to consider the patient eligible to undergo the procedure. A patient will be considered enrolled once the first pulse of the ablation energy has been applied to the tumor. Patients will be hospitalized to undergo the procedure, which will be performed at the study site. Patients will undergo a detailed post-procedure evaluation within 24 hours of the ablation and they will be discharged from the hospital whenever they do not present any complication that requires extended hospitalization. Follow-up evaluation will include imaging assessment, measurement of laboratory parameter levels, including liver enzyme panel, immunological parameters and tumor biomarkers, ablation zone evaluation and record of adverse events.

For safety purposes, this study will take a two (2) step approach:

Step 1:

a. The first subject enrolled will be observed and assessed over a 24-hour period and shall not have experienced any unexpected SAE attributable to the study device.

b. After completion of Step 1a, the second subject enrolled will be observed and assessed over a 24-hour period and shall not have experienced any unexpected SAE attributable to the study device.

Step 2:

- Once Step 1a and 1b are complete, the remaining subjects, after meeting all inclusion (and no exclusion) criteria may be enrolled and treated on a first-come, first-served basis.

Should the first or second subject experience an unexpected serious adverse event attributable to the investigational device, the principal investigator will consult with the Data and Safety Monitoring Board (DSMB), his co-investigator(s) and the sponsor and together determine the appropriate next course of action regarding study. If after such consultation and review, it is determined that the study resume, a third patient will be enrolled and monitored in a similar fashion before commencing with successive enrollment.

An independent DSMB will be used for review of safety data to provide advice to the Sponsor to ensure the safety, protection and well-being of the subjects enrolled in the study. The members of the DSMB will be experts who are independent from the Sponsor. Additional details regarding the structure of the DSMB is provided in Section 9.5.

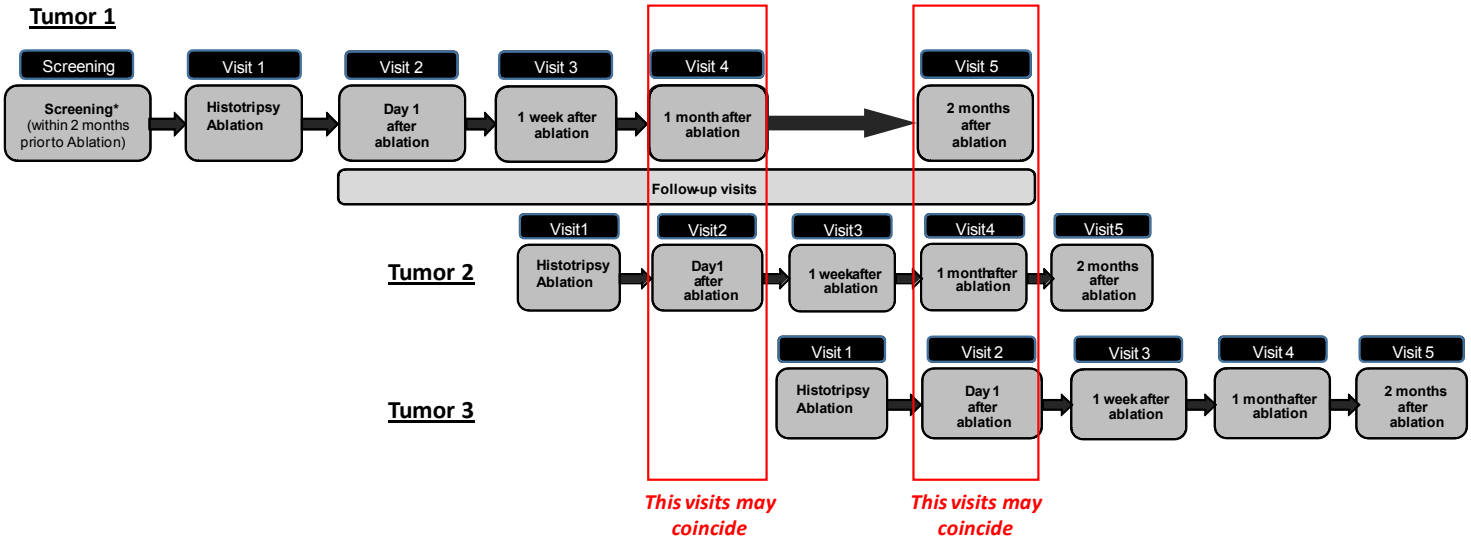
The video recording of the ablation procedure is intended to be performed for all patients participating in the study for research purposes and for feedback for engineering improvements. The video recording will be performed by the investigator and his collaborators during the ablation procedure at the surgery room of the centre. Only the areas restricted to the liver area will be recorded, avoiding, as far as possible, areas of their body by which they can be identified (for example tattoos, scars or recognizable birthmarks). The recording will be identified using numeric codes (no data that identify you will appear), maintaining confidentiality at all times in accordance with current legislation and regulations. The video recording of the procedure is totally voluntary. The patient will be informed about the conditions of the video recording and it will not be performed until the patient has given his/her informed consent for the obtaining and publishing of the video recording for research purposes. The patient could give his/her consent to participate in the study without authorizing the recording of the procedure. The current legislation on the protection of personal data (Data Protection Regulation (EU) 2016/679) will be applied.

During the study it is also intended to collect an extra amount of blood at each study visit when blood samples are taken for hematology and biochemistry evaluation and for immunological and tumor markers assessment as part of the study procedures. These additional blood samples will be sent (along with the blood samples drawn for study laboratory assessments), processed and stored at the central laboratory (Echevarne Laboratory, Barcelona) to be available for additional, originally unforeseen studies and or new scientific evidence arises in this field of research. Additional blood samples collected for research purposes will be sent to the central laboratory according to the instructions included in the Central Laboratory Sampling Manual provided to the investigators. The procedure of blood sample collection, identification of samples (labeling), storage conditions, and shipment details will be included in the Central Laboratory Sampling Manual. Once the clinical trial is completed, all additional blood samples obtained during the study will be stored in the central laboratory where these samples will be preserved for up to 3 years for future investigation. The storage of biological samples will meet the ethical and legal requirements provided in the RD 1716/2011. The biological samples will be handled, stored, and treated according to the Law 14/2007 of biomedical research, which regulates the use of biological samples for such purposes. The collection of these additional blood samples is completely voluntary. The patient will be informed about the conditions of the collection, storage and use of the additional blood samples and they will not be drawn until the patient has given

his/her informed consent for this purpose. The patient could give his/her consent to participate in the study without authorizing the collection of additional blood samples.

Further details on study periods and procedures are provided in section 5.4.

Figure 1. Study design



5.2 TREATMENT ASSIGNMENT OR RANDOMIZATION

There will be no treatment blinding, as this is an open-label study. This is a non-randomized study (no masking).

5.3 STUDY POPULATION

This study will include up to ten (10) subjects meeting all the inclusion criteria described in sections 5.3.1 and none of the exclusion criteria specified in section 5.3.2, and giving their informed consent to participate in the study.

5.3.1 INCLUSION CRITERIA

Subjects are eligible for the study if all of the following criteria are met:

- Written informed consent before any study procedure is performed.
- Subjects of both sexes aged 18 years or older.
- Patients diagnosed with liver cancer, including HCC or liver metastases from breast, lung, pancreas and/or colorectal cancers. If biopsy is required, there will be a minimum of 2-week period after biopsy and before the ablation.
    - HCC patients must meet the United Network for Organ Sharing and Organ Procurement and Transplantation Network (UNOS-OPTN) class 5 criteria for HCC (52).

  - Liver metastases patients must meet minimum criteria of liver biopsy and/or tissue diagnosis of primary tumor or metastatic tumor with new or growing liver tumors radiologically consistent with metastases.

- Patients with liver cancer not candidates for surgical resection and/or not suitable for other locoregional treatments or patients who have not responded to or relapsed from conventional therapies.
- Previous treatment with chemotherapy and/or radiotherapy is permitted provided that these treatments have been discontinued more than 2 weeks before the ablation of the targeted tumor and whenever patients have recovered from any related toxicity (53).
- Previous treatment with immunotherapies is permitted provided that these therapies have been discontinued at least 4 weeks before the ablation and whenever patients have recovered from any related toxicity.
- Previous ablation/surgery on other tumors different from those that will be targeted with the VORTX Rx® is allowed whenever a minimum of 2 weeks has elapsed since the prior procedure(s).
- Tumor(s) to be targeted for ablation will be clearly separated from other tumors or other critical areas upon investigator´s criteria.
- Largest diameter of targeted tumor(s) ≤3 cm.
- Tumor(s) that will be targeted at a depth <10 cm from the skin surface.
- Must have an adequate acoustic window in the abdominal space to be able to visualize targeted tumor(s) using ultrasound imaging; also must be able to visualize targeted tumor using MRI with optional CT imaging at investigator discretion.
- Patients who can safely undergo general anesthesia.
- Liver function score of Child-Pugh A or Child-Pugh B.
- ECOG PS 0, 1 or 2 at screening.
- Adequate liver function (ALT and AST < 2.5 x upper limit of normal [ULN]), renal function (serum creatinine <2 ULN and/or bilirubin <2.5 UNL) and hematologic function (neutrophil count > 1.0 x 10^9/L and platelet > 50 x 10^9/L).
- An INR <2 within the last 7 days prior to the ablation in patients receiving anticoagulants, and an INR <1.5 for patients not treated with anticoagulants.
- Platelets level >50 x 10^9/L within the last 7 days prior to the ablation.

5.3.2 EXCLUSION CRITERIA

Patients are not eligible for participation in this study if one or more of the following criteria are met:

- Patients who decline or are unable to understand, provide or are unwilling to sign an informed consent form.
- Pregnant or nursing (lactating) women; women of childbearing potential and sexually active that are unwilling to use adequate contraception (such as oral contraceptives, intrauterine contraceptive device or barrier method with spermicide or surgical sterilization).
- Targeted tumor(s) not clearly separated.
- Targeted tumor(s) >3 cm.
- Tumor that will be targeted >10 cm from the skin surface.
- Tumor not clearly visible with diagnostic ultrasound and MRI.
- Liver function score of Child-Pugh C.

- Liver volume reserve <40% as measured by CT Scan (54).
- Major surgical procedure, biopsy or significant traumatic injury <2 weeks prior to the procedure or has not recovered from side effects/complications of such procedure or trauma.
- Patient who has not recovered to grade 1 or better from any AEs (except alopecia, fatigue, nausea, vomiting) related to previous anti neoplastic therapies.
- BMI >30.
- Parkinson's disease.
- History of bleeding disorders (e.g. von Willebrand disease) or patients suspected to have a bleeding disorder.
- Not able to temporarily discontinue warfarin, clopidogrel or any other long-acting anticoagulants at least two weeks before the procedure.
- Initiation of any anticancer treatment during the screening period.
- Life expectancy to be less than 6 months.
- ASA score ≥ 4.
- Unable or unwilling to complete all required screening and/or follow-up assessments.
- Patients under ongoing treatment with an investigational medication or medical device that conflicts with the study device.
- Patients for whom the investigator considers that the ablation is not in the patient's best interest.
- Patients with active alcohol or drug addiction or any other condition that, in the investigator's opinion, would interfere with their ability to comply with the study requirements.
- Patients with any concurrent condition that, in the investigator's opinion, would jeopardize the safety of the patient or compliance with the protocol.
- Patients with known sensitivity to topically applied iodine.

5.3.3 WITHDRAWAL CRITERIA

Patients may voluntarily discontinue from the study or be withdrawn at the discretion of the investigator at any time.

Patients will be informed of their right to discontinue the study at any time and for any reason without incurring any prejudice for further medical attention.

The investigator must withdraw the patient from the study if any of the following occur:

- The study is terminated.
- Consent for study participation is withdrawn.
    - Patients may voluntarily withdraw consent to participate in the study for any reason at any time. Withdrawal of consent occurs only when a patient does not want to participate in the study any longer, and does not want any further visits or assessments or study-related contact.
    - If a patient withdraws consent, the patients must be withdrawn from the study and no further assessments conducted. The investigator will record this decision in the patient's chart and on the appropriate electronic CRF (eCRF) pages. Further attempts to contact the patient are not allowed unless safety findings require communication or follow-up.
- The patient becomes pregnant.

