Azcona 31
28028 Madrid
Tel: 902 88 41 02
Fax: 902 88 41 03

Avinguda Josep Tarradellas 8-10
Planta 5, puerta 4
08029 Barcelona
Tel: 93 351 16 15 / Fax: 93 349 63 41

Dynamic

F-B/10-01-002

Study Theresa

"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)"

Statistical Analysis Plan
Version: 1.0
January 2020

Prepared by: Laura Casas **Date:** **Signature**:
Approved by: **Date:** **Signature**:

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
ADE	Adverse event effect
AE	Adverse Event
CI	Confidence Interval
ECOG	Eastern Cooperative Oncology Group
PAV	Planned ablation volume
Q1	First quartile
Q3	Third quartile

1 INTRODUCTION

The statistical analysis plan proposed below details the necessary aspects to know about the study and the statistical methods to be used to apply them in the final statistical analysis.

2 STUDY RATIONALE

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.

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. 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, 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®.

3 OBJECTIVES AND DESIGN

3.1 Study objectives

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.

Secondary objectives

1. Assessment of the safety profile of the VORTX Rx®.
2. Assessment of local tumor progression by MRI at 1 week, 1 month and 2 months, post-procedure.
3. Assessment of the involution of the ablation zone 1 week, 1 month and 2 months post-procedure.
4. Assessment of liver panel.
5. Immunologic assessment.
6. Evaluation of quality of life.
7. Assessment of pain and analgesic requirements after the ablation procedure.

3.2 Study population

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

Inclusion criteria

1. Written informed consent before any study procedure is performed.
2. Subjects of both sexes aged 18 years or older.
3. 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
   - 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.
4. 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.
5. 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.
6. 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.
7. 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).
8. Tumor(s) to be targeted for ablation will be clearly separated from other tumors or other critical areas upon investigator´s criteria.
9. Largest diameter of targeted tumor(s) ≤3 cm.
10. Tumor(s) that will be targeted at a depth <10 cm from the skin surface.
11. 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.
12. Patients who can safely undergo general anaesthesia.

13. Liver function score of Child-Pugh A or Child-Pugh B.
14. ECOG PS 0, 1 or 2 at screening.
15. 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 109/L and platelet > 50 x 109/L).
16. 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.
17. Platelets level >50 x 109/L within the last 7 days prior to the ablation

Exclusion criteria

1. Patients who decline or are unable to understand, provide or are unwilling to sign an informed consent form.
2. 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).
3. Targeted tumor(s) not clearly separated.
4. Targeted tumor(s) >3 cm.
5. Tumor that will be targeted >10 cm from the skin surface.
6. Tumor not clearly visible with diagnostic ultrasound and MRI.
7. Liver function score of Child-Pugh C.
8. Liver volume reserve <40% as measured by CT Scan.
9. 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.
10. Patient who has not recovered to grade 1 or better from any AEs (except alopecia, fatigue, nausea, vomiting) related to previous anti neoplastic therapies.
11. BMI >30.
12. Parkinson's disease.
13. History of bleeding disorders (e.g. von Willebrand disease) or patients suspected to have a bleeding disorder.
14. Not able to temporarily discontinue warfarin, clopidogrel or any other long-acting anticoagulants at least two weeks before the procedure.
15. Initiation of any anticancer treatment during the screening period.
16. Life expectancy to be less than 6 months.
17. ASA score ≥ 4.

18. Unable or unwilling to complete all required screening and/or follow-up assessments.
19. Patients under ongoing treatment with an investigational medication or medical device that conflicts with the study device.
20. Patients for whom the investigator considers that the ablation is not in the patient's best interest.
21. 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.
22. Patients with any concurrent condition that, in the investigator's opinion, would jeopardize the safety of the patient or compliance with the protocol.
23. Patients with known sensitivity to topically applied iodine.

3.3 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.

4 METHODOLOGY

Quantitative variables will be described with measures of central tendency and dispersion: mean, median, SD (standard deviation), Q1 (first quartile) and Q3 (third quartile), minimum and maximum).

Qualitative variables will be described using absolute and relative frequencies (N, %). Two percentage columns will be presented, total percentage (total %) and valid percentage (valid %), that is, the percentage over the sum of valid responses plus missing values and the percentage over the total of valid responses. When there will be not missing values both of them will be equal, and then only one will be shown.

In the analysis of parameters evolution (ablation zone, liver function parameters, immunological parameters, quality of life scores, VAS scale score), among visits, general linear models of repeated measures will be used. With this methodology, it is necessary that the values of the variable must be available at all visits for all patients.

Where an inferential analysis is required, parametric tests shall be used for continuous variables (T-test) that follow a normal distribution. For variables that do not follow a normal (or non parametric) distribution, Mann Whitney (for unpaired data) or Wilcoxon (for paired data) hypothesis tests shall be used.

