CLINICAL INVESTIGATION PLAN

Radioactive holmium microspheres for the treatment of patients with unresectable liver metastases; a single center, interventional, non-randomized, phase II (HEPAR II) trial

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

^{166}Ho-PLLA-MS	Holmium-166 loaded poly(L-lactic acid) microspheres
^{166}Ho-RE	Holmium-166 radioembolization
5-FU/LV	5-fluorouracil in combination with leucovorin
^{90}Y-MS	Yttrium-90 microspheres
^{90}Y-RE	Yttrium-90 radioembolization
µg	Microgram
µmol	Micromole
AE	Adverse Event
ADE	Adverse Device Effect
ALT	Alanine aminotransferase
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
CA	Competent Authority
CBO	Quality institute for Healthcare
CRCLM	Colorectal cancer liver metastases
CRP	C-reactive protein
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DD	Device Deficiency
e.g.	Exempli gratia
ECG	Electrocardiogram
E-CRF	Electronic Case Report Form
EORTC	European Organization for Research and Treatment of Cancer
EU	European Union
FAS	Full Analysis Set
FDG	Fludeoxyglucose
GBq	Giga Becquerel
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
Gy	Gray
h	Hour(s)
Hb	Hemoglobin
HCT	Hematocrite
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IGZ	Dutch Health Care Inspectorate
IMDD	Investigational Medical Device Dossier
IU	International Units
i.v.	Intravenous
J	Joule
keV	kilo electron volt
kg	Kilogram
km	Kilometer
L	Liter
LDH	Lactate dehydrogenase
LPFS	Liver specific progression free survival
MBq	Megabecquerel
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration

MCV	Mean corpuscular volume
METC	Medical research ethics committee (MREC) (in Dutch: Medisch Ethische ToetsingsCommissie (METC))
MeV	Mega electron volt
mg	Milligram
ml	Millilitre
MRI	Magnetic Resonance Imaging
MTRD	Maximum Tolerated Radiation Dose
NCA	National Competent Authority
NCI	National Cancer Institute
NLPFS	Non-liver specific progression free survival
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NYHA	New York Heart Association
PACS	Picture Archiving and Communication System
PERCIST	PET Response Criteria in Solid Tumours
PES	Post-embolization syndrome
PET	Positron Emission Tomography
PPS	Per Protocol Set
PI	Principal Investigator
PT	prothrombin time
PTC	Protocol
PTT	partial thromboplastin time
QOL	Quality of Life
RDLT	Radiation Dose Limiting Toxicity
RECIST	Response Evaluation Criteria in Solid Tumours
RID	Reactor Institute Delft
RILD	Radiation induced liver disease
SAE	Serious Adverse Event
SADE	Serious Adverse Device Effect
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
SI	Système international d'unités (International System of Units)
SPECT	Single Photon Emission Computed Tomography
SVC	Superior vena cava
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
t	Time
Tc-MAA	Technetrium macroaggregates
TLS	Tumour Lysis Syndrome
TT	Thrombin time
ULN	Upper Limit of Normal
UMC	University Medical Center (in Dutch: Universitair Medisch Centrum)
USADE	Unanticipated Serious Adverse Device Effect
VEGF	Vascular Endothelial Growth Factor
VOD	Veno-occlusive disease
Vx2	virus induced papilloma in rabbits liver
WHO	World Health Organization
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met mensen

SUMMARY

Acronym	**H**olmium **E**mbolization **P**articles for **A**rterial **R**adiotherapy II – HEPAR II trial
Rationale	A significant need for new treatment options for dominant liver metastases is recognized, because survival of patients with unresectable liver disease is poor. Although ^{90}Y-MS therapy is evermore used and considered a safe and effective treatment option for patients with liver dominant disease, these microspheres have a drawback: following administration the actual biodistribution cannot be accurately visualized and the maximum absorbed radiation dose is relatively low. The preclinical phase and the phase I clinical trial of ^{166}Ho-radioembolization (^{166}Ho-RE) have been successfully completed. In the HEPAR I trial, ^{166}Ho-RE was proven to be a safe treatment. The absorbed radiation dose for ^{166}Ho-MS is 1.5 – 2 times higher than for ^{90}Y-MS. Consequently, a phase II study for evaluation of tumour response is warranted.
Objective	*Primary objective*: • To determine target lesions tumour response
	Secondary objectives: • To determine overall tumour response • To determine the liver specific progression-free survival • To determine non-liver specific progression-free survival • To assess overall survival • To evaluate toxicity • To evaluate quality of life • To assess performance status • To evaluate ^{99m}Tc-MAA and ^{166}Ho-PLLA-MS scout and therapy dose on SPECT/CT • To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on MRI • To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on PET/CT
Study design	Interventional, treatment, one group, phase II study with a medical device. The study has a group sequential design, with a first decision point when 30 subjects have completed follow-up for three months and further decision points after each 6 patients with 3 months follow-up, up to a maximum of 48 subjects.
Study population	Patients with liver metastases of miscellaneous origin will be included in this study (n = 30 - 48). These male and female patients must be aged ≥18 years and have dominant liver metastases. All histologies are acceptable, provided no standard therapeutic options are available, such as chemotherapy and surgery.
Intervention	^{166}Ho-PLLA-MS will be administered via a catheter during angiography.

Study endpoints	*Primary endpoint*: • Target lesions tumour response
	Secondary endpoints: • Overall tumour response • Liver specific progression-free survival post ^{166}Ho-RE • Non-liver specific progression-free survival • Overall survival • Toxicity • Quality of life • Performance status • ^{99m}Tc-MAA and ^{166}Ho-PLLA-MS scout and therapy dose SPECT/CTs comparison • ^{166}Ho-PLLA-MS scout dose and total dose MRI comparison • ^{166}Ho-PLLA-MS scout dose and total dose PET/CT comparison
Duration of treatment	The study consists of a screening phase of approximately 2 weeks followed by a treatment phase of approximately 2-3 weeks. Patients will be followed until liver specific tumour progression or death has occurred, to a maximum of 12 months.
Methodology	A first cohort of 30 patients will be treated with ^{166}Ho-RE. After the first cohort, up to 3 additional cohorts of 6 patients will be treated with ^{166}Ho-RE. The total number of patients treated in the HEPAR II trial will therefore be at least 30 and at most 48 patients, depending on the observed number of responses. Early termination at a response interim analysis (after 30, 36 or 42 patients) is determined by pre-defined boundaries on the number of observed responses. The boundary in favour of treatment effect may be crossed before 30 patients are reached, but then the study will continue to at least 30 patients to allow estimation of the key secondary endpoints.
Number of study centers	Single center (UMC Utrecht).
Adverse events	All adverse events will be recorded throughout the study.
Inclusion period	Mar 2012 – Aug 2015
Analysis	Full analysis will be performed after the last patient's last visit. The primary analysis will be to estimate the target lesions tumour response rate (with 95% confidence interval, adjusted for early stopping if indicated). Secondary analysis will be similar estimations of response rates or estimated survival curves (cumulative incidences). Primary and secondary analyses will be based on the Full Analysis Set; analyses on the Per Protocol Set will also be performed and reported. The study will be monitored with stopping boundaries on the number of observed responses. Early termination may occur as a consequence after 30, 36

	or 42 subjects completed 3 months follow-up. Safety interim analyses will be performed every 3 months from the moment the first patient has received ^{166}Ho-RE. All available follow-up data will be included in the safety evaluation.
Manufacturers of the medical device	^{165}Ho-PLLA-MS are manufactured by the radionuclide pharmacy of the UMC Utrecht, The Netherlands. ^{165}Ho-PLLA-MS are neutron activated by the Reactor Institute Delft (Delft, the Netherlands). ^{166}Ho-PLLA-MS doses are prepared by the radionuclide pharmacy of the UMC Utrecht, The Netherlands.

1. INTRODUCTION AND RATIONALE

The liver is the most common site of metastatic spread. As many as 50% of all patients with a primary malignancy will in due course develop hepatic metastases.[1] Metastases confined to the liver most commonly, but not exclusively, occurs from colorectal carcinoma, of which the incidence is very high as well. Each year worldwide approximately one million people develop cancer of the large bowel (colorectal carcinoma).[2] The primary tumour is in general resectable, but unfortunately, in 25% of cases the cancer will have spread to the liver at the time of diagnosis whereas in due time more than 50% of patients will develop hepatic metastases.[3, 4] Subtotal hepatic resection is the treatment of choice, yet only 20-30% of patients are eligible for surgical resection of the liver metastases.[5] If resection is performed with curative intent a 33% 5-year survival is reported.[6] In addition to colorectal cancer, metastasis of other types of malignancy can also be confined to the liver. This comprises breast cancer, neuroendocrine cancers, uveal melanoma, etc. Overall, approximately 70% of all patients with uncontrollable solid malignancy will develop liver metastases.

For several decades, standard first-line chemotherapy for colorectal cancer has consisted of 5-fluorouracil in combination with leucovorin (5-FU/LV). Nowadays, oxaliplatin or irinotecan and the Vascular Endothelial Growth Factor (VEGF) antibody bevacizumab is added to 5-FU/LV which has improved median survival from 12 to about 20 months.[7] Typically, long-term survival for patients with unresectable metastatic disease remains less than 5%. For some other types of cancer, no effective chemotherapy protocol is available at all, e.g. uveal melanoma metastases.[8] A summary of standard treatment options for patients with liver metastases is provided in Appendix XIII.