- Further participation would be injurious to the patient's health or well-being in the investigator's medical judgment.
- Use of any prohibited medication (see Section 7.2).
- Use of ablation methods different from that described in the present investigational plan.
- Major protocol violations.
- The patient is lost to follow-up.
    - For patients whose status is unclear because they fail to appear for study visits, without stating an intention to withdraw consent, the investigator should show due diligence to contact the patient and document it in the source documents. A patient should not be considered lost to follow-up until due diligence has been completed. Patients lost to follow-up should be recorded as such on the appropriate disposition eCRF page.
- Administrative problems.

Should a patient be withdrawn from the study, all efforts will be made to complete and report the observations as thoroughly as possible, including the reason for premature study end (i.e. lost to follow-up (LTFU), consent withdrawn, adverse events, etc.). For LTFU all reasonable effort to ascertain the reason(s) (i.e. telephone calls, mail) while fully respecting the subject's rights (ICH E-6 Good Clinical Practice).

In patients who withdraw from the study before ending the established study period, the assessments and collection of data related to the last visit planned just before withdrawal should be performed. If such withdrawal is due to problems related to the investigational device safety or performance, the investigator shall ask for the subject's permission to follow his/her status/condition outside the clinical investigation. In cases of informed consent withdrawal, no data will be collected after informed consent withdrawal and patients will be followed up as per routine clinical practice.

Patients who are withdrawn from the study are not eligible to re-enter the study.

5.4 STUDY PERIODS AND DURATION

The study will consist of a screening period during the 2-month period before the ablation procedure, which will include a preplanning simulation visit within a week prior to the planned ablation, the ablation procedure (one day), and 4 post-ablation study visits one day, one week and 1 and 2 months post-ablation per tumor as it is specified in Table 1. This study schedule will apply for the each tumor ablated (multiple tumors or retreatment of tumors).

The study visits may coincide considering that a one-month period should be elapsed between ablation sessions (in case of multiple ablation sessions) and taking into account the monthly periodicity of the post-ablation follow-up. Therefore, in case of coincident post-ablation visits, if a laboratory test has been performed within the last week as a part of the post-ablation follow-up, it will not be necessary to repeat this assessment. Similarly, the imaging assessment will be performed once in case of coincident post-ablation visits.

Table 1 lists all assessments and indicates with an "X" the visits when they are performed. All data obtained from these assessments must be supported in the patient's source documentation and transcribed to the electronic case report form (eCRF).

Table 1. Visit evaluation schedule

	Screening	**Ablation for each targeted tumor(s) (baseline)**	**Post-ablation follow-up visits per ablation session (in case of multiple targeted tumors or tumor retreatment)**			
Visits		1	2	3	4	5
Days/weeks/months	(Days -60 to -1)		**1 day post ablation**	**7 days post ablation**	**1 month post ablation**	**2 months post ablation**
				± 2 days	**± 1 week**[29]	**± 1 week**[30]
Informed consent[1]	**X**					
Inclusion/exclusion criteria	**X**					
Demographic data	**X**					
Medical history[2]	**X**					
Data related to liver cancer[3]	**X**					
Physical examination	**X**		**X**	**X**	**X**	**X**
Anthropometric data[4]	**X**		**X**	**X**	**X**	**X**
Vital signs[5]	**X**	**X**	**X**	**X**	**X**	**X**
SO_2		**X**				
ECOG PS[6]	**X**		**X**	**X**	**X**	**X**
Child-Pugh score for hepatopathy assessment[7]	**X**		**X**	**X**	**X**	**X**
Liver volume reserve assessment by CT	**X**					
12 lead ECG	**X**[8]					
Complete blood count	**X**[9]		**X**	**X**	**X**	**X**
Basic metabolic panel	**X**[10]		**X**	**X**	**X**	**X**
Liver panel	**X**[11]		**X**	**X**	**X**	**X**
Urinalysis	**X**[12]		**X**	**X**	**X**	**X**
Immunologic parameters	**X**[13]		**X**	**X**	**X**	**X**
Tumor biomarkers	**X**[14]		**X**	**X**	**X**	**X**
Additional blood samples collection for future research purposes (optional)[15]	**X**		**X**	**X**	**X**	**X**
Serum pregnancy test[16]	**X**					
Imaging assessment of liver tumor [17]	**X**					
American Society of Anesthesiologist score[18]	**X**					
QLQ-C30[19]	**X**				**X**	**X**
Procedure-related data		**X**[20]				
Video recording of ablation procedure (optional)		**X**[21]				
Imaging assessment of ablation zone		**X**[22]	**X**[23]	**X**[23]	**X**[23]	**X**[23]
Ablation zone involution assessment[24]			**X**	**X**	**X**	**X**
Medications administered post ablation[25]		**X**	**X**	**X**	**X**	**X**
Transfusions requirement[26]			**X**	**X**		
Pain assessment (VAS)[27]		**X**	**X**	**X**		
Adverse events[28]		**X**	**X**	**X**	**X**	**X**
Treatment prescribed for cancer management					**X**	**X**

ECOG PS: Eastern Cooperative Oncology Group performance status; ECG: electrocardiogram; SO_2: oxygen saturation ; VAS: visual analogue scale

[1] Informed consent must be obtained before any procedure is performed.

[2] Relevant medical history (in at least the last 5 years) and current medical conditions will be recorded. Special attention will be paid to bleeding disorders. All relevant medications and non-drug therapies received by the patient at the screening visit or interrupted within 7 days prior to the procedure will be collected.

[3] Data related to diagnosis, disease characteristics and prior treatment in patients with HCC or liver metastases will be assessed.

[4] Height, weight and body mass index (BMI) will be assessed at screening. Only weight to calculate BMI will be collected at the post-ablation follow-up visits at 1 week, and 1 and 2 months.

[5] Baseline vital signs assessment includes temperature, diastolic and systolic blood pressure, cardiac frequency and respiratory frequency. Physical examination, including vital signs, will be carried out before ablation of additional tumors (up to 3 ablation sessions) or tumor retreatment is performed.

[6] ECOG PS will be assessed before ablation of additional tumors (up to 3 ablation sessions) or tumor retreatment is performed.

[7] For hepatopathy assessment total bilirubin, serum albumin, prothrombin time, ascites (presence/ absence), and hepatic encephalopathy (presence/absence) will be evaluated.

[8] 12 lead ECG will be only performed during the screening period. In addition, an ECG will be performed as a part of the procedures performed before any ablation in case a period of more than 6 months has elapsed since the last ECG.

[9] Complete blood count includes hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts (basophils, eosinophils, lymphocytes, monocytes, neutrophils, and blasts). The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/procedure. In case of coincident post-ablation visits (in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[10] Metabolic Panel includes glucose, calcium, sodium, potassium, bicarbonate, chloride, blood urea nitrogen (BUN), and creatinine. The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/procedure. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[11] Liver panel includes AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, PT and INR. The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/ procedure. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[12] Urinalysis examinations including: color, pH, bilirubin, blood, glucose, ketones, protein, and specific gravity. The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/procedure. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[13] Immune tests including: CD3+, CD4+, CD8+, CD45+, CD16+, CD56+ and CD19+, C-reactive protein (CRP), complement C3, C4 and CH50, immunoglobulins (IgG, IgM, IgA) and IL-6. The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/procedure. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[14] Tumor biomarkers: carcinoembryonic antigen (CEA), alfa-fetoprotein (AFP), Cancer Antigens CA15-3 (Breast Cancer) and CA 19-9 (Pancreatic Cancer) (50, 51). The blood sample for this assessment must be drawn within the last 7 days before the baseline visit/procedure. In case of coincident post-ablation visits (in case of multiple targeted tumors or tumor retreatment), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment. The collection of additional samples will only be performed for the first ablation performed. In case of multiple targeted tumors or tumor retreatment, additional blood samples with the specified purpose will not be collected.

[15] An extra amount of blood will be collected when blood samples are taken for hematology and biochemistry evaluation as part of the study assessments. These additional blood samples will be centrally processed and stored to be available for additional, originally unforeseen studies and or new scientific evidence arises in this field of research. Additional blood samples collected for research

purposes will be sent to the central laboratory (Laboratorio de análisis Echevarne, Barcelona, Spain) according to the instructions included in the Central Laboratory Sampling Manual provided to the investigators. The procedure of blood sample collection, identification of samples (labeling), storage conditions, and shipment details will be included in the Central Laboratory Sampling Manual. The collection of these additional blood samples is completely voluntary. The patient will be informed about the conditions of the collection, storage and use of the additional blood samples and they will not be drawn until the patient has given his/her informed consent for this purpose. The patient could give his/her consent to participate in the study without authorizing the collection of additional blood samples.

[16] Serum pregnancy test within the last 7 days before the baseline visit.

[17] An imaging assessment on magnetic resonance imaging (MRI) will be performed to determine baseline target tumor size (3 perpendicular diameters and volume), number of baseline tumors, location of target tumor in the liver segments, and expected ablation volume.

[18] Anesthetic risk of patients before surgery will be assessed at screening and before each ablation procedure performed during the study (multiple tumors and/or retreatment of tumors) using the American Society of Anesthesiologist score (Appendix 7).

[19] Patient´s quality of life will be assessed using the EORTC QLQ-C30 questionnaire (Appendix 4) at baseline and 1 and 2 months post ablation. The questionnaire will be delivered and explained to patients by site staff. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if patient´s quality of life has been assessed using the EORTC QLQ-C30 questionnaire within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[20] After the procedure, the investigator should collect the following data: procedure date, total duration of procedure and total duration of anesthesia time~~,~~ number and location of tumor(s) ablated, and vital signs and SO_2 during the procedure. Medications administered and transfusions required will be also collected.

[21] The video recording of the ablation procedure is intended to be performed for all patients participating in the study for research purposes and for feedback for engineering improvements. The video recording will be performed by the investigator and his collaborators during the ablation procedure at the surgery room of the centre. Only the areas restricted to the ablation area will be recorded, avoiding, as far as possible, areas of their body by which the patient can be identified (for example tattoos, scars or recognizable birthmarks). The video recording of the procedure is totally voluntary. The patient will be informed about the conditions of the video recording and it will not be performed until the patient has given his/her informed consent for the obtaining and publishing of the video recording of the procedure for research purposes. The patient could give his/her consent to participate in the study without authorizing the recording of the procedure.

[22] Immediate post-procedure imaging assessments will be performed with ultrasound immediately after the ablation to assess the ablation zone and to identify any complications visible on imaging including the integrity of vital anatomical structures (major vessels and organs).

[23] Post-procedure imaging assessments on MRI and optional CT or ultrasound with or without contrast one day after the procedure: ablation zone assessment. Post-procedure imaging assessments on MRI and optional CT or ultrasound with or without contrast 1-week, 1-month and 2-months post-procedure: radiological evaluation of tumor coverage according to The International Working Group on Tumor Ablation; complications visible on imaging will be identified including the assessment of the integrity of vital anatomical structures (major vessels and organs). The images (MRI and optional CT) will be sent to a central reader, expected to be the Department of Radiology of the University of Wisconsin, United States) for central reading and analysis. In case of coincident post-ablation visits ((in case of multiple targeted tumors or tumor retreatment), if the imaging assessment has been performed within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

[24] Ablation zone involution assessments will be performed according to the International Working Group on Tumor ablation criteria.

[25] Medications administered during the 24-hour period post-procedure, including analgesics for post-operative pain, and/or anticoagulants will be collected. All new medications administered or the changes in prior treatment received by the patient will be recorded.

[26] Blood product transfusion required during the 24 hours period post-procedure and during the 1-week post-procedure period will be recorded.

[27] The potential occurrence of pain post-ablation will be assessed using a 0 to 100 mm VAS visual analog scale (VAS), where 0 is “no pain” and 100 is “the maximum pain possible”. Patients will have to mark the level of their pain on the VAS before the ablation procedure, within the 24 hour period post-procedure and one week after the procedure. The requirement of analgesic treatment will be also recorded at the same time when the pain assessment (VAS) is conducted (Appendix 5).

[28] All adverse events experienced by patients will be captured from the time the informed consent is signed until the end of the study and will be recorded in the CRF. In addition, all SAEs occurring during use of the VORTX Rx and after the procedure through the end of the study will be reported to the sponsor according to the guidelines specified in section 9.4.1 of this investigational plan.

[29] This visit may coincide with post-ablation follow-up of other tumor ablated during the study.

[30] This visit may coincide with post-ablation follow-up of other tumor ablated during the study.