These tests will be used in all bilateral cases and with a level of significance of 0.05. In cases where a p-value less than 0.05 appears, it refers to the existence of statistical significance.

Due to the number of evaluable patient ablations available, any result from an applied statistical test must be interpreted with caution.

5 ANALYSIS SETS

5.1 Full analysis set

The full analysis set includes all of targeted tumors ablated from all patients who have undergone an ablation with the investigational device.

5.2 Safety population

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

5.3 Technical performance population

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

5.4 Patient demographics/other baseline characteristics

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

6 DESCRIPTIVE ANALYSIS

6.1 Screening failures

The reason of screening failures will be provided.

6.2 Demographic data

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Age (it will be calculated on the date of informed consent)

The categorical variables to be described (n, %) are:

- Sex
- Race

6.3 Type of liver tumors

The categorical variable to be described (n, %) is:

- Type of liver tumors

6.4 Data related to liver tumors: Liver Metastases

If **Liver metastasis** was selected on Selection Visit the following analyses will be shown:

6.4.1 Primary tumor

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Time from date of diagnosis to study inclusion: Calculated on the day of signature of informed consent (date of informed consent – date of diagnosis)

The categorical variables to be described (n, %) are:

- Disease stage at diagnosis
- Tumor location
- Surgery, if yes, technique used will be described per all surgeries undergone
- Has the patient received neoadjuvant/adjuvant therapy? if yes, the following variables will be described for all records registered:
    - Neoadjuvant/Adjuvant
    - Treatment (Chemotherapy, Targeted therapy, Radiotherapy)
        - In case of chemotherapy or Targeted therapy, type of treatment will be described

6.4.2 Metastatic disease

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Time from date of diagnosis of metastatic disease to study inclusion: Calculated on the day of signature of informed consent (date of informed consent – date of diagnosis of metastatic disease)

The categorical variables to be described (n, %) are:

- Tumor disease presentation
- Disease stage
- Number of metastasis sites
- Metastasis location:
    - Liver-only metastases
    - Extra-hepatic disease (lung, brain, bone, other)

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Number of liver tumor nodules

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown for all records registered:

- Size or tumor nodules (maximum diameter)

The categorical variable to be described (n, %) is for all records registered:

- Location or tumor nodules: liver segment

The categorical variables to be described (n, %) are:

- Prior liver metastases ablation, if yes, the following variables will be described for all ablations registered:
    - Technique used (n,%)
    - Number of tumor nodules ablated (mean, SD, median, Q_1, Q_3, minimum, maximum):
        - Size for all records registered (mean, SD, median, Q_1, Q_3, minimum, maximum)
        - Location: liver segment for all records registered (n,%)
    - Ablation result (n,%)

- Prior liver metastases resection, if yes, the following variables will be described for all resections registered:
    - Technique used (n,%)
    - Number of metastases resected (mean, SD, median, Q_1, Q_3, minimum, maximum):
        - Location: liver segment (n,%)
        - Ablation result (n,%)
    - Complications after liver resection, if yes:
        - Intra-abdominal infection
        - Wound infection
        - Steatohepatitis
        - Sinusoidal dilatation
        - Other
- Neoadjuvant/adjuvant therapy, if yes, the following variables will be described for all records registered:
    - Neoadjuvant/Adjuvant (n,%)
    - Treatment (Chemotherapy, Targeted therapy, Radiotherapy) (n, %)
    - In case of chemotherapy or Targeted therapy, type of treatment will be described (n,%)
- Has the patient receive any treatment for metastatic disease?, if yes, the following variables will be described for all records registered:
    - Type of treatment (n, %)
    - Response (n,%)
    - Number of cycles administered (mean, SD, median, Q_1, Q_3, minimum, maximum)
- Number of lines of treatment for metastatic disease (mean, SD, median, Q_1, Q_3, minimum, maximum)

6.5 Data related to liver tumors: Hepatocellular carcinoma

If **Hepatocellular carcinoma** was selected on Selection Visit the following analyses will be shown:

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Time from date of diagnosis to study inclusion: Calculated on the day of signature of informed consent (date of informed consent – date of diagnosis)

The categorical variables to be described (n, %) are:

- Disease stage
- Barcelona-Clinic Liver Cancer staging

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Number of liver tumor nodules

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown for all records registered:

- Size or tumor nodules (maximum diameter)

The categorical variable to be described (n, %) is for all records registered:

- Location or tumor nodules: liver segment

The categorical variables to be described (n, %) are:

- Surgery, if yes, technique used will be described per all surgeries undergone
- Prior liver metastases ablation, if yes, the following variables will be described for all ablations registered:
    - Technique used (n,%)
    - Number of tumor nodules ablated (mean, SD, median, Q_1, Q_3, minimum, maximum):
        - Size for all records registered (mean, SD, median, Q_1, Q_3, minimum, maximum)
        - Location: liver segment for all records registered (n,%)
    - Ablation result (n,%)
- Prior chemotherapy, if yes, the following variables will be described for all records registered:
    - Type of treatment (n, %)