There is a significant need for additional treatment options for patients with liver metastases who do not exhibit response (anymore) to chemotherapy or who refuse (further) chemotherapy because of severe side effects. An increasingly applied treatment for this category of patients is yttrium-90 radioembolization (^{90}Y-RE). ^{90}Y-RE consists of injecting radioactive yttrium-90 loaded (glass or resin) microspheres into the hepatic artery through a catheter. A recently performed randomized controlled trial has shown statistically significant improvement in progression-free survival in colorectal cancer liver metastases (CRCLM) patients.[9] ^{90}Y-RE is performed in patients with other types of liver metastases as well.[10, 11]

Although yttrium-90 microspheres (^{90}Y-MS) therapy is evermore used and considered a safe and effective treatment option for patients with liver dominant disease, these microspheres have a drawback: following administration the actual biodistribution cannot be accurately visualized and the maximum absorbed radiation dose is relatively low. For this reason, holmium-166 loaded poly(L-lactic acid) microspheres (^{166}Ho-PLLA-MS) have been developed [12, 13] at the Department of Radiology and Nuclear Medicine of the University Medical Center (UMC) Utrecht. Like yttrium-90, holmium-166 emits high-energy beta particles that can eradicate tumours but this isotope also emits gamma radiation which allows for imaging through single photon emission computed tomography (SPECT). In addition, since holmium is highly paramagnetic it can also be visualized *in vivo* using magnetic resonance imaging (MRI). Assessment of the biodistribution of these microspheres is therefore possible. This is very useful for several reasons. Prior to administration of the therapeutic dose a small scout dose of ^{166}Ho-PLLA-MS can be instilled to predict the distribution of the therapeutic dose. Also, quantitative analysis of the SPECT images allows assessment of the radiation dose delivered on both the tumour(s) and the normal liver (i.e. dosimetry) [14]. Quantitative analysis of the MRI scans is possible as well [15, 16].
In a recent, yet unpublished phantom study by our research group, we found that (a limited number of) 511 keV annihilation photons are emitted during the process of ^{166}Ho decay. This finding implicates that ^{166}Ho-MS can be detected with the use of positron emission tomography (PET). The higher resolution of ^{166}Ho-MS PET compared with ^{166}Ho-MS SPECT,

might lead to a more accurate biodistribution assessment of ^{166}Ho-MS. This may improve the overall predictability and safety of radioembolization. However, the detection qualities of ^{166}Ho-MS PET are still to be investigated in patients. For this reason, we implemented ^{166}Ho-PET/CT imaging in the first 6 patients of the HEPAR II trial.

The pharmaceutical quality of the ^{166}Ho-PLLA-MS has been thoroughly investigated and proven to be satisfactory [17-19]. Several animal studies have been conducted to investigate the intrahepatic distribution (tumour to non-tumour ratio), the toxicity profile/biocompatibility of the ^{166}Ho-PLLA-MS, safety of the administration procedure, and efficacy of these particles. A non-survival biodistribution study in rats was performed in which it was demonstrated that the ^{166}Ho-PLLA-MS deposition was restricted to the liver and that in the tumourous tissue the radioactivity concentration was six times higher than in the non-target tissue [20]. To demonstrate that ^{166}Ho-PLLA-MS injected into the hepatic artery have a tumouricidal effect, an efficacy study in virus induced papilloma in rabbits livers (Vx2) was performed. In all animals that were treated with ^{166}Ho-PLLA-MS tumour growth was arrested and necrosis set in [21]. In a rat study, in order to show that ^{166}Ho-PLLA-MS are biocompatible, rods composed of (decayed) ^{166}Ho-PLLA-MS were implanted into the liver and the animals were terminated between 3 days and 18 months post implantation during which time no biochemical or clinical side effects were observed [22]. Finally, an extensive toxicity study in healthy pigs was conducted [23]. Five animals were administered (non-radioactive) ^{165}Ho-PLLA-MS and in 13 animals (radioactive) ^{166}Ho-PLLA-MS were instilled into the hepatic artery. The animals were injected with ^{166}Ho-PLLA-MS in amounts of radioactivity corresponding with very high absorbed liver doses. Just very mild side effects were seen: slight and transitory loss of appetite and somnolence, which may well have been associated with the anesthetic and analgesic agents that had been given and not necessarily with the microsphere ‘treatment’. A very important adverse event which had occurred in two animals (^{166}Ho-PLLA-MS) was inadvertent deposition of ^{166}Ho-PLLA-MS into the gastroduodenal artery with consequent radioembolization of the gastric wall. To avoid this type of complication, in analogy to what is already customary in yttrium-90 therapy, selected vessels in patients will be occluded by coiling prior to administration of ^{166}Ho-PLLA-MS [24, 25].

^{166}Ho-RE has been investigated clinically as well in the HEPAR I trial (Clinicaltrials.gov identifier NCT01031784). In this phase I trial, 15 patients with liver metastases of miscellaneous origin were included, of which 7 with metastases of ocular melanoma, 5 with metastases of colorectal carcinoma, 2 with metastases of cholangiocarcinoma and 1 with metastases of breast cancer.
The study consisted of four radiation dose cohorts (20 Gray (Gy), 40 Gy, 60 Gy and 80 Gy liver absorbed dose). Gy is the ‘International System of Units’-unit (SI) of the energy absorbed from ionizing radiation, and equals 1 Joule (J) per kilogram tissue. Cohorts consisted of either three or – in case of serious adverse events – six patients [26]. The 20-Gy cohort was extended to 6 patients because one of the patients developed a pulmonary embolism two weeks after the radioembolization procedure. This complication was attributed to the extended duration of the procedure in this particular patient without any post-procedural prophylactic antithrombotic medication. All following cohorts consisted of 3 patients. Clinical toxicity in the first 3 cohorts comprised mainly symptoms belonging to the post embolization syndrome (PES). PES comprises fatigue, nausea, abdominal pain, fever, vomiting and/or anorexia, and is a known phenomenon in radioembolization treatment. These symptoms were transient in all cases and well-controlled by outpatient medication. Laboratory toxicity was mild except for expected grade 3 liver enzymes toxicity and grade 3 haematological toxicity. Overall, treatment was well tolerated by the patients in the first 3 cohorts (20 Gy – 60 Gy) and the toxicity profile was very similar to the toxicity which is encountered in patients treated with ^{90}Y-RE. In the 80-Gy cohort, however, treatment was considerably less well tolerated. Patients had more complaints of abdominal pain and nausea. These symptoms were more severe and lasting longer than the clinical toxicities encountered in the previous cohorts. Furthermore, laboratory toxicity was more severe and in

certain cases progressive with a suspected radiation induced liver disease (RILD) in one patient. Liver toxicity is to some extend intended in radioembolization given the 5-mm reach of the beta emitting holmium. Furthermore, lymphocyte- (up to grade 3), leukocyte- (up to grade 3) and platelet counts (up to grade 4) transiently decreased. These adverse events are most probably caused by activation of hepatic stem cells in the bone marrow due to liver damage [27].These symptoms are suspected to be related to the higher radiation dose instead of an embolic effect of treatment because the amount of microspheres was equal in all cohorts. This study was not designed to demonstrate efficacy of ^{166}Ho-RE, but a number of partial remissions and stable disease were observed. A listing of all adverse events encountered in the HEPAR I trial is given in Appendix XIII

The rationale of the study described and proposed in this protocol, the HEPAR II trial, is to assess treatment response in patients with liver dominant disease.

2. OBJECTIVES

In this single center study, the efficacy of radioactive ^{166}Ho-PLLA-MS, a radioembolization device, is tested in patients with liver metastases. A group of at least 30 and at the most 48 patients will undergo radioembolization with ^{166}Ho-PLLA-MS.

Primary objective:

- To determine target lesions tumour response

Secondary objectives:

- To determine overall tumour response
- To determine the liver specific progression-free survival
- To determine non-liver specific progression-free survival
- To assess overall survival
- To evaluate toxicity
- To evaluate quality of life
- To assess performance status
- To evaluate technetium-99m labeled macroaggregated albumin (^{99m}Tc-MAA) and ^{166}Ho-PLLA-MS scout and therapy dose on SPECT/CT
- To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on MRI
- To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on PET/CT

3. STUDY DESIGN

Study type	Interventional
Study design	Interventional, treatment, one group, phase II study with a medical device.
Study start date	Mar 2012
Estimated study completion date	Aug 2015

This phase II study comprises a group of at least 30 and at the most 48 patients suffering from liver metastases of primary cancers of miscellaneous origin. Patients who will be included have hepatic metastases not amenable for surgical resection or further systemic treatment. A liver absorbed dose of 60 Gy will be used in each patient in this study. A radiation dose of 60 Gy equates to 3.8 GBq ^{166}Ho/kg liver tissue.

The primary outcome is target lesions tumour response at three months follow-up. For this study, this is defined as: 1) complete response on CT at 3 months, or 2) partial response on CT at 3 months or 3) stable disease on Computed Tomography (CT) at 3 months (see chapter 8.3.1 Definition of treatment success).

This single arm open label study will have a sequential design as follows. Patients will be followed for target lesions tumour response up to 12 months or less if liver specific tumour progression or death occurs. Interim analyses for tumour response achieved are based on sequential decision boundaries. Stopping boundaries are determined such that an overall one-side alpha of at the most 0.05 is maintained in case the true tumour response is 20% ($p_0 = 20\%$). Early termination at a response interim analysis (after 30, 36 or 42 patients) is determined by pre-defined boundaries on the number of observed responses. The boundary in favour of treatment effect may be reached or crossed before 30 patients are reached, but then the study will continue to at least 30 patients to allow estimation of the key secondary efficacy endpoints. Early stopping for proven efficacy can only occur after the first 30 patients are evaluated. Boundaries for early stopping due to futility, i.e. absence of a relevant effect on tumour response, are also included, and are in effect even before the number of 30 patients is reached. The sequential design with boundaries as given in Table 1 will have a power of 90% to reach a positive tumour response decision in case the true target lesions tumour response is 40% ($p_1 = 40\%$). The exact overall one sided type I error is 4.5% [28].

Table 1 Stopping boundaries for early termination at interim analysis.[28] Stopping is indicated if the observed number of responses is at the boundary or beyond.

Analysis	***Sample Size***	***Lower Boundary***	***Upper Boundary***
1	30	5	11
2	36	6	13
3	42	7	14
4	48	15	16

4. STUDY POPULATION

4.1 Inclusion criteria

Patients meeting the following criteria may enter the study:

1. Patients must have given written informed consent.
2. Female or male aged 18 years and over.
3. Diagnosis of metastatic malignancy to the liver and no detectable malignant disease outside the liver or diagnosis of metastatic malignancy to the liver with limited disease outside the liver (i.e. liver-dominant disease) defined as the sum of the diameters of all metastases in the liver to be more than 200% of the sum of the diameters of all soft tissue lesions outside the liver.
4. Patient is not amenable for standard therapies (other than radioembolization) or patient refuses standard therapies for reasons of toxicity
5. Life expectancy of 12 weeks or longer.
6. World Health Organisation (WHO) Performance status 0-2 (see Appendix III).
7. One or more measurable lesions at least 10 mm in the longest diameter by spiral CT according to the Response Evaluation Criteria in Solid Tumours (RECIST) 1.1 criteria.
8. Negative pregnancy test for women of childbearing potential.