5.4.1 SCREENING (DAY -60 TO DAY 0)

During the screening period, potential candidates will be provided with the information concerning the study and the patient information sheet along with the informed consent form (ICF). Before any study specific procedures are conducted, informed consent must be obtained, in writing or orally in front of a witness who is independent of the investigator team. No patient will be included in the study until being appropriately informed by the investigator and having provided their informed consent to participate in the study.

Once the informed consent has been obtained, the investigator can proceed with collecting data from the consented individual's medical record and conduct the evaluations required to determine eligibility of the patient for potential participation in this study. These evaluations will be conducted within the 2-month period prior to the procedure. During the screening period (days -60 to 1), it must be confirmed that the patient meets all selection criteria for study participation based on the procedures conducted during this period. Patients will be scheduled for the procedure (baseline visit) by site staff.

Patients who sign an informed consent but fail to undergo an ablation for any reason will be considered a screening failure. The reason for not being enrolled will be entered on the screening log and each patient's demographic information and date of screening failure will be recorded at the screening failure log.

Patient information to be collected at screening will include:

- Demographic data: age, gender and race.
- Relevant medical history (in at least the last 5 years) and current medical conditions. Special attention will be paid to bleeding disorders.
- All relevant medications and non-drug therapies received by the patient at the screening visit or interrupted within 7 days prior to the procedure must be reported in the eCRF.
    - Patients receiving warfarin, clopidogrel, or any other long-acting anticoagulants must discontinue these agents at least two weeks prior to the scheduled procedure.
    - Warfarin patients should have INR draw within the last 7 days prior to the ablation showing INR <2.0 and platelets >50 x 10^9/L.
    - Patients receiving immunotherapies must discontinue these agents 4 weeks prior to the scheduled procedure.
- Data to be collected related to <u>liver tumors</u>:
    - <u>Liver Metastases</u>**:**
        - Primary tumor:
            - Date of diagnosis
            - Disease stage (Tumor/node/metastasis [TNM] classification) at diagnosis
            - Location: e.g. colon, rectum, colon and rectum, breast or pancreas.

- Surgery: yes/no, date, technique
- Neoadjuvant and/or adjuvant treatment: yes/no, start date and completion of treatment
- Adjuvant radiotherapy: yes/no, start and end date

▪ Metastatic disease:

- Date of diagnosis
- Disease stage (TNM classification).
- Tumor disease presentation: synchronous, metachronous
- Number of metastatic sites.
- Metastases location: liver-only metastases, extrahepatic disease (lung, etc.).
- Number and size (maximum diameter) of tumors.
- Location of tumors in the liver segments.
- Prior liver metastases ablation: yes/no
  - Date of ablation
  - Technique used: radiofrequency ablation (RFA), HIFU, Microwave (MWA), Cryo, etc.
  - Tumor ablated: size, location (liver segment).
  - Ablation result: complete or incomplete ablation
- Prior liver metastases resection: yes/no
  - Date of surgery
  - Technique used: one-stage or two-stage hepatectomy, portal vein embolization, etc.
  - Number of metastases resected: number, location (liver segment).
  - Resection rate: complete resection (R0), incomplete resection (R1), not evaluable.
  - Complications after liver resection.
  - Neoadjuvant treatment: yes/no, treatment received (chemotherapy, targeted therapy).
  - Adjuvant therapy: yes/no, treatment received (chemotherapy, targeted therapy, radiotherapy).
- First-, second-, or further line of treatment received for metastatic disease: start and end date, type, number of cycles administered (if applicable), and response to last treatment (last tumor assessment).

▪ HCC:

- Date of diagnosis.
- Disease stage TNM classification/Barcelona-Clinic Liver Cancer (BCLC) staging.
- Number and size of tumors.
- Location of tumors in the liver segments.
- Surgery: yes/no, date, technique used.

- Prior liver ablation: yes/no
    - Date of ablation
    - Technique used: RFA, HIFU, MWA, Cryo etc.
    - Tumors ablated: size, location.
    - Ablation result: complete or incomplete ablation
- Prior chemotherapy: yes/no
    - Start and end date, number of cycles administered.
- Prior intra-arterial treatments: yes/no
    - Type (TACE, Y90, intra-arterial chemotherapy), date.

The following will be conducted to further assess eligibility:

- Physical examination.
- Vital signs: temperature, diastolic and systolic blood pressure, cardiac frequency and respiratory frequency.
- Height, weight and body mass index (BMI).
- ECOG performance status (Appendix 6).
- Child-Pugh score for hepatopathy assessment (total bilirubin, serum albumin, prothrombin time, ascites [presence/absence], hepatic encephalopathy [presence/absence]) and category (A, B, or C). Child-Pugh scoring should be conducted in the 7-14 day period before the ablation procedure day.
- Liver volume reserve (%) measured by CT.
- Standard 12 lead electrocardiogram (ECG).
- Baseline imaging to determine the baseline target tumors according to the criteria of the International Working Group on Tumor Ablation (42, 45):
    - Technique used: MRI (optional CT or ultrasound with or without contrast imaging at discretion of Investigator).
    - The images (MRI and optional CT) will be sent to a central reader, the Department of Radiology of the University of Wisconsin School of Medicine and Public Health (Wisconsin, United States), for central reading and analysis.
- Assessment of liver tumor or liver metastases by MRI (within the last 14 days prior to baseline visit/ablation procedure):
    - Baseline target tumor size (diameter in 3 perpendicular dimensions and volume).
    - Number of baseline tumors identified (confirmatory assessment).
    - Location of baseline tumors in the liver segments (confirmatory assessment).
    - Assessment of the expected ablation volume.
- Procedure simulation (within the last 14 days prior to baseline visit/ablation procedure):
    - Using the ultrasound imaging capabilities of the VORTX Rx® system, a simulation of the ablation procedure will be conducted within the one-week period prior to the planned procedure to confirm the ability of the VORTX Rx® system to visualize the target tumor. Site staff should inform patients to come to the clinic for the simulation after an overnight fast of at least 8 hours.
    - The expected PAV will be assessed by the investigator using the ultrasound imaging of the VORTX Rx® at the session of the procedure simulation.

- Laboratory evaluations (within the last 7 days prior to baseline visit/procedure) as follows:

  Note: Site staff should inform subjects to come to the clinic after an overnight fast of at least 8 hours on the day that blood will be drawn for laboratory assessments.

  - Complete blood count (CBC): Hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts (basophils, eosinophils, lymphocytes, monocytes, neutrophils, and blasts).
  - Metabolic panel: glucose, calcium, sodium, potassium, bicarbonate, chloride, blood urea nitrogen (BUN), creatinine.
  - Liver panel: AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, TP and INR.
  - Urinary analysis: color, pH, bilirubin, blood, glucose, ketones, protein, and specific gravity.
  - Immunologic parameters blood test including:
    - Lymphocyte subset panel: CD3+ (T cells), CD4+ (T helper cells), CD8+ (T suppressor/cytotoxic cells), CD45+ (leucocyte common antigen), CD16+, CD56+ (NKT cells) and CD19+ (B-lymphocyte antigen.
    - C-reactive protein (CRP)
    - Complement C3, C4 and CH50
    - Immunoglobulins (IgG, IgM, IgA)
    - Interleukin 6 (IL-6)
  - Tumor biomarkers: carcinoembryonic antigen (CEA), alfa-fetoprotein (AFP), Cancer Antigens CA15-3 (Breast Cancer) and CA 19-9 (Pancreatic Cancer) (50, 51).
  - Blood samples and urine samples obtained will be sent to the central laboratory (Laboratorio de análisis Echevarne, Barcelona, Spain).
  - An extra amount of blood will be collected when blood samples are taken for hematology and biochemistry evaluation as part of the study assessments. These additional blood samples will be centrally processed and stored to be available for additional, originally unforeseen studies and or new scientific evidence arises in this field of research. Additional blood samples collected will be sent (along with the blood samples and urine samples for study assessments) to the central laboratory where these samples will be preserved for up to three years for future testing as related to this study or future scientific discoveries of the subject's medical condition. The procedure of blood sample collection, identification of samples (labeling), storage conditions, and shipment details will be included in the Central Laboratory Sampling Manual which will be provided to the investigators.
- Serum pregnancy test performed within the last 7 days before the baseline visit/procedure.
- American Society of Anesthesiologist score for anesthetic risk assessment (Appendix 7).
- Quality of life assessment using the EORTC QLQ-C30 questionnaire (Appendix 4).

After completion of all procedures required for subject selection and the subject has been determined to be eligible for the procedure, the investigator/site staff will schedule the procedure and inform the subject to fast at least 8 hours prior to arriving to the site the day that the ablation is scheduled.

5.4.2 VISIT 1: VORTX RX ABLATION PROCEDURE

The investigator must confirm that all the inclusion and none of the exclusion criteria are met the day of the procedure.

Pain assessment must be undertaken. Patients will have to mark the level of their pain on the VAS (Appendix 5) before the ablation procedure.

Data related to the procedure and immediate post-ablation data will be collected:

- Date of procedure.
- Total duration of ablation procedure and total duration of anesthesia.
- Number and size of tumors ablated during the procedure.
- Ablated tumor(s) location: proximity to vital structures (near blood vessels or gallbladder or containing blood vessels or gallbladder [yes/no]).
- Vital signs during ablation and oxygen saturation (SO_2).
- AEs: complications during ablation and immediate post-procedural complications.
    - In addition to the recording of AEs in the CRF, the SAEs must be reported as outlined in the section 9.4.1.
- Medications administered after ablation (if any).
- Transfusion required after ablation: yes/no.

5.4.3 VISIT 2: 24 HOURS POST-ABLATION

- Physical examination, including vital signs.
- Laboratory tests (after an overnight fast of at least 8 hours):
    - CBC: hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts.
    - Metabolic Panel: glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine.
    - Liver panel: AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, PT and INR.
    - Immunologic parameters blood test.
    - Tumor biomarker blood test.
    - Urinalysis: color, pH, bilirubin, blood, glucose, ketones, protein, myoglobin, and specific gravity.
    - Blood samples and urine samples obtained will be sent to the central laboratory (Laboratorio de análisis Echevarne, Barcelona, Spain).
    - Additional blood samples will be drawn and sent to the central laboratory for future testing.
- Assess Child-Pugh score.
- Post procedure imaging using MRI; optional CT or ultrasound with or without contrast imaging at Investigator discretion:
    - Assessment of the ablation zone after the procedure by the central reader: actual ablation zone compared with planned ablation volume (component of success criteria).

  - Complications visible on imaging including assessment of the integrity of vital anatomical structures (major vessels and organs).
  - The images (MRI and optional CT) will be sent to central reader (Department of Radiology of the University of Wisconsin School of Medicine and Public Health, Wisconsin, United States), for central reading and analysis.
- Assessment of AEs occurred within 24 hours post-ablation.
  - In addition to the recording of AEs in the CRF, the SAEs must be reported as outlined in the section 9.4.1.
- Pain assessment by a VAS (Appendix 5).
- Medications administered during the 24-hour period post-procedure, including analgesics (drugs and doses) for post-operative pain, and/or anticoagulants.
- Blood transfusion requirement: yes/no.

5.4.4 Visit 3: One week Post-Ablation

- Hospitalization duration.
- Physical examination, including BMI, and vital signs.
- ECOG PS.
- Laboratory test (Site staff should inform patients to come to the clinic after an overnight fast of at least 8 hours on the day that the blood will be drawn for laboratory evaluations):
  - CBC: hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts.
  - Metabolic Panel: glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine.
  - Liver panel: AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, PT and INR.
  - Urinalysis: color, pH, bilirubin, blood, glucose, ketones, protein, myoglobin, and specific gravity.
  - Immunologic parameters blood test.
  - Tumor biomarkers blood test.
  - Blood samples and urine samples obtained will be sent to the central laboratory.
  - Additional blood samples will be drawn and sent to the central laboratory for future testing.
- Assess Child-Pugh score
- Imaging assessment using MRI and optional CT or ultrasound with or without contrast imaging:
  - Assessment of the ablation zone to evaluate tumor coverage.
  - Evaluation of the ablation zone involution.
  - Complications visible on imaging including assessment of the integrity of vital anatomical structures (major vessels and organs).
  - The images (MRI and optional CT or ultrasound with or without contrast imaging) will be sent to central reader, expected to be the Department of Radiology of the University of Wisconsin School of Medicine and Public Health (Wisconsin, United States), for central reading and analysis.