- Number of cycles administered (mean, SD, median, Q_1, Q_3, minimum, maximum)

- Prior intra-arterial treatments, if yes, the following variables will be described for all records registered:
  - Type (n, %)

6.6 Medical history

The categorical variables to be described (n, %) are:

- Has the patient any previous or current relevant medical condition?
- Description
- Type
- For each type “Does it continue?” will be described
- For each type “The patient is treated for this medical condition” will be described

6.7 Physical examination

6.7.1 Anthropometric data

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Height
- Weight
- BMI

6.7.2 Vital signs

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

6.7.3 Physical examination

The categorical variables to be described (n, %) are:

- Result
  - Clinically significant
  - Not clinically significant

6.7.4 ECOG

The categorical variable to be described (n, %) is:

- ECOG

6.7.5 Standard 12 Lead electrocardiogram

The categorical variables to be described (n, %) are:

- Result
    - Clinically significant
    - Not clinically significant

6.8 Laboratory test

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Haemoglobin
- Erythrocytes
- Leucocytes
- Neutrophils
- Basophils
- Haematocrit
- Lymphocytes
- Monocytes
- Eosinophils
- Platelets
- AST
- ALT
- GGT
- Alkaline phosphatase
- Total bilirubin
- Albumin
- Prothrombine (PT)
- INR
- Partial thromboplastin time
- Glucose
- Calcium
- Sodium

- Potassium
- Bicarbonate
- Chloride
- BUN
- Creatinine
- CD3+
- CD4+
- CD8+
- CD45+
- CD16+
- CD56+
- CD19+
- C reactive protein
- Complement C3
- Complement C4
- Complement CH50
- IgG
- IgM
- IgA
- Interleukin-6
- CEA
- AFP
- Ca 15-3
- Ca 19-9

6.9 Child-Pugh score

Child-Pugh score and Child-Pugh category will be calculated according to the following rules:

Child-Pugh Score and Interpretation			
Classification	**1**	**2**	**3**
Serum bilirubin (µmol/L)	<34	34-51	>51
Serum albumin (g/L)	>35	28-35	<28
Presence of ascites	Absent	Controllable	Refractory
Encephalopathy	Absent	Minimal	Severe
INR	<1.7	1.7-2.3	>2.3
Interpretation			
Points	**Class**	**Life Expectancy**	**Perioperative Mortality**
5-6	A	15-50 yrs	10%
7-9	B	Candidate for transplant	30%
10-15	C	1-3 months	82%

The categorical variable to be described (n, %) is:

- Child-Pugh category

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Child-Pugh score

6.10 Liver volume reserve

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Liver volume reserve

6.11 Anaesthetic risk assessment

The categorical variable to be described (n, %) is:

- American society of anaesthesiologist score

6.12 Baseline imaging of the target tumor

The categorical variables to be described (n, %) are:

- Technique used for tumor imaging:
    - Magnetic resonance imaging
    - Computed tomography

- Ultrasound with contrast
- Ultrasound without contrast

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Number of targeted tumors planned to be ablated

The following analyses will be carried out for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Tumor location: liver segment
- Tumor size:
  - X-axis greatest dimension
  - Y-axis greatest dimension
  - Z-axis greatest dimension
- Volume
- Expected ablation volume

6.13 Quality of life assessment

The score of each dimension of this scale will be calculated according to the established scoring algorithms:

Scoring the EORTC QLQ-C30 version 3.0

	Scale	Number of items	Item range*	**Version 3.0** Item numbers	Function scales
Global health status / QoL					
Global health status/QoL (revised)†	QL2	2	6	29, 30	
Functional scales					
Physical functioning (revised)†	PF2	5	3	1 to 5	F
Role functioning (revised)†	RF2	2	3	6, 7	F
Emotional functioning	EF	4	3	21 to 24	F
Cognitive functioning	CF	2	3	20, 25	F
Social functioning	SF	2	3	26, 27	F
Symptom scales / items					
Fatigue	FA	3	3	10, 12, 18	
Nausea and vomiting	NV	2	3	14, 15	
Pain	PA	2	3	9, 19	
Dyspnoea	DY	1	3	8	
Insomnia	SL	1	3	11	
Appetite loss	AP	1	3	13	
Constipation	CO	1	3	16	
Diarrhoea	DI	1	3	17	
Financial difficulties	FI	1	3	28	

** Item range* is the difference between the possible maximum and the minimum response to individual items; most items take values from 1 to 4, giving *range* = 3.*
† (revised) scales are those that have been changed since version 1.0, and their short names are indicated in this manual by a suffix "2" – for example, PF2.