4.2 Exclusion criteria

Patients meeting any of the following criteria cannot enter the study:

1. Brain metastases or spinal cord compression, unless irradiated at least 4 weeks prior to the date of the experimental treatment and stable without steroid treatment for at least 1 week.
2. Radiation therapy within the last 4 weeks before the start of study therapy.
3. The last dose of prior chemotherapy has been received less than 4 weeks prior the start of study therapy.
4. Major surgery within 4 weeks, or incompletely healed surgical incision before starting study therapy.
5. Any unresolved toxicity greater than National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE version 4.0, see Appendix II) grade 2 from previous anti-cancer therapy.
6. Serum bilirubin > 1.5 x Upper Limit of Normal (ULN).
7. Glomerular filtration rate <35 ml/min, determined according to the Modification of Diet in Renal Disease formula.
8. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) > 5 x ULN.
9. Leukocytes < 4.0 10^9/l and/or platelet count < 150 10^9/l.
10. Significant cardiac event (e.g. myocardial infarction, superior vena cava (SVC) syndrome, New York Heart Association (NYHA) classification of heart disease ≥2 within 3 months before entry, or presence of cardiac disease that in the opinion of the Investigator increases the risk of ventricular arrhythmia.
11. Pregnancy or breast feeding (women of child-bearing potential).
12. Patients suffering from diseases with a increased chance of liver toxicity, such as primary biliairy cirrhosis or xeroderma pigmentosum.
13. Patients suffering from psychic disorders that make a comprehensive judgement impossible, such as psychosis, hallucinations and/or depression.
14. Patients who are declared incompetent.
15. Previous enrolment in the present study or previous treatment with radioembolization.

16. Treated with an investigational agent within 42 days prior to starting study treatment.
17. Female patients who are not using an acceptable method of contraception (oral contraceptives, barrier methods, approved contraceptive implant, long-term injectable contraception, intrauterine device or tubal ligation) OR are less than 1 year postmenopausal or surgically sterile during their participation in this study (from the time they sign the consent form) to prevent pregnancy.
18. Male patients who are not surgically sterile or do not use an acceptable method of contraception during their participation in this study (from the time they sign the consent form) to prevent pregnancy in a partner.
19. Patients with abnormalities of the bile ducts (such as stents) with an increased chance of infections of the bile ducts. Or evidence of extensive portal hypertension, splenomegaly, ascites or active hepatitis (B and/or C).
20. Body weight over 150 kg.
21. Moderate or severe adverse reaction to i.v. contrast (Visipaque®), as defined by the ACR Manual on Contrast Media - Version 9 (2013) (see Appendix XIV).
22. MRI contra-indications: severe claustrophobia, metal shrapnel, implanted pacemaker and/or neurostimulators.
23. Liver tumour involvement ≥70% as quantified on CT

5. TREATMENT OF PATIENTS

5.1 Schedule of procedures

Table 2: Schedule of study procedures

Procedures	Screening	pre-treat-ment	treat-ment	1w	3w	6w	9w	3m	6m	9m	12m
Informed consent	X										
In-/exclusion	X	X	X								
Demographic data	X										
Medical history	X										
Physical exam and WHO performance status	X	X	X		X	X	X	X	X	X	X
Pregnancy test (urine)		X									
EORTC Questionnaire	X			X		X		X	X	X	X
^{18F}FDG-PET/CT[1]	X							X	X	X	X
^{166}Ho-PET/CT[2]			XX								
MRI (diagnosis, response and holmium quantification)[3]			XXX					X	X	X	X
Angiography		X	X								
Tc-99m-MAA administration		X									
Scout dose of ^{166}Ho-PLLA-MS			X								
Therapy dose of ^{166}Ho-PLLA-MS			X								
Scintigraphy (planar and SPECT/CT)		X[4]	X[5]	X[6]							
Radiation exposure rate			X								
ECG	X										
Laboratory examination type A[7]	X	X	X					X	X	X	X
Laboratory examination type B[7]					X	X	X				
Laboratory examination type C[7]			X								
Monitoring of (S)AE's + concomitant med.		X	X		X	X	X	X	X	X	X

1. Positron Emission Tomography (PET) will only be performed in FDG-avid tumors.
2. ^{166}Ho-PET/CT for biodistribution assessment will be performed one hour post scout dose and one hour post treatment dose. These PET-scans will only be performed in the 1st - 6th patient that receives treatment. After the 12th patient, patients will either receive ^{166}Ho-PET or ^{166}Ho-MR imaging
3. MRI for tumor response assessment and quantification one day prior to scoutdose. After scout dose, a non-contrast enhanced MRI of a limited duration (<15min) for quantification. After therapy all MRI's will be for tumor response assessment and quantification. The scout dose MRI will only be performed in the 7th - 12th patient that receives treatment. After the 12th patient, patients will either receive 166Ho-PET or 166Ho-MR imaging
4. Tc-99m-MAA scintigraphy
5. Scout dose scintigraphy
6. Post treatment scintigraphy
7. Laboratory examination type A (blood), B (blood) and C (blood and urine) see Table 3: Laboratory examinations

Table 3: Laboratory examinations

Laboratory examination	Parameters	
Type A (blood)	Hematology	Leukocytes, erythrocytes, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils) and platelet count.
	Coagulation profile	Reagent-independent prothrombin ratio prothrombin time/ partial thromboplastin time (PT/PTT). If PT and/or PTT are out of range, Thrombin time (TT) will be automatically measured. Activated partial thromboplastin time (APTT).
	Serum chemistry	Creatinine, total bilirubin, alkaline phosphatase, SGPT/ALT, SGOT/AST, γGT, glucose, chloride, calcium, potassium, sodium, total protein, albumin, bicarbonate, urea, magnesium, phosphorus, ammonia, LDH, CRP and relevant tumour markers.
		Total: 10 ml
Type B (blood)	Hematology	Leukocytes, erythrocytes, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), differential leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, basophils) and platelet count.
	Serum chemistry	Creatinine, total bilirubin, alkaline phosphatase, SGPT/ALT, SGOT/AST, γGT, glucose, albumin, ammonia, lactate dehydrogenase (LDH) and C-reactive protein (CRP).
		Total: 4 ml
Type C (blood and urine)	Holmium content	Holmium content.
		Total blood: 7 ml

5.2 Study procedures

5.2.1 Screening

The screening visit will take place within 14 days prior to the fist angiography. During this visit the following procedures must be documented and reviewed as a part of the screening process:

- Informed consent
- Inclusion and exclusion criteria
- Demographic data
- Medical and surgical history
- Previous and ongoing medications (within the last 3 months)
- Physical examination including height and weight
- Vital signs, including blood pressure (after at least 3 minutes sitting), pulse and temperature
- WHO performance assessment
- PET/CT (should be performed within 2 weeks prior to the first angiography). (PET/)CT is part of the routine work-up for radioembolization. The PET part of the PET/CT examination will only be performed in FDG-avid tumours. The CT part of the PET/CT examination consists of a low dose CT and a diagnostic 3-phase CT of the liver. In non FDG-avid tumours, only a diagnostic 3-phase CT of the liver will be performed.
- Electrocardiogram (ECG)
- Laboratory examination type A (blood)
- European Organization for Research and Treatment of Cancer (EORTC) Quality of Life (QoL) questionnaire

5.2.2 Pretreatment visit

Patients will be hospitalized on the evening before the day of the pretreatment angiography. They will be discharged approximately 24 hours after the intervention unless complications occur. The screening visit can coincide with the pretreatment and treatment visit, if so, duplicate procedures need not be performed. If the treatment is shortly after the pretreatment, the hospital stay can be extended for the duration of that visit. During this visit, the following must be documented and reviewed as a part of the study:

- Inclusion and exclusion criteria
- Physical examination (incl. vital signs)
- Pregnancy test for women of childbearing age
- WHO performance assessment
- Concomitant medication
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type A (blood)
- Pretreatment angiography, administration of ^{99m}Tc-MAA
- ^{99m}Tc-MAA SPECT/CT

On day 0, the patient is subjected to an angiography of the upper abdominal vessels. At least the celiac axis and superior mesenteric artery are visualised, followed by coiling of relevant vessels, especially branches of the celiac axis supplying non-target organs. This procedure will be performed by a skilled and trained interventional radiologist. The catheter is introduced using the Seldinger technique. Prior to the procedure the patient is offered a tranquilizer. Premedication consists of proton pump inhibitors (pantoprazol 1 dd 40 mg), starting at the day of the intervention. Proton pump inhibitors are prescribed to the patients to be used until 3 months post ^{166}Ho-RE.

After successful angiography and coiling of relevant vasculature, a dose of ^{99m}Tc-MAA will be administered. The patient is subjected to scintigraphy to determine the distribution. Both planar imaging of the thorax and abdomen will be performed, as well as SPECT/CT of the abdomen. The thorax and abdomen images will be evaluated qualitatively and the images of the thorax will be evaluated quantitatively (region of interest analysis for lung shunting) as well. Extrahepatic deposition of activity is a contra-indication for administration of ^{166}Ho-PLLA-MS. If the extrahepatic deposition of ^{99m}Tc-MAA cannot be corrected by means of available radiological interventional techniques, the patient will not be eligible to receive a scout or therapy dose of ^{166}Ho-PLLA-MS. If the lung shunt fraction exceeds 20% of the dose of ^{99m}Tc-MAA, the patient will not be eligible to receive a scout or therapy dose of ^{166}Ho-PLLA-MS.