- Pain assessment by a visual analog scale (VAS) (Appendix 5).
- Relevant medications received.
- Blood transfusion requirement: yes/no.
- Assessment of the evolution of AEs (if any) and AEs occurred since last visit.
    - In addition to the recording of AEs in the CRF, the SAEs must be reported as outlined in the section 9.4.1.

5.4.5 VISIT 4: ONE MONTH POST-ABLATION

- Physical examination: BMI, vital signs.
- ECOG PS.
- Laboratory test (Site staff should inform patients to come to the clinic after an overnight fast of at least 8 hours on the day that the blood will be drawn for laboratory evaluations):
    - CBC: hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts.
    - Metabolic Panel: glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine.
    - Liver panel: AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, PT and INR.
    - Urinalysis: color, pH, bilirubin, blood, glucose, ketones, protein, myoglobin, and specific gravity.
    - Immunologic parameters blood test.
    - Tumor biomarkers blood test.
    - Blood samples and urine samples obtained will be sent to the central laboratory.
    - In case of coincident post-ablation visits (in case of multiple ablation sessions), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor (combination of tumors ablated in the same session), it will not be necessary to repeat this assessment.
    - Additional blood samples will be drawn and sent to the central laboratory for future testing.
- Assess Child-Pugh score
- Imaging assessment using MRI and optional CT or ultrasound with or without contrast imaging:
    - Assessment of the ablation zone to evaluate tumor coverage.
    - Evaluation of the ablation zone involution as measured by comparative 3 dimensional (3D) images.
    - Complications visible on imaging including assessment of the integrity of vital anatomical structures (major vessels and organs).
    - The images (MRI and optional CT or ultrasound with or without contrast imaging) will be sent to central reader for central reading and analysis.
    - In case of coincident monthly post-ablation visits (in case of multiple ablation sessions), if the imaging assessment has been performed within the last week as a part of the post-ablation follow-up of a tumor (combination of tumors ablated in the same session), it will not be necessary to repeat this assessment.
- Relevant treatment received

  - Treatment prescribed for cancer management: any antineoplastic treatment required by the patient, including chemotherapy or targeted biological therapies, will be recorded.

- Assessment of the evolution of complications (if any) and AEs occurred since last visit.
  - In addition to the recording of AEs in the CRF, the SAEs must be reported as outlined in the section 9.4.1.
- Quality of life assessment using the EORTC QLQ-C30 questionnaire (Appendix 4).

  In case of coincident post-ablation visits (in case of multiple ablation sessions), if patient´s quality of life has been assessed using the EORTC QLQ-C30 questionnaire within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

5.4.6 Visit 5: Two months Post-Ablation

- Physical examination: BMI, vital signs.
- ECOG PS.
- Laboratory test (Site staff should inform patients to come to the clinic after an overnight fast of at least 8 hours on the day that the blood will be drawn for laboratory evaluations):
  - CBC: hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts.
  - Metabolic Panel: glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine.
  - Liver panel: AST, ALT, alkaline phosphatase, albumin and bilirubin, GGT, PT and INR.
  - Urinalysis: color, pH, bilirubin, blood, glucose, ketones, protein, myoglobin, and specific gravity.
  - Immunologic parameters blood test.
  - Tumor biomarkers blood test.
  - Blood samples and urine samples obtained will be sent to the central laboratory.
  - In case of coincident post-ablation visits (in case of multiple ablation sessions), if a laboratory test has been performed within the last week as a part of the post-ablation follow-up of a tumor (or combinations of tumors ablated in the same session), it will not be necessary to repeat this assessment.
  - Additional blood samples will be drawn and sent to the central laboratory for future testing.
- Assess Child-Pugh score
- Imaging assessment using MRI and optional CT or ultrasound with or without contrast imaging:
  - Assessment of the ablation zone to evaluate tumor coverage.
  - Evaluation of the ablation zone involution.
  - Complications visible on imaging including assessment of the integrity of vital anatomical structures (major vessels and organs).
  - The images (MRI and optional CT or ultrasound with or without contrast imaging) will be sent to central reader for central reading and analysis.
  - In case of coincident monthly post-ablation visits (in case of multiple ablation sessions), if the imaging assessment has been performed within the last week

as a part of the post-ablation follow-up of a tumor (or combinations of tumors ablated in the same session), it will not be necessary to repeat this assessment.

- Relevant treatment received:
    - Treatment prescribed for cancer management: any antineoplastic treatment required by the patient, including chemotherapy or targeted biological therapies, will be recorded.
- Assessment of the evolution of complications (if any) and AEs occurred since last visit.
    - In addition to the recording of AEs in the CRF, the SAEs must be reported as outlined in the section 9.4.1.
- Quality of life assessment using the EORTC QLQ-C30 questionnaire (Appendix 4).

  In case of coincident post-ablation visits (in case of multiple ablation sessions), if patient´s quality of life has been assessed using the EORTC QLQ-C30 questionnaire within the last week as a part of the post-ablation follow-up of a tumor, it will not be necessary to repeat this assessment.

5.4.7 End of the study or termination

The end of study (at data closure after last patient last visit (LPLV)) will occur after all subjects in the study have completed their last assessment as per protocol.

The study can be terminated at any time for any reason by the Sponsor or regulatory authorities when there were risks for subjects or other reasons that justify such a decision. A participating site can be closed when there is evidence of non-compliance with this study protocol. Both the Sponsor and investigator will have the right to early terminate the study at any moment. Should this occur they both will revise and decide the procedures to follow in each case, due consideration will be taken to the protection of subjects participating in the study. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the subjects' interests. The sponsor will be responsible for informing the CEIm (for its initials in Spanish, *Comité Ético de la Investigación con medicamentos*) of the early termination of the trial.

6 Ablation

6.1 Medical device

6.1.1 Medical device overview

Investigational medical device: VORTX Rx®

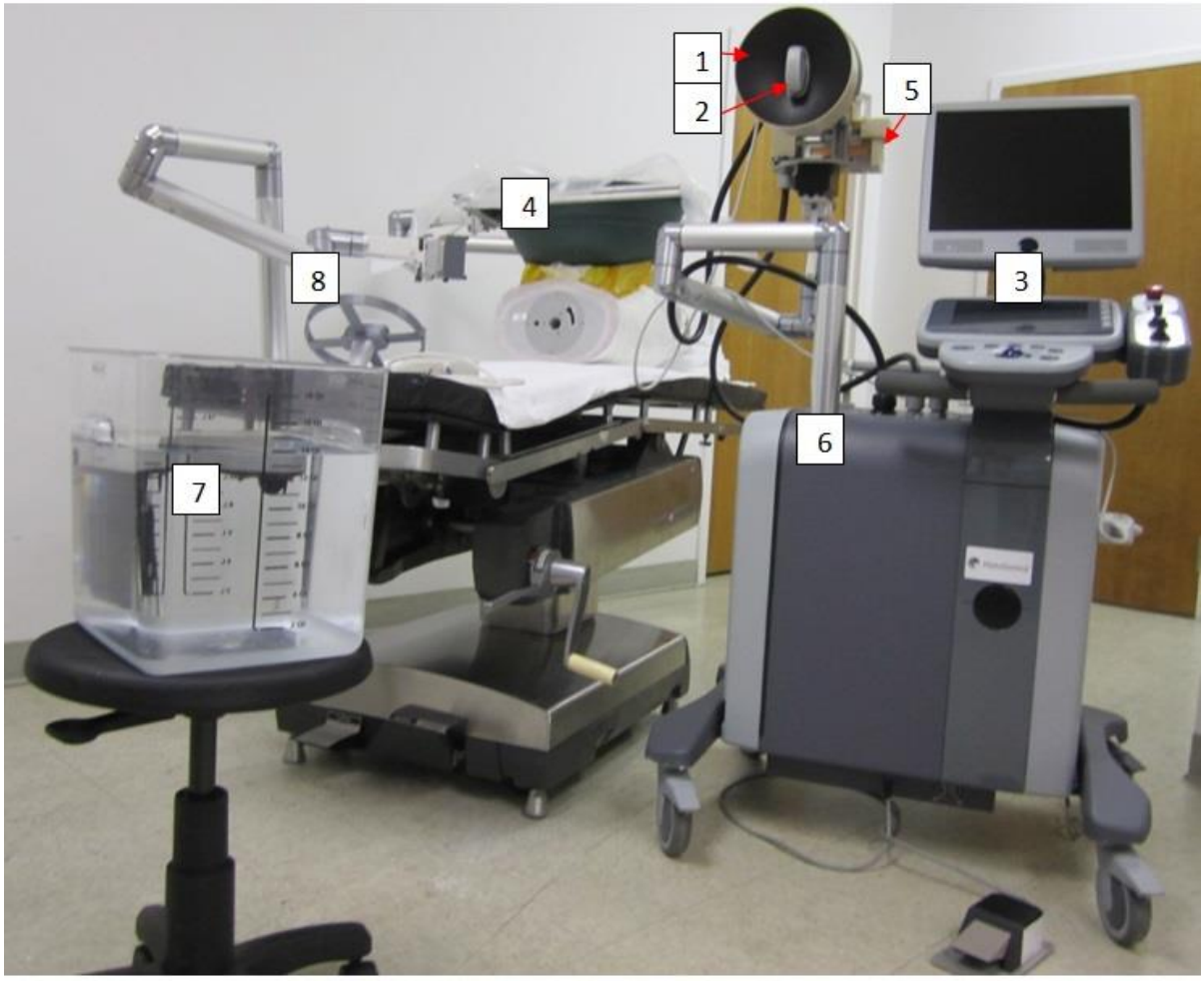
The VORTX Rx® is a platform device for non-invasive, non-thermal, image-guided histotripsy ablation delivery. The system as configured is a portable device designed to treat soft tissues using very low duty cycle ultrasound pulses from outside the patient's body. The low duty cycle refers to the fact that the microsecond histotripsy pulses are only on approximately 1% of the time. These pulses form an acoustic cavitation bubble cloud at the focal area which mechanically destroys the cellular structure of the soft tissue with no remaining intact cells. The bubble cloud is positioned in the tissue of the liver by the clinician.

The VORTX Rx® ultrasound system is a mobile (AC-powered) designed to treat soft tissue (e.g., liver) through the extracorporeal application of high intensity focused ultrasound. It consists of a

high frequency ultrasound therapy transducer (generator), a micro-positioner, an Ultrasound Medium Coupler (UMC) bowl to provide a water coupling (e.g. degassed water and chilling probe) and 3M Ioban™ drape, a hand-held imaging probe (e.g., a piezoelectric transducer), operator's console (cart), monitoring systems, e-stop button, software, and a foot-switch. See figure below (Figure 2) or the User Manual for further details.

This device is for *Investigational Purposes Only* and will NOT be CE Marked. The proposed conformity assessment of the VORTX RX® System is a Class IIb, rule 9 device according to Annex IX of the Council Directive 93/42/EEC, The device rules include administration of energy in a potentially hazardous way (VORTX RX primary mode of action is to ablate soft tissue through mechanical focused ultrasound cavitation). It is an active, non-implantable medical device. The device has software controls. None of the special rules (rules 13 to 18) apply.

Figure 2. VORTX Rx® system



1 Therapy Transducer
2 Imaging Probe
3 Console & Micropositioner Control (Software)
4 Ultrasound Medium Container (UMC): bowl, non-sterile 3M Ioban™ drape & holder
5 Micropositioner
6 Cart and Arm
7 Coupling Medium (degassed water container)
8 Laser Alignment Tool & Positioning Tool

6.2 ABLATION PROCEDURE USING THE VORTX RX

The study investigator will be fully trained on the VORTX Rx® device and will be supported during the procedure by qualified and trained engineering personnel from HistoSonics, Inc. Site personnel will be qualified and trained prior to the study. A copy of the User Manual will be provided to the site personnel.

Within 7-14 days prior to the procedure day, MRI imaging of the targeted tumor will be conducted to identify the location and dimensions of the targeted tumor(s). In addition, within a week prior to each ablation procedure, a procedure simulation will be carried out using VORTX Rx® ultrasound imaging of the liver to assess the targeted tumor(s) prior to the procedure to ensure adequate and clear visualization using ultrasound-image guidance.

Patients will be admitted to the hospital for the procedure. The ablation will be performed in the surgery room of the center(s). Patients will be placed under general anesthesia to reduce patient discomfort and provide for investigator complete control, including patient breathing and motion during the procedure.