For all scales, the *RawScore*, *RS*, is the mean of the component items:
RawScore =*RS*= (*I1*+ *I2*+….+*In*) /*n*

Then for **Functional scales**:

$$Score = [1 - ((RS-1)/range)] * 100$$

and for **Symptom scales / items** and **Global health status / QoL:**
Score = {(*RS* -1) / *range*}*100

Examples:

Emotional functioning:

$$RawScore = (Q_{21} + Q_{22} + Q_{23} + Q_{24})/4$$

$$\text{EF } Score = \{1 - (RawScore - 1) / 3\} \times 100$$

Fatigue:

$$RawScore = (Q_{10} + Q_{12} + Q_{18})/3$$

$$\text{FA } Score = \{(RawScore - 1) /3\} \times 100$$

Missing items:

• Have at least half of the items from the scale been answered?

• If *Yes*, use all the items that were completed, and apply the standard equations given on the previous pages for calculating the scale scores; ignore any items with missing values when making the calculations.

• If *No*, set scale score to missing.

• For single-item measures, set score to missing.

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Global health status/QoL (revised)
- Physical functioning (revised)
- Role functioning (revised)
- Emotional functioning
- Cognitive functioning
- Social functioning
- Fatigue
- Nausea and vomiting
- Pain
- Dyspnoea
- Insomnia
- Appetite loss
- Constipation
- Diarrhoea
- Financial difficulties

7 DESCRIPTIVE ANALYSIS: VISIT 1: ABLATION PROCEDURE

The categorical variables to be described (n, %) are:

- Number of ablations procedures
- Number or targeted tumors per ablation procedure
- Number of re-treatments

7.1 Pain assessment

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- VAS score

7.2 Data related to the ablation procedure

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variable to be described (n, %) is:

- Has a test pulse energy been applied?

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Total duration of the procedure (hours, minutes)
- Total duration of the anaesthesia (hours, minutes)

The following analyses will be carried out separately for for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

The categorical variables to be described (n, %) are:

- Could the ablation be completed?
    - If not, reason will be described

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Total treatment duration (hours, minutes)
- Treatment mean amplitude

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Outer contour diameters:
    - X-axis greatest dimension
    - Y-axis greatest dimension
    - Z-axis greatest dimension
- Planned ablation volume
- Diameter D
- Tumor depth ((from skin surface)

The categorical variables to be described (n, %) are:

- Proximity to vital structures, if yes:
    - Near blood vessels
    - Near gallbladder
    - Near Bile ducts
    - Other
- Containing vital structures, if yes:
    - Containing blood vessels
    - Containing gallbladder
    - Other

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Total PAV in case multiple tumors

7.3 Vital signs

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

8 DESCRIPTIVE ANALYSIS: VISIT 2: 24 HOURS POST-ABLATION

The categorical variable to be described (n, %) is:

- Number of re-treatments

8.1 Anthropometric data

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Weight

8.2 Vital signs

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

8.3 Physical examination

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3) and:

The categorical variables to be described (n, %) are:

- Result
    - Clinically significant
    - Not clinically significant

8.4 Laboratory test

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Haemoglobin
- Erythrocytes
- Leucocytes
- Neutrophils
- Basophils
- Haematocrit
- Lymphocytes
- Monocytes
- Eosinophils
- Platelets
- AST
- ALT
- GGT
- Alkaline phosphatase
- Total bilirubin
- Albumin
- Prothrombine (PT)
- INR
- Partial thromboplastin time
- Glucose
- Calcium
- Sodium
- Potassium
- Bicarbonate
- Chloride
- BUN
- Creatinine
- CD3+
- CD4+
- CD8+
- CD45+
- CD16+
- CD56+
- CD19+
- C reactive protein

- Complement C3
- Complement C4
- Complement CH50
- IgG
- IgM
- IgA
- Interleukin-6
- CEA
- AFP
- Ca 15-3
- Ca 19-9

8.5 Child-Pugh score

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Child-Pugh score and Child-Pugh category will be calculated according to the following rules:

Child-Pugh Score and Interpretation			
Classification	**1**	**2**	**3**
Serum bilirubin (μmol/L)	<34	34-51	>51
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Presence of ascites	Absent	Controllable	Refractory
Encephalopathy	Absent	Minimal	Severe
INR	<1.7	1.7-2.3	>2.3
Interpretation			
Points	**Class**	**Life Expectancy**	**Perioperative Mortality**
5-6	A	15-50 yrs	10%
7-9	B	Candidate for transplant	30%
10-15	C	1-3 months	82%

The categorical variable to be described (n, %) is:

- Child-Pugh category

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Child-Pugh score

8.6 Post-procedure imaging

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Technique used for tumor imaging:
  - Magnetic resonance imaging
  - Computed tomography
  - Ultrasound with contrast
  - Ultrasound without contrast
- Damage to the integrity of vital anatomical structures, if yes
  - Mayor vessels, specify
  - Organs, specify

The following analyses will be carried out separately for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- X-axis greatest dimension
- Y-axis greatest dimension
- Z-axis greatest dimension
- Diameter D
- Ablation zone volume