5.2.3 Treatment visit

The second intervention takes place around 1 week after the first intervention but not later than 3 weeks after. Patients will be hospitalized on the evening before the day of treatment. They will be discharged approximately 24 hours after the intervention unless complications occur. During this visit, the following must be documented and reviewed as a part of the study:

- Inclusion and exclusion criteria
- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- Pretreatment MRI
- Treatment angiography, administration of scout dose of ^{166}Ho-PLLA-MS
- Post scout dose SPECT/CT

- Post scout dose MRI or post scout- and post therapy dose ^{166}Ho-PET/CT
- Treatment angiography, administration of therapy dose of ^{166}Ho-PLLA-MS
- Post treatment MRI
- Monitoring of occurrence of (serious) adverse events
- Collecting of 24-hours urine for holmium content (using either a condom-catheter or an Foley catheter)
- Laboratory examination type A and C (blood and urine)
- Radiation exposure rate

Prior to the procedure, the patient is offered a tranquilizer and a Foley catheter is inserted or a condom-catheter is applied for 24-hours urine collection. If a Foley catheter is used, the patient will receive a single dose of antibiotics 3 hours post ^{166}Ho-RE (therapy dose). An experienced interventional radiologist will administer the scout dose of ^{166}Ho-PLLA-MS through a catheter inside the hepatic artery at the position as planned during the first intervention. At the end of this angiography, the vascular sheath will remain in situ (femoral artery). This sheath will be used for the treatment angiography later that day. The vascular access site will be covered with a sterile patch. In addition, NaCl 0.9% under pressure will be connected to the sheath to prevent from clot formation. The patient will remain in supine position in his/her bed and will be transferred to the MRI / PET and SPECT scanners. In order to detect inadvertent administration to the lungs or other non-target organs (e.g. stomach, duodenum, pancreas) scintigraphy will be performed after administration of the scout dose of ^{166}Ho-PLLA-MS to assess its distribution. Both planar imaging of the thorax and abdomen will be performed, as well as SPECT of the abdomen. If the ^{166}Ho-PLLA-MS scout dose SPECT is suspect for extrahepatic deposition, after a week a second ^{99m}Tc-MAA procedure will be started. In this procedure the blood vessels that probably cause the extrahepatic deposition will be identified if possible. Based on the findings, the interventional-radiologist might adapt the catheter position and/or coil the culprit extrahepatic vessels. In case the second ^{99m}Tc-MAA SPECT does not show extrahepatic deposition, a new ^{166}Ho-PLLA-MS scout dose will be administered. In case this second ^{166}Ho-PLLA-MS scout dose SPECT does not show extrahepatic deposition the ^{166}Ho-PLLA-MS therapy dose will be given. If again extrahepatic ^{166}Ho-PLLA-MS scout dose deposition occurs, the treatment will be terminated.
In order to obtain detailed information on the distribution of the ^{166}Ho-PLLA-MS, **either** MRI will be performed after the scout dose **or** ^{166}Ho-PET/CT will be performed both after the scout dose and the therapy dose. ^{166}Ho-PET/CT will be performed in the 1st – 6th patient that receive ^{166}Ho-RE. In the 7th – 12th patient, ^{166}Ho-PET/CT will not be performed. Instead, these patients will receive an MRI of the ^{166}Ho-MS scout dose. After the first 12 patients, patients will either receive an MRI of the ^{166}Ho-MS scout dose or a ^{166}Ho-PET/CT scan of the scout dose and a ^{166}Ho-PET/CT of the therapy dose. MRI will be performed after the therapy dose in all patients of the HEPAR II trial.
The MRI after the scout dose will be a non-contrast enhanced MRI of limited duration (less than 15 minutes acquisition time). The ^{166}Ho-PET/CT will be performed with a low-dose, non-contrast enhanced CT scan for anatomical reference, and will be of limited duration (approximately 30 minutes). Because the 1st – 6th patient will undergo different imaging than the 7th – 12th patient, there are two versions of the patient information letter in which either ^{166}Ho-PET/CT or ^{166}Ho-MS scout dose MRI is embedded.
The target liver absorbed dose is fixed to 60 Gy and, therefore, the total amount of radioactivity of the therapy dose varies with the liver weight (see chapter 6.5, Table 4). An experienced nuclear medicine physician will administer both the scout and therapy dose assisted by the interventional radiologist.

5.2.4 Visit week 1

This visit will take place at the outpatient clinic. During this visit, the following must be documented and reviewed as a part of the study:

- Post therapy scintigraphy (scan will be acquired when the administered dose will have decayed to 500 megabecquerel (MBq) or less, which will take around 3-5 days, depending on the administered dose)
- QoL

5.2.5 Visit week 3

This visit will take place at the outpatient clinic. During this visit, the following must be documented and reviewed as a part of the study:

- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type B (blood)

5.2.6 Visit week 6

These visits will take place at the outpatient clinic. During these visits, the following must be documented and reviewed as a part of the study:

- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- QoL
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type B (blood)

5.2.7 Visit week 9

This visit will take place at the outpatient clinic. During this visit, the following must be documented and reviewed as a part of the study:

- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type B (blood)

5.2.8 Visit 3 months

This visit will take place at the outpatient clinic at 3 months post treatment ± 7 days. During this visit, the following must be documented and reviewed as a part of the study:

- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- QoL
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type A (blood)
- PET/CT, MRI

5.2.9 Visit 6, 9, and 12 months

This visit will take place at the outpatient clinic at 6, 9, and 12 months post treatment ± 14 days. During this visit, the following must be documented and reviewed as a part of the study:

- Physical examination (incl. vital signs)
- WHO performance assessment
- Concomitant medication
- QoL
- Monitoring of occurrence of (serious) adverse events
- Laboratory examination type A
- PET/CT, MRI

5.3 Holmium content

Pooled urine samples will be collected from -5-0 hours (the beginning of the safety dose angiography until the end of the therapy dose angiography), 0-3 hours (end of therapy dose angiography until 3 hours afterwards), 3-6 hours, and 6-24 hours following therapy dose administration. The date and time of the start and the end of the collection period, and whether the collection was complete or not, will be noted in the E-Case Report Form (CRF). Measurement of holmium content in urine and blood will be performed. The date and the time of measurement and the results will be reported in the E-CRF.

During the hospitalization for treatment (at t = 0, 3, 6, and 24 hours following ^{166}Ho-PLLA-MS administration) blood will be drawn for measuring the holmium content in the blood. T=0 measurement is performed when the patient has returned to the ward after treatment angiography.

5.4 Laboratory examinations

Blood samples for safety parameters will be taken using an indwelling canula or by single vein puncture. During the follow-up visits with the investigator, blood samples for safety parameters will be drawn by a research nurse and delivered to the Laboratory of Clinical Chemistry and Haematology (see Table 3).

5.5 Radiation exposure rate

During the hospitalization for treatment, the radiation exposure rate will be measured at t = 0, and 24 hours from 1 meter distance (see Table 2). T=0 measurement is performed when the patient has returned to the ward after treatment angiography.

5.6 Use of co-medication / prophylactic measures

All patients that are eligible for the experimental treatment will receive proton pump inhibitors (pantoprazol 1 dd 40 mg) starting the day before the catheterisation, which will be continued for 6 weeks.

All patients that are eligible for the experimental treatment will receive anti-emetic drugs (ondansetron i.v. 8mg) and prevention of contrast allergy (dexamethason 16mg p.o.and 10mg i.v. and clemastine 1mg p.o.) prior to catheterisation.

During the vascular intervention the patient will receive heparin (1000 IU/ml in saline, up to 5000 IU), to avoid the formation of thromboembolism during the intervention.

The vascular intervention will be performed under x-ray guidance. To be able to visualise the blood vessels during the procedure, a non-ionogenic x-ray contrast agent (jodixanol, Visipaque®) will be administered to the patient.

Should women of childbearing age require microsphere therapy, non-pregnancy needs to be ascertained prior to treatment. Therefore, proper contraceptive measures should be used, such as the birth control pill.

As a prophylactic measure for thromboembolic events, all patients will be provided with pressure stockings at the day of each angiography. Patients will be advised to wear these pressure stockings until the end of hospitalization or until the moment that patient is able to mobilise.

After the angiography, when the urinary catheter is removed, patients receive a single gift of amoxicillin/clavulanic acid (1000/200mg i.v.) for prevention of urinary tract infections.

5.7 Escape medication

All escape medication mentioned below is equal to the standard escape medication for treatment with ^{90}Y-RE.

Patients are offered intravenous analgesics (morphine 7.5 mg – 15 mg/24 h) prior to the procedure. Patients may receive oral analgesics (paracetamol up to 4000 mg/24 h) for relief of fever and pain after the administration of microspheres. To reduce nausea and vomiting, patients will receive anti-emetics (ondansetron up to 3 dd 8 mg) during the first 24 hours after administration of microspheres. In the case of persisting nausea, metoclopramide (up to 300 mg/24 h) will be used.

The vascular contrast agent jodixanol (Visipaque®) can cause renal insufficiency in poorly hydrated patients. Therefore, patients will be hydrated according to the CBO-directive “Richtlijn Voorzorgsmaatregelen bij jodiumhoudende contrastmiddelen”, page 23-26 (see Appendix VI).
Inadvertent shunting of microspheres to non-target organs, including the lungs, stomach, pancreas, duodenum and the gall bladder, can be associated with serious side effects. To reduce toxicity of the radioactive microspheres in this case, amifostine (Ethyol®, up to 200 mg i.v./m^2 for 7 days) can be administered.

In case patients with neuroendocrine tumours receive this experimental treatment, the release of neuroendocrine factors may give rise to the so called ‘carcinoid syndrome’ (see 7.1.8). This syndrome can be prevented to some extent with octreotide (300 µg i.v./24 h).

6. INVESTIGATIONAL MEDICAL DEVICE

6.1 Name and description of investigational medical product

The device under investigation comprises radioactive particles dedicated for treatment of hepatic malignancies. The particles, called microspheres, contain the radionuclide holmium-166, which emits gamma rays (81 kilo electron volt (keV)) and high-energy (1.8 mega electron volt (MeV)) beta particles. The beta particles are responsible for the therapeutic effect of the device, the gamma ray can be used for nuclear imaging purposes.

Liver metastases are preferably supplied by the hepatic artery. This selective vascularisation allows the use of the hepatic artery for selective administration to the metastases without compromising hepatic flow by the portal vein. The microspheres are locally administered by means of selective catheterisation of the hepatic artery by a trained intervention radiologist.

6.2 Animal studies

Several animal studies have been performed with ^{166}Ho-PLLA-MS. These studies were aimed to get insight into the toxic effect, the method/technique of administration, efficacy, safety and in vivo stability of the microspheres. The studies have been performed on rats, rabbits, and pigs. The animal studies showed positive results. An overview and summary of the animal studies performed is given in the Investigational Medical Device Dossier (IMDD, page 13, 18 and Appendix 6).

6.3 Clinical studies

In the HEPAR I trial, 15 patients with liver metastases from miscellaneous primary cancers have received radioembolization with escalating doses of ^{166}Ho-PLLA-MS (20-40-60-80 Gy). Cohorts consisted of 3-6 patients. The treated patients with liver metastases consisted of 7 patients with metastases of ocular melanoma, 5 with metastases of colorectal carcinoma, 2 with metastases of cholangiocarcinoma and 1 with metastases of breast cancer.
The 20 Gy-cohort was extended to 6 patients because one patient had developed a pulmonary embolism post ^{166}Ho-RE. This serious adverse event was ascribed to the lengthy procedure in this patient. The last cohort (80 Gy) consisted of 3 patients and was stopped because of dose limiting toxicity in two patients, as was prospectively described in the study protocol. The other two cohorts (40 Gy and 60 Gy) comprised 3 patients each.