The ablation will be performed using the initial system settings determined by pre-clinical studies, as detailed in the User Manual. Prior to the procedure, the device will perform a system check on itself (not involving a patient) to ensure device performance and alignment of the bubble cloud to the focal position of the therapy transducer. The therapy transducer with co-axially aligned ultrasound imager will then be coupled to the patient's chest or abdomen using the ultrasound medium coupling device (UMC), which will be applied to the patient. The investigator will use the mechanical arm to grossly position the therapy transducer to the center of the targeted tumor volume. Once the center of the tumor has been identified on ultrasound imaging, the investigator will lock the arm in place to fix the position of the therapy transducer for pre-ablation planning.

Prior to starting the ablation, the investigator will use the system software to align the target tissue contours and margin contours to cover the entire target region. Once these parameters are set, the investigator will scan through the PAV using the system micro-positioner in order to verify that the target contour diameters match the desired target in every imaging plane. The investigator can then make minor adjustments to the plan as needed, prior to proceeding to the ablation test pulse stage of the workflow.

Once the investigator has confirmed the treatment plan, a test pulse will be delivered in order to generate a bubble cloud at the center of the tumor. To accomplish this, the investigator will adjust the therapy system output until a bubble cloud is achieved and sustained at the focus. The investigator will confirm that the bubble cloud location matches the expected ablation location (i.e. crosshairs) and make any minor modifications to the crosshairs as needed. Once test pulse energy has been applied, the subject will be considered enrolled.

After successful completion of the test pulse, the investigator will move to the treatment stage of the workflow and initiate the ablation. In this process, the system will mechanically scan through the entire targeted volume within the contours, including the margin, set during the planning stage. During ablation, the investigator will monitor the procedure using real-time ultrasound imaging to ensure the bubble cloud remains in the expected location in the target throughout the procedure. The system provides visual indicators of treated and untreated points during the procedure. The investigator will have the ability to visualize the need to stop and restart the ablation at any time, such as to correct for any unexpected motion or mis-alignment of the bubble cloud that may occur.

After the entire ablation is finished, the investigator will review the ablated zone using ultrasound imaging to determine if any further ablation is required. The investigator will then confirm the ablation is completed and remove the therapy system from the patient. The overall procedure

time is expected to last less than 4 hours. After the procedure, patients are expected to be hospitalized for up to 24 hours. They will be discharged within 24 hours whenever patients have not experienced any complication requiring extended hospitalization.

More details on the procedure to be performed using the investigational device is provided in the IB.

6.3 MEANS OF SUPPLY OF THE MEDICAL DEVICE

The VORTX Rx® medical device used in this study will be supplied by HistoSonics, Inc. (Ann Arbor, Michigan, USA). This is an investigational device that is a not CE-marked and will be imported into the European Union using an importation company dedicated to that purpose.

7 CONCOMITANT MEDICATIONS

All medications and non-drug therapies (including blood transfusions) administered during the study will be listed in the eCRF. The generic drug name, the reason for prescription, and the initiation and completion dates will be recorded.

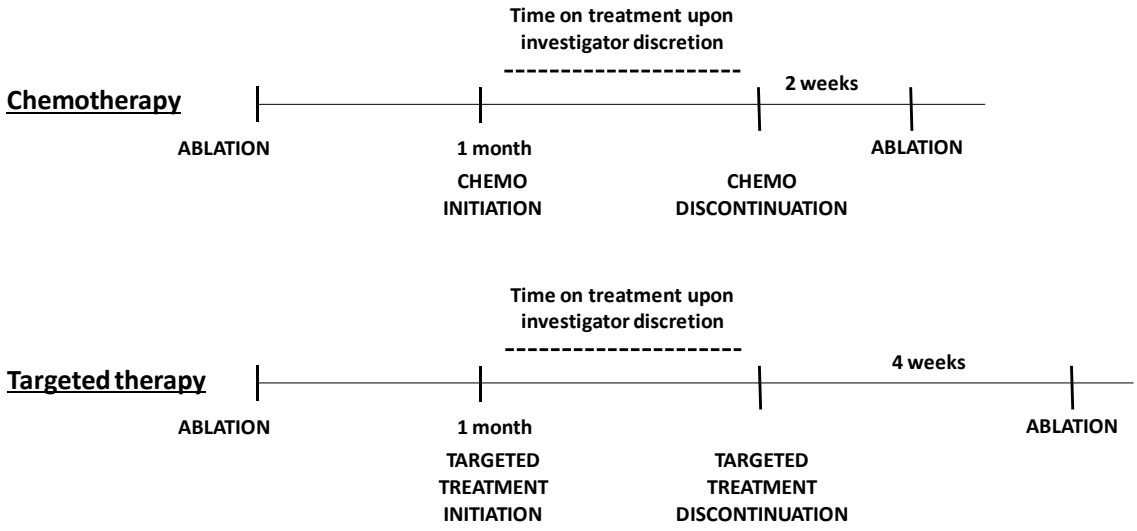
The patient will be told to notify the investigator about any new medications he/she takes during the study.

7.1 PERMITTED CONCOMITANT MEDICATIONS

The use of any medication needed by patients for proper clinical management is allowed at the discretion of the investigator, with the exception of those detailed in section 7.2 whose use would require patient´s withdrawal.

Chemotherapy and targeted therapies are allowed to be used during the study provided that a one month period has elapsed since the ablation was performed. In addition, a subsequent ablation may be performed 2 weeks after chemotherapy completion/discontinuation and after 4 weeks following any targeted treatment completion. A maximum of 3 treatments (chemotherapy or targeted therapies) will be allowed during the patient follow up. Each treatment received by the patient during his/her participation in the study will be restricted to the timing specified per protocol (minimum 1 month between ablation procedures, minimum 2 weeks post any chemotherapy with said chemotherapy not to begin before 1 month post any ablation) (Figure 3).

Figure 3. Chemotherapy and/or targeted therapy administration



The use of analgesics for pain is allowed after ablation whenever these agents are prescribed by the investigator. The use of analgesics during the study will be recorded in the eCRF.

7.2 Prohibited concomitant medications/interventions

The following medications are prohibited during the study:

- Any investigational drug administration or use of any investigational medical device.
- Clopidogrel and warfarin or any other long-acting anticoagulants.

8 Assessments

8.1 Ablation assessments

Radiologic evaluation of the ablation zone will be conducted according to The International Working Group on Image-guided Tumor Ablation (42, 45) to determine technical success (primary endpoint) 1 day after the ablation procedure and to evaluate local tumor progression and involution of the ablation zone on each subsequent follow-up visit (1 week and 1 and 2 months post-ablation).

Post-ablation imaging will utilize contrast-enhanced dynamic MRI using a 1.5T MR scanner with maximum slice thickness of 5 mm. Optional contrast-enhanced multidetector CT, with at least 64 slices and a maximum slice thickness of 5 mm or ultrasound with or without contrast, may be used at investigator discretion. The same technique used for evaluation of target tumors for patient selection (baseline evaluation prior the procedure) will be used for evaluation of the ablation zone. Both MRI and optional CT imaging modalities will be performed with pre- and post-dynamic intravenous contrast enhancement. MRI is the primary modality for imaging follow-up, given that this technique provides excellent soft tissue resolution with multiplanar imaging capability and lacks ionizing radiation. However, if a contraindication so warrants or if additional data is desired, CT or ultrasound with or without contrast imaging may be performed in some cases at the discretion of the Investigator.

Radiologic evaluation of acute technical success and the ablation zone progression during the study will be conducted by an independent central-reader radiologist. All images (MRI and CT) will be sent through the eCRF or other common secured imaging portal or via secure media to a central reader, expected to be the Department of Radiology of the University of Wisconsin School of Medicine and Public Health (Wisconsin, United States), where the data sets will be analyzed.

8.1.1 Technical Performance assessments

Acute technical performance will be evaluated on the basis of technical success of each tumor ablation assessed by contrast enhanced MRI 1-day post-procedure.

Technical success of the ablation is defined as the ability of the investigational device to generate an ablation zone per the PAV (a three-dimensional volume defined by the investigator using the VORTX Rx® in the planning phase of the software workflow). Technical success will be determined by assessing if the post-procedure ablation zone corresponds to the PAV, as measured by a multi-dimensional measurement of the 1-day post-procedure ablation zone and characterized qualitatively in the radiological report. The acute technical success of up to 3 separate tumor ablations in any individual patient will be assessed.

8.1.2 Tumor Progression Assessment

Local tumor progression will be assessed by radiological evaluation according to the International Working Group on Image-guided Tumor Ablation Group at 1-week, 1-month and 2-months post-ablation.

Local tumor progression is defined as the appearance of tumor foci at the edge of the ablation zone after ablation has been achieved.

Baseline imaging will be used to obtain measurements of the target tumor and will be used as a comparator to the ablation zone at follow-up imaging time points.

8.1.3 Involution of the Ablation Zone

Involution of the ablation zone during the study will be assessed by the radiological evaluation of the ablation zone at 1-week, 1-month and 2-months post-ablation. The 1-day post-procedure MRI will serve as the baseline for the radiological evaluation of the involution of the ablation zone.

8.2 Safety assessments

Safety will be monitored by assessing and collecting AEs, SAEs, laboratory evaluations, physical examinations, vital signs, ECOG performance status, ECG, and imaging studies.

Safety will be evaluated from the point of ablation initiation (first ablation performed) through to each post-ablation follow-up visits during patient participation. The investigator will assess the appearance of AEs from procedure initiation and will record it in the eCRF, together with concomitant medications and/or blood product transfusions received over this time and any antineoplastic therapies used through study completion.

An independent DSMB will be used for review of safety data to provide advice to the sponsor regarding actions the board recommends to ensure the safety, protection and well-being of the subjects enrolled in the study. Additional details regarding the structure of the DSMB is provided in Section 9.5.

For details on AE collection and reporting, refer to Section 9.4.

8.2.1 PHYSICAL EXAMINATION

A physical examination will be performed at screening (and before any ablation procedure performed) and at every follow-up visits. It will include the examination of general appearance and body systems as indicated by patient symptoms, AEs, or any other findings.

Significant findings that were present prior to the signing of informed consent must be included in the eCRF. Significant new findings that begin or worsen after informed consent is signed must be recorded as AEs in the eCRF.

8.2.2 VITAL SIGNS

Vital signs will be collected in all study visits. Vital signs will be taken with the patient in the sitting position after 5 minutes of rest.

8.2.3 HEIGHT AND WEIGHT

Height in centimeters (cm) will be measured only at screening. Body weight in kilograms (to the nearest 0.1 kilogram [kg] in indoor clothing, but without shoes) will be measured during the screening visit and at subsequent follow-up visits to assess BMI.

8.2.4 ECOG PERFORMANCE STATUS

The performance status will be assessed according to the ECOG performance status at screening, and at post-ablation follow-up visits 1 week, 1 and 2 months post-ablation.

The ECOG performance status is graded on a 6-point scale that ranges from 0 to 5 (Appendix 6).

8.2.5 LABORATORY EVALUATIONS

All laboratory assessments will be performed at a central qualified laboratory. Site staff should inform patients to come to the clinic after an overnight fast of at least 8 hours on the day that the blood will be drawn for laboratory evaluations.

In case of coincident post-ablation visits when more than one ablation procedure has been performed, it will not be necessary to repeat the laboratory test if this assessment has been performed within the last week as a part of the post-ablation follow-up of a tumor.