The categorical variable to be described (n, %) is:

- Technical success

8.7 Pain assessment

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- VAS score

9 DESCRIPTIVE ANALYSIS: VISIT 3: ONE WEEK POST-ABLATION

The categorical variable to be described (n, %) is:

- Number of re-treatments

9.1 Anthropometric data

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Weight

9.2 Vital signs

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

9.3 Physical examination

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Result
    - Clinically significant
    - Not clinically significant

9.4 ECOG

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variable to be described (n, %) is:

- ECOG

9.5 Laboratory test

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Haemoglobin
- Erythrocytes
- Leucocytes
- Neutrophils
- Basophils
- Haematocrit
- Lymphocytes
- Monocytes
- Eosinophils
- Platelets
- AST
- ALT
- GGT
- Alkaline phosphatase
- Total bilirubin
- Albumin
- Prothrombine (PT)
- INR
- Partial thromboplastin time
- Glucose
- Calcium
- Sodium

- Potassium
- Bicarbonate
- Chloride
- BUN
- Creatinine
- CD3+
- CD4+
- CD8+
- CD45+
- CD16+
- CD56+
- CD19+
- C reactive protein
- Complement C3
- Complement C4
- Complement CH50
- IgG
- IgM
- IgA
- Interleukin-6
- CEA
- AFP
- Ca 15-3
- Ca 19-9

9.6 Child-Pugh score

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Child-Pugh score and Child-Pugh category will be calculated according to the following rules:

Child-Pugh Score and Interpretation			
Classification	**1**	**2**	**3**
Serum bilirubin (µmol/L)	<34	34-51	>51
Serum albumin (g/L)	>35	28-35	<28
Presence of ascites	Absent	Controllable	Refractory
Encephalopathy	Absent	Minimal	Severe
INR	<1.7	1.7-2.3	>2.3
Interpretation			
Points	**Class**	**Life Expectancy**	**Perioperative Mortality**
5-6	A	15-50 yrs	10%
7-9	B	Candidate for transplant	30%
10-15	C	1-3 months	82%

The categorical variable to be described (n, %) is:

- Child-Pugh category

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Child-Pugh score

9.7 Post-procedure imaging

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Technique used for tumor imaging:
    - Magnetic resonance imaging
    - Computed tomography
    - Ultrasound with contrast
    - Ultrasound without contrast
- Damage to the integrity of vital anatomical structures, if yes
    - Mayor vessels, specify

- Organs, specify

The following analyses will be carried out separately for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- X-axis greatest dimension
- Y-axis greatest dimension
- Z-axis greatest dimension
- Ablation zone volume

The categorical variable to be described (n, %) is:

- Local tumor progression by MRI

9.8 Pain assessment

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- VAS score

10 DESCRIPTIVE ANALYSIS: VISIT 4: 1 MONTH POST-ABLATION

The categorical variable to be described (n, %) is:

- Number of re-treatments

10.1 Anthropometric data

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Weight

10.2 Vital signs

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

10.3 Physical examination

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Result
    - Clinically significant
    - Not clinically significant

10.4 ECOG

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variable to be described (n, %) is:

- ECOG

10.5 Laboratory test

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Haemoglobin
- Erythrocytes
- Leucocytes
- Neutrophils
- Basophils
- Haematocrit
- Lymphocytes
- Monocyte
- Eosinophils
- Platelets
- AST
- ALT
- GGT
- Alkaline phosphatase
- Total bilirubin
- Albumin
- Prothrombine (PT)
- INR
- Partial thromboplastin time
- Glucose
- Calcium
- Sodium
- Potassium
- Bicarbonate
- Chloride
- BUN
- Creatinine
- CD3+
- CD4+
- CD8+

- CD45+
- CD16+
- CD56+
- CD19+
- C reactive protein
- Complement C3
- Complement C4
- Complement CH50
- IgG
- IgM
- IgA
- Interleukin-6
- CEA
- AFP
- Ca 15-3
- Ca 19-9

10.6 Child-Pugh score

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Child-Pugh score and Child-Pugh category will be calculated according to the following rules:

Child-Pugh Score and Interpretation			
Classification	**1**	**2**	**3**
Serum bilirubin (μmol/L)	<34	34-51	>51
Serum albumin (g/L)	>35	28-35	<28
Presence of ascites	Absent	Controllable	Refractory
Encephalopathy	Absent	Minimal	Severe
INR	<1.7	1.7-2.3	>2.3
Interpretation			
Points	**Class**	**Life Expectancy**	**Perioperative Mortality**
5-6	A	15-50 yrs	10%
7-9	B	Candidate for transplant	30%
10-15	C	1-3 months	82%

The categorical variable to be described (n, %) is:

- Child-Pugh category

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Child-Pugh score

10.7 Post-procedure imaging

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Technique used for tumor imaging:
    - Magnetic resonance imaging
    - Computed tomography
    - Ultrasound with contrast
    - Ultrasound without contrast
- Damage to the integrity of vital anatomical structures, if yes
    - Mayor vessels, specify
    - Organs, specify

The following analyses will be carried out separately for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- X-axis greatest dimension
- Y-axis greatest dimension
- Z-axis greatest dimension
- Ablation zone volume

The categorical variable to be described (n, %) is:

- Local tumor progression by MRI

10.8 Quality of life assessment

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The score of each dimension of this scale will be calculated according to the established scoring algorithms:

Scoring the EORTC QLQ-C30 version 3.0

	Scale	Number of items	Item range*	**Version 3.0** Item numbers	Function scales
Global health status / QoL					
Global health status/QoL (revised)†	QL2	2	6	29, 30	
Functional scales					
Physical functioning (revised)†	PF2	5	3	1 to 5	F
Role functioning (revised)†	RF2	2	3	6, 7	F
Emotional functioning	EF	4	3	21 to 24	F
Cognitive functioning	CF	2	3	20, 25	F
Social functioning	SF	2	3	26, 27	F
Symptom scales / items					
Fatigue	FA	3	3	10, 12, 18	
Nausea and vomiting	NV	2	3	14, 15	
Pain	PA	2	3	9, 19	
Dyspnoea	DY	1	3	8	
Insomnia	SL	1	3	11	
Appetite loss	AP	1	3	13	
Constipation	CO	1	3	16	
Diarrhoea	DI	1	3	17	
Financial difficulties	FI	1	3	28	

* *Item range* is the difference between the possible maximum and the minimum response to individual items; most items take values from 1 to 4, giving *range* = 3.
† (revised) scales are those that have been changed since version 1.0, and their short names are indicated in this manual by a suffix "2" – for example, PF2.

For all scales, the *RawScore*, *RS*, is the mean of the component items:
RawScore =*RS*= (*I1*+ *I2*+….+*In*) /*n*

Then for **Functional scales**:

$$Score = [1 - ((RS-1)/range)] * 100$$

and for **Symptom scales / items** and **Global health status / QoL:**
Score = {(*RS* -1) / *range*}*100

Examples:

Emotional functioning:

$$RawScore = (Q_{21} + Q_{22} + Q_{23} + Q_{24})/4$$

$$\text{EF } Score = \{1 - (RawScore - 1) / 3\} \times 100$$

Fatigue:

$$RawScore = (Q_{10} + Q_{12} + Q_{18})/3$$

$$\text{FA } Score = \{(RawScore - 1)/3\} \times 100$$

Missing items:

• Have at least half of the items from the scale been answered?

• If *Yes*, use all the items that were completed, and apply the standard equations given on the previous pages for calculating the scale scores; ignore any items with missing values when making the calculations.

• If *No*, set scale score to missing.

• For single-item measures, set score to missing.

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Global health status/QoL (revised)
- Physical functioning (revised)
- Role functioning (revised)
- Emotional functioning
- Cognitive functioning
- Social functioning
- Fatigue
- Nausea and vomiting
- Pain
- Dyspnoea
- Insomnia
- Appetite loss
- Constipation
- Diarrhoea
- Financial difficulties

11 DESCRIPTIVE ANALYSIS: VISIT 5: 2 MONTHS POST-ABLATION

The categorical variable to be described (n, %) is:

- Number of re-treatments

11.1 Anthropometric data

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Weight

11.2 Vital signs

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Temperature
- DBP/SBP
- Cardiac frequency
- Respiratory frequency

11.3 Physical examination

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Result
    - Clinically significant
    - Not clinically significant

11.4 ECOG

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variable to be described (n, %) is:

- ECOG

11.5 Laboratory test

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Haemoglobin
- Erythrocytes
- Leucocytes
- Neutrophils
- Basophils
- Haematocrit
- Lymphocytes
- Monocyte
- Eosinophils
- Platelets
- AST
- ALT
- GGT
- Alkaline phosphatase
- Total bilirubin
- Albumin
- Prothrombine (PT)
- INR
- Partial thromboplastin time
- Glucose
- Calcium
- Sodium

- Potassium
- Bicarbonate
- Chloride
- BUN
- Creatinine
- CD3+
- CD4+
- CD8+
- CD45+
- CD16+
- CD56+
- CD19+
- C reactive protein
- Complement C3
- Complement C4
- Complement CH50
- IgG
- IgM
- IgA
- Interleukin-6
- CEA
- AFP
- Ca 15-3
- Ca 19-9

11.6 Child-Pugh score

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

Child-Pugh score and Child-Pugh category will be calculated according to the following rules:

Child-Pugh Score and Interpretation			
Classification	**1**	**2**	**3**
Serum bilirubin (µmol/L)	<34	34-51	>51
Serum albumin (g/L)	>35	28-35	<28
Presence of ascites	Absent	Controllable	Refractory
Encephalopathy	Absent	Minimal	Severe
INR	<1.7	1.7-2.3	>2.3
Interpretation			
Points	**Class**	**Life Expectancy**	**Perioperative Mortality**
5-6	A	15-50 yrs	10%
7-9	B	Candidate for transplant	30%
10-15	C	1-3 months	82%

The categorical variable to be described (n, %) is:

- Child-Pugh category

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variable will be shown:

- Child-Pugh score

11.7 Post-procedure imaging

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The categorical variables to be described (n, %) are:

- Technique used for tumor imaging:
    - Magnetic resonance imaging
    - Computed tomography
    - Ultrasound with contrast
    - Ultrasound without contrast
- Damage to the integrity of vital anatomical structures, if yes
    - Mayor vessels, specify
    - Organs, specify

The following analyses will be carried out separately for total targeted tumors (tumor 1 + tumor 2 + tumor 3….):

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- X-axis greatest dimension
- Y-axis greatest dimension
- Z-axis greatest dimension
- Ablation zone volume

The categorical variable to be described (n, %) is:

- Local tumor progression by MRI

11.8 Quality of life assessment

The following analyses will be carried out separately for each ablation procedure (ablation 1, ablation 2 and ablation 3):

The score of each dimension of this scale will be calculated according to the established scoring algorithms:

Scoring the EORTC QLQ-C30 version 3.0

	Scale	Number of items	Item range*	**Version 3.0** Item numbers	Function scales
Global health status / QoL					
Global health status/QoL (revised)†	QL2	2	6	29, 30	
Functional scales					
Physical functioning (revised)†	PF2	5	3	1 to 5	F
Role functioning (revised)†	RF2	2	3	6, 7	F
Emotional functioning	EF	4	3	21 to 24	F
Cognitive functioning	CF	2	3	20, 25	F
Social functioning	SF	2	3	26, 27	F
Symptom scales / items					
Fatigue	FA	3	3	10, 12, 18	
Nausea and vomiting	NV	2	3	14, 15	
Pain	PA	2	3	9, 19	
Dyspnoea	DY	1	3	8	
Insomnia	SL	1	3	11	
Appetite loss	AP	1	3	13	
Constipation	CO	1	3	16	
Diarrhoea	DI	1	3	17	
Financial difficulties	FI	1	3	28	

* *Item range* is the difference between the possible maximum and the minimum response to individual items; most items take values from 1 to 4, giving *range* = 3.
† (revised) scales are those that have been changed since version 1.0, and their short names are indicated in this manual by a suffix "2" – for example, PF2.

For all scales, the *RawScore*, *RS*, is the mean of the component items:
RawScore =*RS*= (*I1*+ *I2*+….+*In*) /*n*

Then for **Functional scales**:

$$Score = [1 - ((RS-1)/range)] * 100$$

and for **Symptom scales / items** and **Global health status / QoL:**
Score = {(*RS* -1) / *range*}*100

Examples:

Emotional functioning:

$$RawScore = (Q_{21} + Q_{22} + Q_{23} + Q_{24}) / 4$$

$$\text{EF } Score = \{1 - (RawScore - 1) / 3\} \times 100$$

Fatigue:

$$RawScore = (Q_{10} + Q_{12} + Q_{18}) / 3$$

$$\text{FA } Score = \{(RawScore - 1) / 3\} \times 100$$

Missing items:

• **Have at least half of the items from the scale been answered?**
• If *Yes*, use all the items that were completed, and apply the standard equations given on the previous pages for calculating the scale scores; ignore any items with missing values when making the calculations.
• If *No*, set scale score to missing.
• For single-item measures, set score to missing.

Descriptive statistics (mean, SD, median, Q_1, Q_3, minimum, maximum) of the following continuous variables will be shown:

- Global health status/QoL (revised)
- Physical functioning (revised)
- Role functioning (revised)
- Emotional functioning
- Cognitive functioning

- Social functioning
- Fatigue
- Nausea and vomiting
- Pain
- Dyspnoea
- Insomnia
- Appetite loss
- Constipation
- Diarrhoea
- Financial difficulties

12 ADVERSE EVENTS

12.1 Adverse events

To obtain adverse events per patient, the maximum severity will be calculated for each of the adverse events recorded and the following analysis will be described:

- Number of patients with at least one adverse event.
- Number of patients with at least one adverse device effect.
- Number of patients with different adverse events reported according to severity (GI, GII, GIII, GIV and GV).

12.2 Related to investigational device

All adverse events related to investigational device will be considered, i.e., all events specified as relationship “yes” to investigational device and “possibly related”, “probably related” and “related”.

This analysis will be provided per patient. To obtain related events per patient, the maximum severity will be calculated for each of the events recorded and the following analysis will be described:

- Number of patients with at least one related adverse event.
- Number of patients with different related adverse events reported according to severity (GI, GII, GIII, GIV and GV).