Clinical toxicity in the first 3 cohorts consisted mainly of symptoms belonging to the post embolization syndrome (PES)[29]. PES comprises fatigue, nausea, abdominal pain, fever, vomiting and/or anorexia, and is a known phenomenon in radioembolization treatment. These symptoms were transient in all cases and well-controlled by outpatient medication. Laboratory toxicity was mild except for expected grade 3 liver enzymes toxicity and grade 3 haematological toxicity. Overall, treatment was well tolerated by the patients in the first 3 cohorts (20 Gy – 60 Gy) and the toxicity profile was very similar to the toxicity which is encountered in patients treated with ^{90}Y-RE [29-31]. In the 80-Gy cohort, however, treatment was considerably less well tolerated. Patients had more complaints of abdominal pain and nausea. These symptoms were more severe and lasting longer than the clinical toxicities encountered in the previous cohorts. Furthermore, laboratory toxicity was more severe and in certain cases progressive with a suspected radiation induced liver disease (RILD) in one patient. Liver toxicity is to some extend intended in radioembolization given the 5-mm reach of the beta emitting holmium. Furthermore, lymphocyte- (up to grade 3), leukocyte- (up to grade 3) and platelet counts (up to grade 4) transiently decreased. These adverse events are most probably caused by activation of hepatic stem cells in the bone marrow due to liver damage [27].These symptoms are suspected to be related to the higher radiation dose instead of an embolic effect of treatment because the amount of microspheres was equal in

all cohorts. As a consequence, dose limiting toxicity was reached in the 80 Gy-cohort and the decision was taken to stop the study and continue with 60 Gy as a target dose for the HEPAR phase II study. A listing of all adverse events encountered in the HEPAR I trial is given in Appendix XIII. This study was not designed to demonstrate efficacy of ^{166}Ho-RE, but a number of partial remissions and stable disease were observed.

A summary of the HEPAR I trial is given in the IMDD (page 14).

6.4 Summary of known and potential risks and benefits

6.4.1 Potential risks

Based on the literature on ^{90}Y-MS and animal studies on ^{166}Ho-PLLA-MS and the clinical data derived from the HEPAR I trial, it is concluded that, if the ^{166}Ho-PLLA-MS are administered correctly, the risk of complications is low. Although a potential risk described in the literature, no complications associated with non-target delivery of ^{166}Ho-PLLA-MS were observed in the HEPAR I trial. It has been described in the literature that, due to excessive radiation doses delivered to the liver parenchyma, “radiation induced liver disease” or “radiation hepatitis” is known to occur [32, 33]. This veno-occlusive disease can usually be managed by steroid treatment but in some cases will result in fulminant liver failure. However, the incidence of this complication is very low and has not been observed in any of the patient treated with ^{166}Ho-RE in the safety study. The risk of mild side effects associated with the macroembolic effect of radioembolization is significant but transient, and usually well managed by outpatient medication (see Chapter 7)

6.4.2 Potential benefits

In short, the use of radioactive microspheres administered intra-arterially as radionuclide therapy for liver malignancies can overcome disadvantages of external beam radiation, which is limited by the radiosensitivity of healthy liver tissue. Microspheres have the potential to provide treatment to previously untreatable patients.

The preclinical data (see IMDD page 22) as well as the clinical data obtained from the HEPAR I trial demonstrate that RE with ^{166}Ho-PLLA-MS is suitable for treatment of patients with liver malignancies and may have a beneficial effect on QoL and survival. However, the latter variable was not a primary endpoint of this safety study and must be further investigated in the HEPAR II trial.

6.5 Dose

The amount of ^{166}Ho radioactivity (A) that must be administered to a patient to deliver the desired absorbed liver dose can be calculated according to the following formula [23]:

$$A_{Ho166}\ (MBq)/LW\ (kg) = Liver\ Dose\ (Gy)/15.87 \times 10^{-3}(J/MBq) = Liver\ Dose\ (Gy) \times 63\ (MBq/J)$$

where *LW* is the liver weight of the patient which is determined on CT or MRI.

In this study, the patients will receive a mean liver absorbed dose of 60 Gy. The required amount of ^{166}Ho activity may then be calculated as follows:

$$A_{Ho166}\ (MBq)/LW\ (kg) = 60\ (Gy)/15.87 \times 10^{-3}(J/MBq)$$

$$A_{Ho166}\ (MBq)/LW\ (kg) = 3781(Gy \times MBq/J)$$

$$A_{Ho166}\ (MBq) = 3781 \times LW\ (kg)$$

The calculated dose is based on the assumption that all administered activity will be equally distributed over the whole liver. This is not a realistic assumption but a rather conservative and safe approach. Using this approach, only mild adverse events were encountered in healthy pigs treated with calculated whole liver doses up to 150 Gy [23]. The administered activity will show heterogeneous distribution over the liver enabling regeneration of relatively spared liver tissue. There were notable differences in laboratory and clinical toxicity between the 20-, 40-, and 60-Gy cohorts and the 80-Gy cohort in the HEPAR I trial.

In Table 4, "Dose (Gy) and activity (MBq) relation of holmium-166 treatment", examples of amounts of activity for typical liver weights are given for a liver absorbed dose of 60 Gy, which equates to 3.8 GBq/kg (liver weight).

Table 4. Liver weight and total radioactivity relation

	LW (kg)				
	1	1.5	2	2.5	3
Liver dose (Gy)	A (MBq)	A (MBq)	A (MBq)	A (MBq)	A (MBq)
60	**3780**	**5670**	**7560**	**9450**	**11340**

6.6 Accountability of radioactive device

The following flowchart demonstrates the route from preparation to disposition of the radioactive device ^{166}Ho-PLLA-MS.

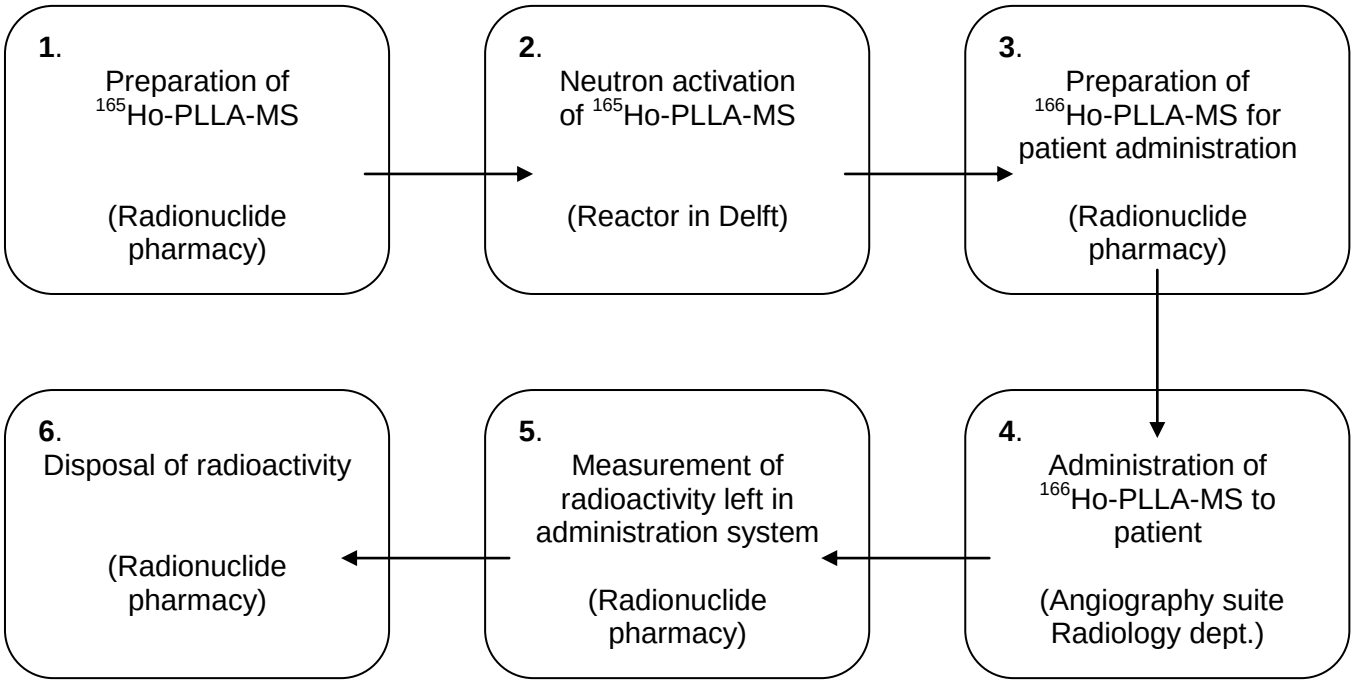

Figure 1. ^{166}Ho-PLLA-MS flowchart, route from preparation to disposition

Step 1 – Preparation of ^{165}Ho-PLLA-MS is carried out at the Good Manufacturing Practice (GMP) facility (room E.02.4.11) of the radionuclide pharmacy of the UMC Utrecht. After passing quality control, batches of ^{165}Ho-PLLA-MS are stored in the radionuclide pharmacy at room temperature in room E.02.411 in a vacuum dessicator.

Before neutron activation, patient dose vials are engraved with a unique identification number. A complete description of the preparation, labelling and release of ^{165}Ho-PLLA-MS is given in the IMDD (page 8-10, Appendix 2 and 3, PTC-01 and PTC-02).

Step 2 – Neutron activation is carried out at the Reactor Institute Delft (Delft, the Netherlands). A complete description of neutron activation is given in the IMDD (page 11, Appendix 4 and 7 and PTC-04).

Step 3 – Preparation of ^{166}Ho-PLLA-MS for patient administration is carried out at the GMP facility (room E.02.4.11) of the radionuclide pharmacy of the UMC Utrecht. A complete description of the preparation of ^{166}Ho-PLLA-MS is given in the IMDD (Appendix 4, PTC-01 and PTC-05).

Step 4 – Administration of ^{166}Ho-PLLA-MS to the patient is carried out at the angiography suite of the radiology department. A complete description of the method of administration of ^{166}Ho-PLLA-MS is given in the IMDD (page 19-20).