Table 2. Local clinical laboratory parameters collection plan

Test	Parameters to be tested	Visit
Complete blood count	Hematocrit, hemoglobin, platelets, red blood cells, white blood cells, differential counts (basophils, eosinophils, lymphocytes, monocytes, neutrophils, and % of blasts).	During the screening period (7 days before the baseline visit) and at every follow-up visit (1 day, 1 week and 1 and 2 months post-ablation).
Chemistry	Sodium, potassium, calcium, chloride, bicarbonate, glucose, total protein, BUN, creatinine, alkaline phosphatase, ALT (SGPT), AST (SGOT), GGT, albumin, total bilirubin	During the screening period (7 days before the baseline visit) and at every follow-up visit (1 day, 1 week and 1 and 2 months post-ablation).
Coagulation	PT, PTT and INR.	During the screening period (7 days before the

		baseline visit) and at every follow-up visit (1 day, 1 week and 1 and 2 months post-ablation).
Immune tests	Lymphocyte subset panel: CD3+ (T cells), CD4+ (T helper cells), CD8+ (T suppressor/cytotoxic cells), CD45+ (leucocyte common antigen), CD16+, CD56+ (NKT cells) and CD19+ (B-lymphocyte antigen), CRP, complement C3, C4 and CH56, immunoglobulins (IgG, IgM, IgA) and IL-6.	At screening (7 days before the baseline visit) and at every follow-up visits performed, 1 day, 1 week, 1 and 2 months post-ablation.
Urinalysis	Dipstick urinalysis. Macroscopic panel: color, pH, bilirubin, blood, glucose, ketones, protein, and specific gravity.	During the screening period (7 days before the baseline visit) and at every follow-up visit (1 day, 1 week and 1 and 2 months post-ablation).
Pregnancy test	Serum pregnancy test.	During the screening period (within the last 14 days before the baseline visit) and at any moment if necessary.
Other laboratory analysis	Not applicable	Not applicable
Tumor biomarkers	Carcinoembryonic antigen (CEA), alfa-fetoprotein (AFP), Cancer Antigens CA15-3 (Breast Cancer) and CA 19-9 (Pancreatic Cancer) (50, 51)	At screening (7 days before the baseline visit) and at follow-up visits performed 1 week, 1 and 2 months post-ablation.

8.2.6 Pregnancy test

All female patients (regardless of childbearing potential) must undergo a serum pregnancy test at screening to confirm eligibility in the trial.

In case an additional pregnancy test is indicated during the trial, a serum test should be performed. In case of pregnancy, the patient must be immediately withdrawn from the trial, and the pregnancy must be reported immediately following the same procedure as for SAE. Pregnancy, suspected pregnancy, or a positive pregnancy test should be reported within 24 hours to the CRO Dynamic Science S.L. using the Pregnancy Report Form and following the same criteria than those established for SAE reporting (Section 9.4.1).

8.3 Other assessments

8.3.1 Screening purpose

8.3.1.1 American Society of Anesthesiologist Score

Anesthetic risk will be assessed at screening using the American Society of Anesthesiologist (ASA) score (55) (Appendix 7). It also referred to as ASA physical status (ASA-PS). The ASA physical status class risk stratification system is based on comorbid conditions that are a threat to life or that limit activity and thus helps in predicting preoperative risks.

The ASA score will be used a global score to subjectively assess the physical status of patients before the procedure and estimate pre-operative risk. All patients must be assessed for their ASA-PS at screening to confirm eligibility in the study and before each ablation procedure performed during the study.

8.3.1.2 Electrocardiogram

A standard 12 lead ECG will be performed at screening. In addition, an ECG will be performed as a part of the procedures performed before any ablation in case a period of more than 6 months has elapsed since the last ECG.

Interpretation of the tracing must be documented in the eCRF. Each ECG tracing should be labeled with the study code, patient number, date, and kept in the source documents at the study site.

Clinically significant abnormalities present after the patient signed informed consent should be reported in the eCRF as medical history. Clinically significant findings must be discussed with the Sponsor prior to enrolling the patient in the study. New or worsened clinically significant findings occurring after informed consent must be recorded as AEs in the eCRF.

8.3.2 Patient Reported Outcome assessment

8.3.2.1 Pain assessment

The potential occurrence of pain post-ablation will be assessed using a 0 to 100 mm VAS, where 0 is “no pain” and 100 is “the maximum pain possible” (Appendix 5). Patients will be asked to mark the level of their pain on the 100 mm VAS at the day of the ablation (before the procedure), and within the 24 hours period post-ablation and one week after the procedure. The requirement of analgesic treatment will be also recorded at the same time when the pain assessment (VAS) is conducted. The use of analgesic will be recorded in the eCRF.

8.3.2.2 Quality of life assessment

The quality of life of patients (QoL) will be evaluated using the validated Spanish version of the EORT QLQ-C30 questionnaire version 3.0 (56) (Appendix 4).

The patients will be asked to complete the first pre-procedure questionnaire at the clinic and then to complete the questionnaire for the second time while they attend to the clinic 1 and 2 months following the procedure. The questionnaire will be delivered to patients by site staff for self-completion at the clinic. The patients will be given brief instructions on how the questionnaire should be completed.

The EORTC QLQ-C30 is a 30-item generic health-related QoL instrument designed to assess cancer patients’ physical, psychological and social functioning. It is composed of 9 multi-item scales (5 functional scales [physical, role, cognitive, emotional, and social], a global QoL scale [GQoL], and 3 symptom scales [fatigue, pain, and nausea and vomiting]), 5 single-item symptom scales assessing additional symptoms commonly reported by cancer patients (dyspnoea, loss of appetite, insomnia, constipation and diarrhea), and an item on the perceived financial impact of the disease.

All of the scales and single-item measures range in score from 0–100. A high scale score represents a higher response level. Thus, a high score for a functional scale represents a high/healthy level of functioning. A high score for the global health status/QoL represents a high QoL. However, a high score for a symptom scale/item represents a high level of symptomatology/problems.

9 SAFETY MONITORING AND REPORTING

9.1 EXPECTED ADVERSE EVENTS

The expected adverse events associated with the device when used clinically for liver tumor ablation include potential risks typically associated with other established ablation methods, ultrasound-energy-delivery related risks, patient positioning-related complications and potential cytotoxicity associated to the skin contact with the device transducer. In addition, patients will be under general anesthesia during the procedures, as respiration and motion needs to be controlled. The potential occurrence of anesthesia-related AEs must therefore be considered.

Taking into consideration risks associated with other established ablation methods, ultrasound-energy delivery, patient positioning, anesthesia, and preclinical experience with the device, the following potential clinical risks have been identified for non-invasive VORTX Rx® system and related procedure:

Intervening tissue thermal injury, skin burn/irritation, rib burn, ,major blood vessel stenosis, major bile duct stenosis, lung damage, intestinal perforation, gallbladder damage/perforation, intervening tissue mechanical damage, petechial hemorrhage, intra-hepatic hematoma, hemothorax, intractable pleural effusion, tumor implantation, large blood vessel rupture/excessive bleeding, major bile duct rupture/biloma, excessive hemolysis, transient liver dysfunction/hepatic failure, ascites, chronic liver dysfunction/hepatic failure, liver abscess, peritoneal bleeding, diaphragmatic injury, unnecessary exposure to anesthesia, portal vein thrombosis, venous thrombosis, pulmonary embolism, cardiac arrest, post-ablation syndrome (delayed pain, nausea, fever, vomiting), pain, and death.

9.2 SAFETY PLAN

The VORTX Rx® was developed using HistoSonics' International Organization for Standardization (ISO) 13485 - Quality Management System (QMS) procedures and to United States 21 Code of Federal Regulations (CFR) Part 820 Quality System Regulation, ISO 14971 Medical Devices – Application of Risk Management to Medical Devices and European (EU) Council Directive 93/42/EEC 2007 and the essential requirements for investigational devices in Annex X and MEDDEV 2.7/3 rev. 3 Clinical Investigations: Serious Adverse Event Reporting.

The risk management plan is fully integrated with HistoSonics' product realization procedures and design controls with the intent of eliminating, or minimizing, real and potential risks associated with the device to acceptable levels when compared with the potential benefits for the indicated use/intended purpose. The product risk analysis includes three types of development related analyses: 1) use hazard analysis (includes usability/human factors), 2) product design risk analysis, and 3) process risk analysis (manufacture and assembly to include safety testing), as well as a clinical risk analysis. The entire process results in a risk management report that summarizes the risk mitigation efforts, details the residual risks, and documents risk management acceptance.

The VORTX Rx® is a currently a production-equivalent device intended for investigational use only. The risk management plan is based on the clinical experience available with the device used in the feasibility study for BPH treatment, the proposed indications for use for liver, and the available preclinical experience in animal models and ongoing studies for soft tissue ablation. Please refer to the IB for a complete summary of safety information.

Several measures will be taken to ensure the safety of patients participating in this study. Careful selection of patients to exclude patients at higher risk for toxicities, a detailed pre-procedure patients' assessment, participation by only qualified investigators, and close monitoring during the procedure and post-ablation follow-up are measures that will be employed

to minimize potential risks. Patients will undergo careful safety monitoring throughout the study, including assessments of the nature, frequency and severity of AEs.

Clinical risks must be minimized by detailed assessment of the subject, especially in regard to coagulopathy, underlying hepatic reserve, and tumor proximity to major structures such as the bile duct or intestine, prior to, during and after the procedure by qualified investigators. In addition, close post-ablation follow-up of patients may minimize risks associated with the medical device and the procedure that may not have been anticipated and will allow for rapid response to any conditions that are presented.

An independent DSMB will be used for review of safety data to provide advice to the sponsor regarding actions the board recommends to ensure the safety, protection and well-being of the subjects enrolled in the study. The members of the DSMB will be experts who are independent from the Sponsor. Additional details regarding the structure of the DSMB is provided in Section 9.5.

9.3 Adverse events

9.3.1 Definitions

9.3.1.1 Adverse Event (AE)

An AE is defined as any untoward medical occurrence, unintended disease or injury or any untoward clinical sign(s), symptom(s), or medical condition(s) (including abnormal laboratory finding if considered clinically significant by the investigator), in patients participating in a clinical investigation with a medical device, whether or not related to the investigational medical device.

Pre-existing conditions will not be reported during the study unless exacerbated. Conditions that were already present at the time of informed consent will be recorded as medical history in the eCRF. Worsening of any pre-existing condition will be reported an AE, or as a SAE if the event meets the criteria for a SAE (see Section 9.3.1.2).

Laboratory abnormalities and abnormal vital signs that are definitely or probably device related will be considered an AE if they are clinically significant in the investigator´s judgment, induce clinical signs or symptoms, or require a medical intervention or change in concomitant therapy. Medical and scientific judgment will be exercised in deciding whether an isolate laboratory or vital sign abnormality should be classified as an AE.

The investigator will provide the study monitor with the normal laboratory values before the study initiation.

AEs (including laboratory abnormalities that constitute an AE) will be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms (e.g. anemia instead of low hemoglobin). When a clear diagnosis cannot be identified, each sign or symptom will be reported as a separate AE.

The presence of post-procedure pain will not be recorded as an AE during the immediate follow-up visits 1 day and 1 week after the procedure, as post-procedure pain will be expected data collected at these visits. However, pain will be recorded as an AE thereafter.

The presence of complications from the point of ablation initiation will be recorded as AEs in the CRF, and it will be reported as a SAE if the event meets the seriousness criteria specified below.

9.3.1.2 Serious adverse events

A serious adverse event is defined as any AE that meets at least one of the following criteria:

- It is fatal (death is a result, it is not an event)
- It is life-threatening (It refers to an AE in which the patient was at risk of death at the time of the event; It does not refer to an event which hypothetically might have caused death if it had been more serious).
- Requires inpatient hospitalization or prolongation of existing hospitalization (unplanned hospitalization during at least one night)
- Results in persistent or significant disability/incapacity.
- Constitutes a congenital anomaly/birth defect.
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.
- Note that planned hospitalization for pre-existing condition, or a procedure required by the Clinical Investigation Plan, without serious deterioration in health is not considered a SAE.

9.3.1.3 Adverse device effect (ADE)

An adverse device effect (ADE) includes any AE resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device. This includes any event that is a result of a use error or intentional misuse.

9.3.1.4 Unanticipated serious adverse Device Effects (UADE)

Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.3.1.5 Serious adverse device effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a SAE.

9.3.1.6 Unanticipated serious adverse Device Effects (USADE)

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.

NOTE: Anticipated SADE (ASADE): an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report.

9.3.2 Causality

The relationship between the use of the medical device and the occurrence of each AE shall be assessed and categorized. During causality assessment activity, clinical judgement shall be used and the relevant documents, such as the IB and the Clinical Investigational Plan (CIP) shall be consulted. The presence of confounding factors, such as concomitant

medication/treatment, the natural history of the underlying disease, other concurrent illness or risk factors shall also be considered.

The investigator will assess the reasonable possibility that the study medical device either caused or contributed to the AE.