12.3 Related to Ablation procedure

All adverse events related to ablation procedure will be considered, i.e., all events specified as relationship “yes” to ablation procedure and “possibly related”, “probably related” and “related”.

This analysis will be provided per patient. To obtain related events per patient, the maximum severity will be calculated for each of the events recorded and the following analysis will be described:

- Number of patients with at least one related adverse event.
- Number of patients with different related adverse events reported according to severity (GI, GII, GIII, GIV and GV).

12.4 Serious adverse events (SAE/SADE)

The following variables will be described:

- Number of patients with at least one serious adverse event (SAE/SADE).
- A list by patient of all serious adverse event recorded will be provided as well serious adverse events characteristics.

13 CONCOMITANT MEDICATION

The categorical variables to be described (n, %) are:

- Number of patients with at least one drug due to Post-procedure pain. A list with drugs recorded will be provided.
- Number of patients with at least one drug due to Metastatic disease. A list with drugs recorded will be provided.

14 END OF STUDY/PREMATURE WITHDRAWAL

The categorical variables to be described (n, %) are

- Has the patient completed the study according to protocol? if yes:
    - Reason for premature withdrawal
- If patient´s death, a list with reasons and if the adverse event has been related will be provided.

15 OBJECTIVES ANALYSES

15.1 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.

The following analyses will be carried out to respond to this objective:

The number of targeted tumors in whom technical success is achieved (Visit 2: 24 hours post-ablation; Technical success) 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 per patient.

15.2 Secondary objectives

1. **Assessment of the safety profile of the VORTX Rx®.**

This objective is given the answer with the analyses proposed in sections 6.7.2, 6.7.3, 6.7.4, 6.7.5, 6.8, 8.2, 8.3, 8.4, 9.2, 9.3, 9.4, 9.5, 10.2, 10.3, 10.4, 10.5, 11.2, 11.3, 11.4, 11.5 and 12 of this document.

2. **Assessment of local tumor progression by MRI at 1 week, 1 month and 2 months, post-procedure.**

The following analyses will be carried out to respond to this objective:

The number of patients who have indicated local tumor progression **in at least one visit** (1 week, 1 month, 2 months) for each tumor ablated in ablation 1, ablation 2, and ablation 3 will be provided.

3. **Assessment of the involution of the ablation zone 1 week, 1 month and 2 months post-procedure.**

The following analyses will be carried out separately for each tumor (tumor 1, tumor 2, tumor 3….) to respond to this objective:

- Ablation zone volume at 24 hours post-ablation, 1-week, 1 month and 2 months post-ablation will be compared among visit using a general linear model of repeated measures.
  In addition:
  - Ablation zone volume at 24 hours post ablation vs ablation zone volume at 1 week will be compared using Paired T-Test or Wilcoxon depending on variable distribution.
  - Ablation zone volume at 24 hours post ablation vs ablation zone volume at 1 month will be compared using Paired T-Test or Wilcoxon depending on variable distribution.
  - Ablation zone volume at 24 hours post ablation vs ablation zone volume at 2 months will be compared using Paired T-Test or Wilcoxon depending on variable distribution.

4. **Assessment of liver panel.**

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2, ablation 3) to respond to this objective:

- AST at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- ALT at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- GGT at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

- Alkaline phosphatase at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Total bilirubin at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Albumin at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Prothrombine (PT) at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- INR at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

5. **Immunologic assessment.**

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2, ablation 3) to respond to this objective:

- CD3+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CD4+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CD8+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CD45+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CD16+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

- CD56+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CD19+ at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- C reactive protein at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Complement C3 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Complement C4 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Complement CH50 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- IgG at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- IgM at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- IgA at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Interleudin-6 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CEA at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- AFP at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

- CA 15-3 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- CA 19-9 at Screening and at 24 hours post-procedure and the follow-up visits performed at 1 week, and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

6. **Evaluation of quality of life.**

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2, ablation 3) to respond to this objective:

- Global health status/QoL (revised) at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Physical functioning (revised) at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Role functioning (revised) at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Emotional functioning at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Cognitive functioning at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Social functioning at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Fatigue at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Nausea and vomiting at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Pain at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Dyspnoea at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Insomnia at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Appetite loss at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

- Constipation at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Diarrhoea at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.
- Financial difficulties at Screening and 1 and 2 months post-procedure will be compared among visit using a general linear model of repeated measures.

7. **Assessment of pain and analgesic requirements after the ablation procedure.**

The following analyses will be carried out separately for each ablation (ablation 1, ablation 2, ablation 3) to respond to this objective:

- VAS score at screening, at 24 hours post-procedure and the follow-up visits performed at 1 week, will be compared among visit using a general linear model of repeated measures.

In addition, the following categorical variable will be described (n, %) (this analysis will be carried out for the total patients):

- Number of patients with at least one drug due to Post-procedure pain (concomitant medication form).