Step 5 – Measurement of radioactivity left in the administration materials is carried out at the GMP facility (room E.02.4.11) of the radionuclide pharmacy of the UMC Utrecht. Measurement is performed to calculate the net activity administered to the patient.

Step 6 – Disposal of radioactivity is carried out in room E.00.2.19. A complete description of the disposal of radioactivity is given in the IMDD (PTC.300.02).

7. SAFETY PROFILE

The following effects are expected based on literature on treatment with ^{90}Y-MS [34-36] and form an exhaustive list of side effects observed in thousands of patients. A small selection of the following side effects has been observed in patients in the HEPAR I trial (7.1.1; 7.1.2; 7.1.3; 7.1.4). The safety profile is divided in general and technique related effects.

7.1 General side effects and complications

When the patient is treated with the proper technique, without excessive radiation to any organ, the common adverse events after receiving radioactive microspheres are fever, abdominal pain, nausea, vomiting, diarrhoea and fatigue. An abnormality of liver function tests is likely to occur. This may be up to grade 3 or 4 (CTCAE vs 4) in the case of AST/SGOT, ALT/SGPT, γGT, ALP and LDH, without direct clinical relevance. In general these effects are transient [25]. In the HEPAR I trial, we observed AST, ALT or GGT serum level elevations up to grade 3 in 13/15 patients (87%).

7.1.1 Fatigue

Fatigue is often observed in patients. In general it does not exceed grade 2. To date, we observed fatigue in 10/15 patients (67%) treated with ^{166}Ho-RE.

7.1.2 Fever

Fever can be observed immediately after the embolization (as part of the post-embolization syndrome) or later in the follow-up. It can last for one week. The occurrence of fever later in the follow-up may be caused by the development of hepatic abscesses. To date, fever was observed in 6/15 patients (40%) following ^{166}Ho-RE, typically starting on the day of treatment, lasting 1-3 days, but may last for several weeks.

7.1.3 Abdominal pain

Right upper quadrant abdominal pain is frequently observed in patients undergoing radio-embolization, but easily managed by outpatient medication. In the HEPAR I trial, most patients experienced acute abdominal pain, which was managed with paracetamol and occasionally opiate medication. To date, one patient (7%) has been readmitted because of abdominal pain in the HEPAR I trial.

7.1.4 Gastrointestinal toxicity

Nausea and/or vomiting are known side effects of RE. Both may be controlled with anti-emetic therapy. Ondansetron (up to 3 dd 8 mg) is recommended the first 24 hours after the administration. Subsequently, metoclopramide (up to 300 mg/24 h) can be prescribed. Diarrhoea should be treated with adequate doses of loperamide (up to 16 mg/24 h). To date in the HEPAR I trial, nausea was present in 13/15 patients (87%). A selection, 8/15 patients (53%) progressed to vomitus. Diarrhoea was observed in one patient.

7.1.5 Tumour Lysis Syndrome

A major but very rare complication is Tumour Lysis Syndrome (TLS), which is caused by rapid necrosis of the tumour. Laboratory tests show high serum levels of LDH, high uric acid, high serum potassium, high phosphate and low serum calcium. In patients with solid tumours, this side effect is extremely rare. Supportive care including fluids is recommended. TLS has not been observed in patients treated in the HEPAR I trial.

7.1.6 Radiation hepatitis

The frequency of radiation hepatitis is very low in radioembolization due to the inhomogeneous distribution of the microsphere dose. Management of this serious complication, in case hepatic insufficiency occurs, consists of the use of high doses of lactulose, steroids, and, if appropriate, assessing brain pressure and relieving increased brain pressure. Hepatic insufficiency may occur in <1% of all treated patients. Radiation hepatitis (or Radiation induced liver disease) was suspected in one patient of the HEPAR I trial who had received 80 Gy.

7.1.7 Veno occlusive disease

Veno occlusive disease (VOD) may occur by radiation damage to the portal veins. This is caused by activation of the coagulation system probably due to endothelial damage. Anticoagulants such as low molecular heparin (Fraxiparin in therapeutic dosages) have been shown to be beneficial. To date, VOD has not been observed in patients in the HEPAR I trial.

7.1.8 Carcinoid crisis

In case patients with neuroendocrine tumours receive this experimental treatment, the release of neuroendocrine factors may give rise to the so called ‘carcinoid syndrome’. This consists of high blood pressure, flushing and diarrhoea. This syndrome can be prevented to some extent with octreotide (300 µg i.v./24 h). Carcinoid crisis has not occurred in the HEPAR I trial.

7.1.9 Leuko- and thrombopenia

In a number of cases, transient and reversible leuko- and thrombopenia has been observed both in case of ^{90}Y-RE and ^{166}Ho-RE. The nadir of the laboratory toxicities can be up to grade 4.

7.2 Inadvertent non-target radioembolization

The following technique related effects are directly related to inadvertent deposition of the microspheres in organs other than the liver. None of the following events have occurred in any of the HEPAR I patients.

7.2.1 Peptic ulceration

A peptic ulcer may occur in <10% of all patients undergoing RE. The development of acute peptic ulceration is suggested by the symptoms of gastric ulcers and diagnosed by endoscopy. If this complication occurs, the patient should be treated using best standard care, including pain relief, gastric acid blocking drugs (pantoprazol i.v. up to 80 mg) and intravenous fluids. Treatment is the same as for any cause of peptic ulceration.

7.2.2 Pancreatitis

Pancreatitis may occur in 1% of all treated patients, according to the literature on RE. The post treatment nuclear images will reveal if any radioactivity may have lodged in the pancreas. Additional tests such as serum amylase are indicated if pancreatitis is suspected. If this occurs, the patient should be treated using best standard care, including pain relief, gastric acid blocking drugs (pantoprazol i.v. up to 80 mg), and intravenous fluids.

7.2.3 Radiation pneumonitis

High levels of implanted radiation and/or excessive shunting to the lung may lead to radiation pneumonitis. This may be suspected if patients develop a non-productive cough several days or weeks after the implantation of the microspheres and is diagnosed by plain X-ray or CT of the thorax. Patients should be treated with systemic corticosteroids (1 mg/kg/day) and supportive care until the condition has subsided.

7.2.4 Radiation induced cholecystitis

A rare complication is radiation induced cholecystitis, which may occur in 1-2% of all treated patients. This side effect can be expected a few weeks after the intervention. Complaints are local pain in the liver area, sometimes colicky in nature, elevated bilirubin serum levels and increased CRP. Treatment may consist of a cholecystectomy.

7.2.5 Medical treatment for inadvertent non-target radioembolization

In case a considerable portion of the dose of radioactive microspheres are deposited outside the liver, the potential toxicity may be decreased by starting treatment with the radiation scavenger amifostine (up to 200 mg i.v./m^2 for 7 days). It is imperative to start this treatment as soon as possible.

7.3 Other technique related complications

The following technique related complications have been reported in the literature following RE.

7.3.1 Spurious aneurysm and hematoma

As a complication of arterial catheterization in the groin, a false aneurysm (spurious aneurysm/pseudoaneurysm) may develop at the vascular access site within a few days after the procedure. This can be suspected in case of local pain and swelling in the groin, and is diagnosed with the use of ultrasound. In most cases, ultrasound-guided compression will be effective. Pseudoaneurysms less than 2 cm in size can be managed conservatively and monitored by serial imaging to confirm spontaneous resolution. First-line treatment consists of injection of bovine thrombin [37]. If the aneurysm is becoming very large and threatens or causes skin necrosis, or is expanding rapidly as it may be infected, surgical intervention should be performed. In case of vascular access site hematoma, conservative treatment is advised, because hematomas are known to resolve spontaneously in most cases within days.

7.3.2 Infection or inflammation of the arterial puncture wound

As a complication of arterial catheterization in the groin an infection/inflammation of the arterial puncture wound may develop. Adequate antibacterial treatment is warranted.

7.3.3 Iatrogenic arterial dissection and/or inadvertent embolization

Iatrogenic arterial dissection is a well-known risk of endovascular catheter treatment. Manoeuvring of a foreign object (catheter) within the vascular system comprises a risk of damage to the intimal layer of the arterial wall. As a result, an intimal dissection can occur which may or may not become symptomatic. If dissection of an (abdominal) artery becomes symptomatic, this can be managed in general by medical treatment (such as lowering of the blood pressure). Alternatively, if the dissection occurs during endovascular treatment, direct

measures can be taken to reduce the dissection by means of balloon inflation or deployment of a bare-metal stent. If these measures fail, or if the dissection becomes evident after endovascular treatment, operative surgery should be considered. Another consequence of iatrogenic arterial catheterization might be inadvertent embolization leading to (partial) infarction of the upper abdominal organs.

7.3.4 Contrast-induced renal insufficiency

Although the frequency of contrast-induced renal insufficiency has decreased by the use of non-ionogenic contrast, care should be taken to hydrate the patient adequately. Particularly in patients with an impaired food intake and or diabetes mellitus this will also require adequate hydration (>2 l of fluid/24 h) prior to all angiographic procedures. If renal insuffiency develops, patients will complain about asthenia, vomiting may occur and oedema can be observed. The diagnosis is made by laboratory testing. Rehydration is generally sufficient. Care should be taken to prevent hyperkalemia to avoid cardiac rhythm abnormalities. To prevent nephrotoxicity, patients will be pre- and posthydrated according to the CBO-directive “Richtlijn Voorzorgsmaatregelen bij jodiumhoudende contrastmiddelen”, page 23-26.

7.3.5 Thromboembolic events

It is known that patients with advanced malignant disease have an increased risk on the development of thromboembolic events especially in case of prolonged immobilization such as surgery or angiographic procedures. Prophylactic pressure stockings will be provided for all patients during the hospitalization period. No standard prophylactic anticoagulants will be provided due to the inherent risk of haemorrhages during invasive procedures.