The Sponsor and the investigators will use the following definitions to assess the relationship of the AE to the investigational medical device:

- Not related: relationship to the device or procedures can be excluded when:
    - the event is not a known side effect of the product category the device belongs to or of similar devices;
    - the event has no temporal relationship with the use of the investigational device or the procedures;
    - the discontinuation of the medical device application does not impact on the event:
    - the event involves a body-site or an organ not expected to be affected by the device.
    - the serious event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors);
    - harms to the subject are not clearly due to use error;
    - In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the event.
- Unlikely: the relationship with the use of the device seems not relevant and/or the event can be reasonably explained by another cause, but additional information may be obtained.
- Possible: the relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.
- Probable: the relationship with the use of the investigational device seems relevant and/or the event cannot reasonably be explained by another cause, but additional information may be obtained.
- Causal relationship: the adverse event is associated with the investigational device or with procedures beyond reasonable doubt when:
    - the event is a known side effect of the product category the device belongs to or of similar devices and procedures;
    - the event has a temporal relationship with investigational device use/application or procedures;
    - the event involves a body-site or organ that
        - the investigational device is applied to
        - the investigational device has an effect on;
    - the event follows a known response pattern to the medical device (if the response pattern is previously known);
    - other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out;
    - harm to the subject is due to error in use;

- To establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device and the serious event.

The Sponsor and the investigators will distinguish between the AEs related to the investigational device and those related to the procedures (any procedure specific to the clinical investigation). An AE can be related both to procedures and the investigational device. Complications of procedures are considered not related if the said procedures would have been applied to the patients also in the absence of investigational device use/application.

In some cases, the event may be not adequately assessed because information is insufficient or contradictory and/or the data cannot be verified or supplemented. The Sponsor and the Investigators will make the maximum effort to define and categorize the event and avoid these situations. Where the Sponsor remains uncertain about classifying the serious event, it should not exclude the relatedness and classify the event as "possible".

9.3.3 SEVERITY

The severity of the AEs will be assessed according to the NCI CTCAE version 4.0 (44) (Appendix 2). If NCI CTCAE grading does not exist for an AE, the severity of mild, moderate, severe, life-threatening and death, corresponding to grades 1 - 5, will be used:

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a].
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL[b,c]
4	Life-threatening consequences; urgent intervention indicated [d].
5	Death related to AE [d].

Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

If an event is assessed as "medically significant" it must be reported as a SAE.

Grade 5 AE must be reported as a SAE.

9.4 COLLECTING AND REPORTING OF ADVERSE EVENTS

The investigator must record all AEs, whether or not related to the use of the study medical device in the CRF.

The occurrence of AEs should be sought by questioning of the patient during the screening process after signing informed consent and at each visit during the study. AEs also may be detected when they are volunteered by the patient during the screening process or between visits, or through physical examination, laboratory test, or other assessments.

As far as possible, the following information will be collected for each AE in the CRF:

- The severity grade according to the NCI CTCAE version 4.0 (Appendix 2).
- Its duration (start and end dates, or ongoing at end of study).
- Its suspected relationship to the investigational device (VORTX Rx®)
- Whether any medication or therapy was given due to the AE. If a concomitant medication or non-drug therapy is given, this action should be recorded on the eCRF.
- Whether it is serious.
- Its outcome.

Once an AE is detected, it should be followed until its resolution or until it is judged to be stabilized; an assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the medical device, the interventions required to treat it, and the outcome.

9.4.1 Reporting of serious adverse events

9.4.1.1 Report by the investigator to the sponsor

Every SAE, regardless of suspected causality, occurring after ablation initiation, will be reported to the Sponsor within 24 hours of learning of its occurrence. The new findings/updates to the already reported SAE will be notified as follow-up to the original episode within 24 hours of the investigator after awareness by the investigator.

Information about all SAEs will be collected and recorded on the SAE Form where all applicable sections of the form must be completed. The investigator will assess and record the relationship of each SAE to the study medical device or the procedure, complete the SAE Form in English, and send the completed, signed form by fax within 24 hours to the CRO Dynamic Science S.L. (Fax: +34 914561126), who will communicate the SAEs to the Sponsor. The original copy of the SAE Form and the fax confirmation sheet will be kept with the eCRF documentation at the study site.

9.4.1.2 Report by the sponsor to the Competent Authorities

SAE reporting to the National Competent Authorities will be conducted according to the MEDDEV 2.7/3 revision 3 (May 2015) guideline for SAEs reporting in clinical investigations with medical devices in line with the requirements of Annex 7 of Directive 90/385/EEC and Annex X of Directive 93/42/EEC, as amended by Directive 2007/47/EC, and the Circular 07/2004 regulating clinical investigations with medical devices. The sponsor will report all SAEs to Spanish Agency of Medicines and Medical Devices (Agencia Española de Medicamentos y Productos Sanitarios - AEMPS acronym in Spanish) and to the applicable CEIm.

The Sponsor will notify the AEMPS of all SAEs experienced since commencement of the procedure, regardless of suspected causal relationship no later than 7 days after occurrence. For SAEs which indicate an imminent risk of death, serious injury, or serious illness, that requires prompt remedial action for other patients/subjects, users or other persons* or a new finding to it, the SAE reporting to the AEMPS will be performed immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event.

The completed SAE report form will be sent by email in Excel to the AEMPS, or an equivalent format which allows using the inserted filters.

The SAE reporting will be updated and transmitted to the AEMPS each time a new reportable event or a new finding/update to an already reported event is to be reported. More detailed information will be provided on request of the AEMPS, if so requested by using the individual

reporting form. The Sponsor shall identify the new/updated information in the status column of the tabular form as:

a = added = new reportable event;

m = modified =new finding/update to an already reported event;

u = unchanged

Changes in a line should be highlighted in bold and/or color in the respective column.

* This includes: A) events that are of significant and unexpected nature such that they become alarming as a potential public health hazard, e.g. human immunodeficiency virus (HIV) or Creutzfeldt-Jacob Disease (CJD). These concerns may be identified by either the NCA or the manufacturer. B) the possibility of multiple deaths occurring at short intervals.

9.5 DATA AND SAFETY MONITORING BOARD

The Data and Safety Monitoring Board (DSMB) is an independent group of experts that advises study team, sponsor and the study investigators. The members of the DSMB serve in an individual capacity and provide their expertise and recommendations. The members of the DSMB will be experts who are independent from the Sponsor.

This board will convene at regular intervals to review any SAEs and device malfunctions that occur during the study. The DSMB will serve as a data monitor and make recommendations based on the data regarding the safety of patients utilizing a risk/benefit ratio in relation to the study and ablation outcomes. The DSMB will make recommendations to the study sponsor regarding the safety of the study, and whether the study should continue under the current protocol, under a revised protocol or be stopped due to safety concerns. The DSMB may also recommend halting enrollment to perform a detailed review of a device or patient situation/AE that is unexpected or in a higher frequency than has previously been reported. Patient safety is the highest priority for this study, involving this device and procedure. A DSMB charter will be developed, which will outline the guidelines the DSMB will use to review and make recommendations to the Sponsor regarding study continuation.

DSMB Composition: The composition DSMB for this study will be included in the DSMB Charter.

DSMB meetings/review frequency: It is anticipated the DSMB will meet monthly for this study; however, meetings may be postponed or cancelled due to lack of new data.

The DSMB will review the research protocol and plans for data and safety monitoring. Once monthly, the DSMB will review the study's data manager's report that will include the following information:

- The number of participants consented for the study
- The reasons for withdrawal (if any) from the study
- Any safety concerns revealed during the course of the study
- Summary of AEs and serious AEs
- Summary of UADEs
- The current, approved consent form

Any material presented to the DSMB will be presented in a way to preserve patient confidentiality. The DSMB will also review the investigator's summary of any new data or evidence that might alter the risk/benefit ratio for participating in the study. After reviewing this information, the DSMB will hold a meeting with the Study Sponsor and report its summary of any serious and unexpected AEs, UADEs, or other unanticipated problems that involve risk to

study participants, and whether these appear related to the study-based interventions. Minutes of such meeting will be prepared to document the DSMB's communication to the study sponsor.

The membership of the DSMB should reflect the disciplines and medical specialties necessary to interpret the data from the clinical study and to fully evaluate participant safety. The number of DSMB members, will be based on the complexity in design and analysis, and potential level of risk but generally consists of three to seven members with desired participation from:

- Expert(s) in the clinical aspects of the disease/patient population being studied;
- A biostatistician; and,
- Investigators with expertise in current clinical study conduct and methodology

Ad hoc specialists may be invited to participate as non-voting members at any time if additional expertise is desired.

Staff without direct involvement in study implementation and who meet other membership criteria may participate as *ex officio*, non-voting members. Staff serving in these positions must have a current confidential financial disclosure report on file. Representatives of the sponsor (industry) of the test device or any other individual with vested interests in the outcome of the study are not eligible to serve on the DSMB although they may attend open sessions of the DSMB meetings.

No member of the DSMB should have direct involvement in the conduct of the study.

There will be regular, ongoing communication between the study sponsor, the investigator and the DSMB. The investigator will take responsibility for reporting any serious and unexpected AEs or other unanticipated study problems to the study sponsor, and, where required, the Competent Authority. A copy of each report will be sent to the DSMB. Actions taken by the ethics committee in response to AE reports will be immediately reported to the DSMB, investigators and study sponsor, which will review all SAEs, as they arise.

10 STATISTICAL METHODS AND DATA ANALYSIS

10.1 SAMPLE CALCULATION

No formal statistical power calculations to determine sample size is performed for this study due to the nature of this study design and patient population.

Due to the nature of this study in which the investigational device will be used for the first time for soft tissue ablation of liver tumors in humans, the approximate number of patients planned to be included in this study is 10.

10.2 OVERALL CONSIDERATIONS

Upon completion of the study, after the data for the final study patient have been recorded, and once all possible data discrepancies have been resolved, the study database will be locked and transferred to the person responsible for statistical analysis.

A detailed statistical analysis plan will be prepared at the appropriate time, which will be approved by the sponsor, to guide the analysis of the data and justify any change in the original plan.

A general description will be given of the variables included in the study and non-parametric tests will be performed if applicable.

There are no plans to allocate unavailable data, which will simply be described as missing data.

The statistical analysis of the study data will be carried by the biometrics department of Dynamic Science S.L.

No interim analyses are planned.

10.3 Analysis sets

10.3.1 Full analysis set

The full analysis set includes all patients who have undergone an ablation with the investigational device. The full analysis set will be used for all listings of raw data.

10.3.2 Safety population

The study population that will be included in the safety analysis consist of all patients who have undergone an ablation with the investigational device.

10.3.3 Technical Performance population

The technical performance population set includes all patients who have undergone an ablation with the investigational device and have an assessment of technical performance.

10.4 Patient demographics/other baseline characteristics

Demographic and other baseline data will be summarized descriptively for the full analysis set.

10.5 Primary objective/Endpoint

The primary objective of the study is to evaluate the acute technical performance of the VORTX Rx® medical device for the ablation of primary and metastatic liver tumors. The ability of the investigational device to create an ablation zone that correspond to the PAV (a three-dimensional volume defined by the investigator using the VORTX Rx® in the planning phase of the software workflow) will be assessed by multi-dimensional measurement of the 1 day post-procedure ablation zone and accompanied by general radiological findings characterized (qualitatively) in radiology report from a central radiologist reader applying The International Working Group Image-guided Tumor Ablation guidance (42, 45). The acute technical success of up to 3 separate tumor ablations will be assessed in each patient.

The number of targeted tumors in whom technical success is achieved (ablation zone corresponds to PAV) will be divided by the number of targeted tumors in which the ablation has been initiated with the investigational device to calculate technical success percentage.

10.6 Secondary Objectives

- Safety profile of the investigational medical device will be assessed on the basis of all AEs (serious and non-serious, and probably or definitively related to the medical device) experienced during the procedure conducted with this medical device and all observed and volunteered AEs and abnormal findings on physical examination, vital signs, laboratory and imaging tests throughout the study.

  The safety analysis will be performed in the “safety population” which includes all patients who have undergone an ablation with the VORTX Rx® medical device.

  All AEs recorded during the study will be listed and summarized. All SAEs will be listed by patients. Any other safety information will be listed and tabulated as appropriate. This

includes analysis of all AE and SAEs throughout the study, physical examinations, changes in vital signs, ECOG performance status, laboratory values, and ECG.

Summary tables for AEs should include only AEs that started or worsened during the ablation and post-ablation follow-up period (2 months).

The incidence of ablation-emergent AEs (new or worsening since ablation initiation) will be summarized by system organ class and or preferred term, severity (based on CTCAE version 4.0 grades), type of AE, whether they were SAEs or not, and relation to the medical device under study.