8. METHODS

8.1 Study endpoints

8.1.1 Primary study endpoint

- The primary outcome is target lesions tumour response at three months follow-up. For this study, this is defined as: 1) complete response on CT at 3 months, or 2) partial response on CT at 3 months or 3) stable disease on CT at 3 months (see chapter 8.3.1 Definition of treatment success). Tumour size will be assessed on the portovenous phase of the dynamic contrast-enhanced CT images. At fixed intervals in time (3, 6, 9, and 12 months post ^{166}Ho-RE), the largest liver lesions are selected, to a maximum of two, and the maximum diameter in axial plane is measured. The sum of the maximum diameter of the lesions is recorded.
    - Complete Response (CR): disappearance of all lesions
    - Partial Response (PR): ≥ 30% decrease in the sum of the longest diameters of target lesions, with the baseline measurements taken as reference
    - Stable Disease (SD): <30% decrease and <20% increase
    - Progressive Disease (PD): ≥20% increase in the sum of the longest diameters of target lesions, with the baseline measurements taken as reference or appearance of new lesions

8.1.2 Secondary study endpoints

- To determine overall tumour response: (PET)CT will be used to quantify overall tumour response in time according to RECIST 1.1 guidelines.
  Antitumoural effect will also be assessed by relevant tumour markers (e.g. CEA for colorectal carcinoma and chromogranine A for neuroendocrine tumours) levels. Tumour marker changes will be expressed as a percentage of the pre-treatment values.
- To determine liver specific progression-free survival (LPFS). LPFS equals the interval between ^{166}Ho-RE and tumour progression in the liver according to RECIST 1.1. The protocol dictates that tumour response is measured on CT at 3, 6, 9, and 12 months post ^{166}Ho-RE. However, to obtain a true LPFS, CT of the liver for tumour response assessment can be performed at the physician's discretion in case of suspected tumour progression. LPFS is assessed overall (i.e., all patients treated in this study), and may be assessed separately for each primary tumour type
- To determine non-liver specific progression-free survival (NLPFS): at fixed intervals in time (3, 6, 9, and 12 months post ^{166}Ho-RE), and at the physician's discretion, (PET/)CT is performed for tumour response assessment. NLPFS is the time between ^{166}Ho-RE and tumour progression according to RECIST 1.1 for both intra- and extrahepatic tumours. NLPFS is assessed overall (i.e., all patients treated in this study), and may be assessed separately for each primary tumour type
- To assess overall survival. Date of death will be documented during the course of the study. After completion of study follow-up, survival will be checked through the central registration of the UMC Utrecht (Dutch: afdeling Zorgadministratie & -informatie) and / or the patient's general physician.
- To evaluate toxicity: the toxicity profile was the primary endpoint of the HEPAR I trial. In patients treated in the HEPAR II trial, the safety and toxicity of ^{166}Ho-RE will be further evaluated
- To evaluate QoL: Quality of live is evaluated using the EORTC questionnaire QLQ-C30 with colorectal liver metastases module QLQ-LMC21 (see Appendix VII). QoL is assessed at baseline, at 1 week, and at1, 3, 6, and 12 months post ^{166}Ho-RE

- To assess performance status: using WHO performance status criteria. Performance status is assessed at every visit except for week 2
- To evaluate ^{99m}Tc-MAA and ^{166}Ho-PLLA-MS scout and therapy dose on SPECT/CT
- To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on MRI. ^{166}Ho-PLLA-MS scout dose MRI will only be performed in the 7th – 12th patient undergoing treatment. After the 12th patient, patients will either receive ^{166}Ho-PET/CT imaging of the scout and therapy dose or ^{166}Ho-MR imaging of the scout dose
- To evaluate ^{166}Ho-PLLA-MS scout dose and ^{166}Ho-PLLA-MS total dose on PET/CT. ^{166}Ho-PET/CT will only be performed in the 1st – 6th patient. After the 12th patient, patients will either receive ^{166}Ho-PET/CT imaging of the scout and therapy dose or ^{166}Ho-MR imaging of the scout dose

8.2 Withdrawal of individual patients

Patients can abandon the study at any time for any reason if they wish to do so without any consequences for treatment or care. The investigator can decide to withdraw a patient from the study for urgent medical reasons.

Patients will be withdrawn from the study if:

- The investigator considers it in the best interest of the patient that he/she be withdrawn
- The patient withdraws consent
- The patient is unable to comply with protocol procedures

The date and reason for withdrawal must be recorded. If the patient withdraws consent after the therapy dose of ^{166}Ho-PLLA-MS has been administered, the patient will be advised to agree to follow-up safety investigations.

8.2.1 Replacement of withdrawn patients

All patients registered in the study will be accounted for. Patients who do not receive the therapy dose of ^{166}Ho-PLLA-MS will be replaced by another patient, and will not be included in any of the analyses. Based on the (sequential) design and criteria, recruitment and treatment will continue for the first cohort until 30 patients can be evaluated for tumour response. All patients that received ^{166}Ho-RE will be analysed for safety and secondary endpoints, regardless of their availability for tumour response evaluation. For example, a patient that drops out of the study 2 months post treatment will be evaluated for safety and secondary endpoints. However, this patient does not count as one of the 30 patients in whom tumour response needs to be evaluated in the first cohort. Therefore, the patient needs to be replaced. Consequently, if the study continues beyond 30 patients, recruitment will continue in cohorts of 6 patients that can be evaluated for tumour response.

8.3 Premature termination of the study

8.3.1 Definition of treatment success

The number of successes obtained in this study is based on the target lesions tumour response at 3 months. The following 3 categories of patients will be regarded as treatment success:

1. Patients with complete response on CT (according to RECIST 1.1[38])
2. Patients with partial response on CT (according to RECIST 1.1)
3. Patients with stable disease on CT (according to RECIST 1.1)

8.3.2 Determination of ineffective therapy

Therapy for an individual patient is regarded ineffective if target lesions tumour response assessment at 3 months indicates progressive disease according to RECIST 1.1 or if death occurs within this period.

Early termination of the study for lack of efficacy (futility) will be guided by the lower group sequential boundary on the number of patients with treatment success (see 8.3.1). Advice to stop early will be given by the Independent Data Monitoring Committee, that may include other considerations (safety) as well.

8.4 Independent Data Monitoring Committee (IDMC)

An IDMC is established. The IDMC is an independent group comprising internal members (from within the UMCU) and external members. This phase II study is open label and patients are individually closely monitored for safety, as was done in the HEPAR I study. The main objective is to decide whether or not to embark on a larger phase III trial. Hence it was considered adequate to establish an IDMC with both (independent) internal UMCU members as well as an external member. In case of a future phase III trial, a fully external IDMC is strongly recommended. The IDMC includes the following members:

- Dr. J.M.H. de Klerk, Meander Medical Center Amersfoort; nuclear medicine physician
- Dr. F.P. Vleggaar, University Medical Center Utrecht; gastro-enterologist
- Dr. O.E.H. Elgersma, Albert Schweitzer ziekenhuis; interventional radiologist
- Dr. I. van der Tweel, Julius Center Health Sciences and Primary Care Utrecht; biostatistician

The IDMC will analyse preliminary study results on tumour response after the first 30 patients who are amenable for tumour response assessment at 3 months (i.e. PPS), and every 6 patients who are amenable for tumour response assessment at 3 months thereafter (see Table 5). Waiting for the three-month follow-up of all 30/36/42/48 patients is only required if, based on the available follow-up in the other patients, it remains uncertain whether the stopping boundaries will be reached.

The IDMC analyse preliminary study results on safety as well (see Table 5). In contrast to the tumour response analyses, the safety analyses will include all patients that received treatment within a time span of 3 months (regardless of their availability for tumour response assessment, i.e. FAS), and therefore, these reviews may not run in sync with the IDMC tumour response analyses. Patient inclusion and treatment may continue during IDMC safety analyses. In its safety analyses, the IDMC will consider specifically the reasons and potential consequences on treatment benefit of the patients that discontinued before the 3 months assessment. The IDMC will receive reports on a regular basis on all SAEs reported for this trial and a complete summary safety report for each safety analysis. The IDMC will work according to the IDMC charter (see Appendix VIII) Recruitment will not be interrupted unless otherwise requested by the IDMC Chairman.

The responsibilities of the IDMC include:
- To safeguard the safety of current and future participating patients
- To safeguard the continued scientific merit of the trial
- To evaluate whether the efficacy data comply with the stopping criteria, and advice accordingly taking all data into account
- To make recommendations for changes in study processes where appropriate
- To advice on continuation of the study

The IDMC will report to the Principal Investigator (on behalf of the sponsor), who is responsible of communicating to others (e.g. the METC) as appropriate or required by regulation.

Assessing safety signals for concern in this population is a complex matter, taking into account the adverse events caused by the disease as well as weighing safety versus the potential benefit in this population with poor prognosis. This is further compounded by the lack of comparative group in this design. Treatment related adverse events and serious adverse events in this heavily pretreated and advanced patient population are difficult to distinguish from adverse events caused by the underlying disease and comorbidities. Life-threatening toxicity should always be carefully analysed and weighed against treatment effect, but given the population, grade 3-4 toxicities will occur in a substantial proportion of study patients. Liver enzyme toxicity, for instance, occurs frequently in this patient category, but is only considered life-threatening if the metabolic function and/or the productive function of the liver are compromised. Therefore, only bilirubin grade 3-4 and/or factor V grade 3-4 toxicity occurring in >30% of the study population, must be regarded as a clear sign that the toxicity of ^{166}Ho-RE may outweigh the potential palliative benefits. This guideline assumes analysis of at least 12 patients in order to base this conclusion on sufficient data. All other CTCAE grade 3-4 events, excluding liver enzyme toxicity, may occur in up to 50% of the study population, this also implies analysis of at least twelve patients. Grade 3-4 events which can clearly be attributed to disease or comorbidity, are excluded from this guidance.

Table 5. IDMC review scheme

IDMC tumour response analysis*	Patient series	IDMC safety analysis**
No. 1	1-30	Every 3 months
No. 2	30-36	
No. 3	36-42	
No. 4	42-48	

* IDMC tumour response analyses will be held after the first 30 patients and every 6 patients thereafter. Primarily, patients who are available for tumour response assessment 3 months post treatment are evaluated (PPS). FAS data will be analysed during IDMC reviews of safety
** IDMC safety analyses will be held every 3 months after treatment of the first patient. All patients that received ^{166}Ho-RE will be evaluated (FAS). Safety analysis will at least be held after treatment of 30 patients.

9. SAFETY REPORTING

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the patients and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study inclusion will be suspended pending further review by the METC, except insofar as suspension would jeopardise the patients' health. The investigator will take care that all patients are kept informed.