The incidence of the following AEs, if definitively related to the study medical device, will be evaluated:

1. Major bleeding that requires transfusion within 48 hours
2. Visceral perforation (bowel or stomach)
3. Major biliary duct injury
4. Death

The safety profile of the study will be deemed favorable if there are:

- No deaths due to the ablation, and
- No deaths from visceral perforation (bowel or stomach), and
- 20% or lower incidence of major bleeding requiring transfusion within 48h, and
- 20% or lower incidence major bile duct injury.

- To assess the local tumor progression.
    - Assessment of the ablation zone of each tumor will be conducted by a central-reader radiologist based on local tumor progression according to the International Working Group Image-guided Tumor Ablation (42, 45) (Appendix 3) at 1-week, 1 month and 2 months post-ablation.
- To assess the involution of the ablation zone.
    - The size of each tumor ablation zone on MRI and optional CT will be captured at each post-ablation follow up visit will be assessed and compared at 1-week, 1 month and 2 months post-ablation.
- To assess liver panel post-ablation.
    - The change in the levels of the liver function parameters (AST, ALT, alkaline phosphatase, albumin, bilirubin, GGT, PT and INR) will be calculated from baseline to the post-procedure evaluation performed 24 hours post-ablation and the follow-up visits performed 1 week, and 1 and 2 months post-procedure.
- To evaluate immunological response of ablation
    - The change in the levels from immune tests (Immune tests including: CD3+, CD4+, CD8+, CD45+, CD16+, CD56+ and CD19+, CRP, complement C3, C4 and CH50, immunoglobulins (IgG, IgM, IgA)), IL-6, and tumor biomarkers (CEA, AFP, Cancer Antigens CA15-3 (Breast Cancer) and CA 19-9 (Pancreatic Cancer) (50, 51) will be calculated from baseline to the post-procedure evaluation performed 24 hours post-procedure and the follow-up visits performed 1 week, and 1 and 2 months post-procedure.
- To evaluate patient quality of life of patient's post-procedure.
    - For the analysis of change of quality of life, the descriptive statistics of the score of the dimensions and the total score of the QLQ-C30 questionnaire will be performed, and the change from the screening to the visits at 1 and 2 months post-procedure will be calculated.

  - QLQ-C30 scores will be generated according to the EORTC Scoring Manual (Appendix 4). These scores vary from 0 (worst) to 100 (best) for the GHS and functional scales, and from 0 (best) to 100 (worst) for symptomatic scales.

- To assess pain and analgesic requirements post-procedure.
  - The VAS scale score will be calculated 24 hours and 1 week post-procedure and the change in pain score will be analyzed (Appendix 5).
  - The number of patients requiring analgesics 24 hours and 1 week post-procedure will be calculated and compared.

11 ETHICAL CONSIDERATIONS

11.1 REGULATORY AND ETHICAL COMPLIANCE

This clinical trial will be conducted in accordance with the protocol, the principles established in the current revised version of the Declaration of Helsinki (Fortaleza, Brazil; October 2013), and in accordance with applicable regulatory requirements for investigations with medical devices, in particular the Circular 7/2004 of the AEMPS regulating the clinical investigations with medical devices.

By signing the protocol, the investigator agrees to adhere to the instructions and procedures described in the protocol and thereby to adhere to the principles of good clinical practice for medical device trials that it conforms to.

The sponsor will submit the pertinent documentation to the CEIm. The study may not be started until approval by the CEIm and the AEMPS has been obtained.

Handling, communication and transfer of the personal data for all the participating subjects will comply with the stipulations of the Regulation (EU) 2016/679 on Personal Data Protection. The investigator and study data will be entered and processed in a file owned by the sponsor, which will be held by Dynamic Science, in accordance with the aforementioned Organic Law.

The study staff involved in the conduct of this trial will be appropriately qualified education, training and experience to perform the assigned tasks.

11.2 INFORMED CONSENT PROCEDURES

Each subject who is asked to participate in the study will be informed about the same both orally and in writing providing them with a document called “Patient Information Sheet”, which will contain the necessary relevant information for the patient in a form that is entirely legible and comprehensible to decide if he/she wishes to participate in the trial (Appendix 1).

It is the responsibility of the investigator to obtain informed consent from each patient participating in the study after explaining the objectives, methods, potential benefits and risks of the study deriving from their participation in the study, the confidentiality of personal data and the contact details for the doctor responsible for the study. Consent must be obtained before performing any study-specific procedure. Each patient should be explained in a clear and unequivocal manner that he/she is free to refuse to participate in the study and that he/she may withdraw his/her consent at any time for any reason without penalty or refusal of the best treatment as the investigator’s discretion. The investigators must keep the signed informed consent in the study file and it must be documented in the eCRF and in the patients’ medical records. The investigator will obtain the subject’s informed consent (preferably in writing, or if not feasible, orally before witnesses independent of the research team). In addition, the subject participating in the study must be given a copy of the informed consent.

11.3 CONFIDENTIALITY

The information disclosed and obtained during this study will be considered confidential and must be treated as such at all times.

The study data and materials may not be disclosed in part or in full by the investigator or his/her staff to any unauthorized person without the prior formal written consent of the sponsor.

All materials, information (oral or written) and unpublished documentation provided to the investigators, including this protocol and the CRFs (electronic and/or paper), and all documents generated during the study and the database, will be considered the property of the sponsor, and will be protected from non-permitted uses by persons unrelated to the research and, therefore, will be considered strictly confidential and will not be disclosed to third parties except for those specified in the subsequent paragraph.

The investigator and the institution will permit direct access to the source data and documents for the performance of monitoring, auditing, review by the medicinal product research ethics committee, and trial inspection by the health authorities. This access will be restricted to the sponsor, monitor, study doctors and their research staff, the AEMPS, the competent health authorities of the autonomous communities, the CEIm, and the sponsor or personnel authorized by the sponsor, when necessary to verify the study data and procedures, but always maintaining their confidentiality in accordance to current legislation.

Only medical history data that are related to the study will be subject to verification. This verification will be done to the extent possible in the presence of the principal investigator/coinvestigators, and the confidentiality of all personal data of the subjects participating in the clinical trial will be maintained at all times in accordance with the Regulation (EU) 2016/679 on Personal Data Protection.

Handling, communication and transfer of the personal data for all the participating subjects will comply with the stipulations of the Regulation (EU) 2016/679 on Personal Data Protection. The investigator and study data will be entered and processed in a file owned by the sponsor, which will be held by Dynamic Science, in accordance with the aforementioned Organic Law.

12 PRACTICAL CONSIDERATIONS

12.1 ARCHIVING

For the purpose of the clinical trial, a file will be generated with all the study documentation, including the protocol, CRF, signed informed consents, reports of SAEs and authorizations of National Competent Authorities. One copy of which will be kept by the investigator at his/her site (investigator file) and another by the sponsor (sponsor file). The content of these files will comply with the requirements for study documentation in applicable legislation.

The study documentation will be kept for the time required by currently applicable legislation.

12.2 SOURCE DOCUMENTS

The CRF will be considered the source document for the VAS scale for pain assessment and the quality of life questionnaire QLQ-C30, since these specific study procedures are not performed under clinical practice conditions.

Access to source documents

The study will be monitored by regular visits and phone calls to the investigator. During visits to the study site, the monitor must review the original patient records, medication records and document storage. The monitor must also evaluate the study procedures and discuss any possible problems with the investigator. During the course of the study, auditing visits to the participating sites may be performed.

In compliance with applicable regulations, the investigator and his/her team as well as the study site are required to permit authorized representatives of the study sponsor, regulatory agencies and the CEIm direct access for review of the data/documents of the patients' original medical records for verification of the study procedures and study-related data. This direct access includes examination, analysis, verification and reproduction of any record or report that is important for evaluation of the study. The investigator is obliged to inform and obtain consent from the subject to permit access by designated representative to the subject's study-related records without violating the confidentiality of the subject.

13 QUALITY CONTROL

13.1 STUDY MONITORING

The accuracy of the data recorded in the eCRF will be verified by study monitor by verifying the recorded data. During the study, monitoring visits will be performed to ensure the study complies with all aspects of the protocol, good clinical practice (GCP) guidelines and applicable legislation. The source documents (original documents, data and records) will be reviewed to verify the information recorded in the eCRFs. The investigator and the site will ensure the sponsor or its representative (including the monitor), the CEIm and the health authorities have access to the study documents. This access includes the investigator file, eCRF, patient information sheet and informed consent, medical histories of the subjects and other source documents. It is important that the investigator and study staff be available during the monitoring visits and devotes sufficient time to the process.

14 FUNDING

All financial aspects related to the clinical trial will be specified in a contract between the sponsor and the sites where the trial is to be conducted. The financial budget of the study will be made available to the CEim for its evaluation.

The sponsor will fund the study in accordance with the guidelines of the present protocol. This funding includes all materials necessary to conduct the study; the cost of the authorization and control processes with the research and health authorities; design, maintenance and management of the database; and statistical analysis of the information generated. In any event, the funding shall be independent of the results of the study.

As required by current legislation and in particular by the Circular 07/2004, the sponsor has taken out an insurance policy with the company Chubb European Group Limited, with an address at Paseo de la Castellana 141, 6 Planta 28046 Madrid, España and with policy number ESCAIA21218, which covers the liabilities of the sponsor/principal investigator, the research staff and the sites where the clinical trial is conducted, in the event of any damage or injury to the health of the patient resulting from this research, conducted strictly in accordance with both the scientific protocol and applicable law in Spain and professional standards, during the conduct of the study and for one year following termination of the study, unless otherwise proven.

15 PUBLICATION GUIDELINES

All information related to the study is considered confidential and property of the sponsor until its publication. It must not be revealed to others without prior written consent from the sponsor.

Once the study has been completed and the statistical report has been performed, the sponsor, in conjunction with the investigators, will prepare the final study report, which will be submitted to the investigators, the research ethics committees, the AEMPS and the health authorities, if so requested. This final report will be the basis for the preparation of the manuscripts that are desired to be published in medical journals.

The sponsor is obliged to publish the results, whether positive or negative, in scientific journals, meetings and/or congresses, before being disclosed to the non-healthcare public, indicating the funding obtained for conduct of the trial and ensuring at all times the anonymity of the participating subjects.

The results or conclusions of this study will be reported at scientific congresses and published in scientific journals. The investigator agrees to maintain this information in strict confidence, and will not use it for any other purpose without the written authorization of the sponsor.

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17 APPENDICES

APPENDIX 1. Patient information sheet and informed consent form (ICF)

Attached as separate documents.

APPENDIX 2. NCI-CTCAE (Version 4.0)

Attached as a separate document.

APPENDIX 3. International Working Group on Image-Guided Tumor Ablation Guidance Document

Attached as a separate document.

APPENDIX 4. EORTC QLQ-C30 questionnaire

Attached as a separate document.

APPENDIX 5. VAS for pain assessment

Attached as a separate document.

APPENDIX 6. Eastern Cooperative Oncology Group performance status (ECOG)

Grade	Performance 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.
Source: Oken et al (1982).	

APPENDIX 7. American Society of Anesthesiologist score

ASA PHYSICAL STATUS CLASSIFICATION SYSTEM

Last approved by the ASA House of Delegates on October 15, 2014

Current definitions (NO CHANGE) and Examples (NEW)

ASA PS Classification	Definition	Examples, including, but not limited to:
ASA I	A normal healthy patient	Healthy, non-smoking, no or minimal alcohol use
ASA II	A patient with mild systemic disease	Mild diseases only without substantive functional limitations. Examples include (but not limited to): current smoker, social alcohol drinker, pregnancy, obesity (30 < BMI < 40), well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	Substantive functional limitations; One or more moderate to severe diseases. Examples include (but not limited to): poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, premature infant PCA < 60 weeks, history (>3 months) of MI, CVA, TIA, or CAD/stents.
ASA IV	A patient with severe systemic disease that is a constant threat to life	Examples include (but not limited to): recent (< 3 months) MI, CVA, TIA, or CAD/stents, ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis
ASA V	A moribund patient who is not expected to survive without the operation	Examples include (but not limited to): ruptured abdominal/thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischemic bowel in the face of significant cardiac pathology or multiple organ/system dysfunction
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes	

*The addition of “E” denotes Emergency surgery: (An emergency is defined as existing when delay in treatment of the patient would lead to a significant increase in the threat to life or body part)