9.2 Definitions in safety reporting

According to EU Directive 90/385/EEC, 93/42/EEC (last amended Directive 2007/47/EC), and 'Guidelines on medical device: MEDDEV 2.7/3', the following definitions are used in the safety reporting:

9.2.1 Investigational medical device

Medical device (Ho-166 poly lactic microspheres) being assessed for safety or performance in this clinical investigation (HEPAR-II)

9.2.2 Device Deficiency (DD)

Inadequacy of the medical device related to its identity, quality, durability, reliability, safety or performance, such as malfunction, misuse or use error and inadequate labelling.

9.2.3 Adverse events (AEs)

Adverse events are defined as any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational device (Ho-166 poly lactic acid microspheres). This also includes events related to the procedures involved (any procedure specified in the clinical investigation plan).

9.2.1 Adverse Device Effects (ADEs)

If an Adverse Event is considered to be related to the use of the investigational medical device then it will be designated an Adverse Device Effect (ADE). This includes any adverse event resulting from insufficiencies or inadequacies in the instruction(s) for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical devise. This also includes any event that is a result of a use error or intentional misuse.

9.2.2 Serious adverse events

A serious adverse event is an Adverse Event that:

- led to a death;
- led to a serious deterioration in health that either:
- resulted in life-threatening illness or injury or
  resulted in a permanent impairment of a body structure or a body function, or
- required in-patient hospitalization or prolongation of existing hospitalization or

- resulted in medical or surgical intervention to prevent life threatening illness or injury or permanent impairment to a body structure or body function
- led to fetal distress, fetal death or a congenital abnormality or birth defect.

This includes Device Deficiencies that might have led to a Serious Adverse Event if a suitable action had not been taken or the intervention had not been made or if circumstances had been less fortunate.

Death of one of the patients who is no longer included in the study will only be reported as a SAE if death occurs within 30 days of the end of the study participation.

Note: A planned hospitalization for a pre-existing condition, or a procedure required by the Clinical Investigation Plan without a serious deterioration in health is not considered to be a serious adverse event.

9.2.3 Serious Adverse Device Effect (SADE)

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event.

9.2.4 Suspected unexpected serious adverse reactions (SUSAR)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information.

9.2.5 Unanticipated Serious Adverse Device Effect (USADE)

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

9.3 Safety reporting

9.3.1 Recording

All Adverse (Device) Events reported spontaneously by the patient or observed by the investigator or his staff will be recorded.

9.3.2 Report by the investigator to the sponsor

The investigator will report any reportable event to the sponsor within 3 calendar days after the awareness of the occurrence of the event.

9.3.3 Responsible for reporting SA(D)E

In the HEPAR II study the sponsor (Dr M. Hendriks) and/or the authorized persons, principal investigator (Dr B.A. Zonnenberg) and the study coordinator (T. Bosma) will be responsible for reporting.

9.3.4 Reportable events

The following events are considered to be reportable events: any SA(D)E (including SUSAR and/or USADE), any new findings/updates in relation to already reported events

9.3.5 Timelines of reporting

If a SA(D)E indicates an imminent risk of death, serious injury, or serious illness and requires prompt remedial action for other patients/subject, users or other persons or a new finding to it, it will be reported immediately but no later than 2 calendar days after awareness by the sponsor of a new reportable event or of new information in relation with an already reported event. The event will be reported to the accredited METC that approved the protocol. At the same time SA(D)E's will be reported to the members of the IDMC and the national competent authority (IGZ).

All other SA(D)Es will be reported within 7 calendar days after the sponsor has first knowledge of the SA(D)E. At the same time these other SA(D)Es will be reported to the members of the IDMC. The NCA (IGZ) will be reported of other SA(D)E's once every quarter year.

The remaining SUSARs and USADES are also recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs and USADES from the study device, accompanied by a brief report highlighting the main points of concern.

9.3.6 Reporting standards

The METC will be notified through the web portal ToetsingOnline. The IDMC will receive a copy of this correspondence. The NCA will receive an updated version of the reporting form as required in MEDDEV 2.7/3.

9.4 Documentation

Attention is to be paid to the occurrence of AEs at all stages of the examination. Thus, the patient should be closely observed by the investigator both during and after the treatment. The recording phase for AEs will start with the first study drug administration and will end the last day of the follow-up period. AEs related to hematological or renal toxicity which are still present at the end of the follow-up period will be followed up until complete resolution as assessed by the investigator. Any adverse events have to be documented in detail as indicated on the E-CRF. The following information is required:

- The date and **time of onset** of any AEs
- The **duration** (the entire duration of an event or symptom, calculated from date of onset and date of end)
- The maximum **intensity** (mild, moderate or severe; for definitions, see below).
- The **drug relationship** of the AE to the investigational product (for definitions, see below)
- Any **study drug action taken** and any other **action taken** by the investigator to resolve the adverse events (entered in free text)
- The **outcome** of the adverse event (recovered completely, recovered with residual effects, continuing).
- An assessment of the **seriousness** of the event will be made by the investigator.

9.4.1 Intensity

Toxicities listed in the NCI CTCAE v4.0 are graded on a scale of 0 to 4. If a specific adverse event is not included in the toxicity scale, the investigator is to classify its intensity according to the following definitions:

Table 6. Intensity grading of AEs not described in NCI CTCAE v4.0

Intensity	Definition
Mild	The patient is aware of signs or symptoms, but they are easily tolerated. Usually does not require additional therapy or discontinuation of study treatment.
Moderate	The signs and symptoms are sufficient to restrict, but do not prevent usual activity; possibly requires additional therapy but usually does not require discontinuation of study treatment.
Severe	The patient is unable to perform usual activities and usually requires discontinuation of study treatment.

9.4.2 Treatment relationship

The investigator will be asked whether an AE is related to the administration of ^{166}Ho-PLLA-MS, or whether he is not capable to define a clear relationship to the study device. The investigator will also be asked whether an AE is related to inadvertent deposition of the microspheres in organs other than the liver, and should therefore be classified as technique related.

Table 7. categories of treatment relationship of AEs

Categories	Definition
None	The time course between administration of ^{166}Ho-PLLA-MS and occurrence or worsening of the adverse event rules out a causal relationship **and/or** another cause is confirmed and no indication of involvement of ^{166}Ho-PLLA-MS in the occurrence/worsening of the adverse event exists.
Unlikely	The time course between administration of ^{166}Ho-PLLA-MS and occurrence or worsening of the adverse event makes a causal relationship unlikely **and/or** the known effects of ^{166}Ho-PLLA-MS or of the substance class provide no indication of involvement in occurrence/worsening of the adverse event and another cause adequately explaining the adverse event is known **and/or** regarding the occurrence/worsening of the adverse event a plausible causal chain may be deduced from the known effects of ^{166}Ho-PLLA-MS or the substance class, but another cause is much more probable **and/or** another cause is confirmed and involvement of ^{166}Ho-PLLA-MS in the occurrence/worsening of the adverse event is unlikely.
Possible	Regarding the occurrence/worsening of the adverse event, a plausible causal chain may be deduced from the pharmacological properties of ^{166}Ho-PLLA-MS or the substance class, but another cause just as likely to be involved is also known **or** although the physical properties of ^{166}Ho-PLLA-MS or the substance class provide no indication of involvement in the occurrence/worsening of the adverse event, no other cause gives adequate explanation
Probable	The physical properties of ^{166}Ho-PLLA-MS or of the substance class **and/or** the course of the adverse event after dechallenge and, if applicable, after rechallenge **and/or** specific tests suggest involvement of ^{166}Ho-PLLA-MS in the occurrence/worsening of the adverse event, although another cause cannot be ruled out.
Definite	The physical properties of ^{166}Ho-PLLA-MS or of the substance class **and** the course of the adverse event after dechallenge and, if applicable, after rechallenge **and** specific tests indicate involvement of ^{166}Ho-PLLA-MS in the occurrence/worsening of the adverse event and no indication of other causes exists.
Unclassified [only used for SAE]	The available information is not sufficient for causality assessment.

9.5 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached, or until completion of study follow up period. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

10. STATISTICAL ANALYSIS

Two sets of study data will be evaluated: the Full Analysis Set (FAS) and the Per Protocol Set (PPS). The FAS is defined as the set of data generated from all included patients who received the therapy dose. The PPS is defined as the set of data generated from the included patients who complied with the protocol up to at least 3 months of follow-up. All analyses will be performed for the FAS. Analyses to assess tumour response and explore and understand the direct effects of therapy will be based on the PPS.

The primary analysis will be the evaluation of the number and percentage of patients with target lesions tumour response, according to the group sequential decision criteria as given in chapter 3. In addition, the estimated response percentage and appropriate 95% confidence interval will be presented. Further description in numbers and percentages with complete response, partial response, stable disease and progressive disease will be provided. For the tumour response decision, this analysis is primarily based on the PPS set as defined above. However, the analysis based on the FAS will also be performed. In that case, a conservative imputation on target lesions tumour response will be done for those patients that did not reach their 3 months of follow-up (e.g., if a patient died before 3 months or stopped because of disability reasons it will be classified as failure).

10.1 Secondary outcome parameters

Progression free survival (both overall and liver specific) and overall survival will be described using Kaplan Meier and cumulative incidence curves. These analyses will be based on the FAS.

10.2 Safety

Frequency counts and percentages of SAEs and toxicity events (CTC graded) will be presented. Further exploratory summaries and graphs of relevant safety (laboratory) parameters will be presented.

11. ETHICALCONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version 2008) (see Appendix I) and in accordance with the Medical Research Involving Human Patients Act (WMO), the requirements of International Conference on Harmonization - Good Clinical Practice (ICH-GCP) and this protocol. The protocol is submitted to the Independent Ethics Committee (IEC) and to the Radiation Protection Committee of the UMC Utrecht. The study will not start until written approval of the IEC of the UMC Utrecht has been received by the investigator.

11.1.1 Obligations of the investigator

An updated copy of the curriculum vitae of each investigator and co-investigator will be provided to the ethics committee. For the purpose of ensuring compliance with GCP and regulatory guidelines, Health Authorities may conduct a site audit or an inspection. By signing this protocol the investigator agrees to allow regulatory agencies to have direct access to the study records for review.

11.2 Recruitment and consent

Patients with dominant liver metastases with no standard therapeutic options available, such as chemotherapy and surgery, will be referred to the Principal Investigator (PI). The PI will inform every patient and obtain their informed consent.

11.2.1 Consent

Before enrolment into the study the PI will inform every patient, verbally and in writing (Appendix X) about the nature of the study, its purpose, procedures, expected duration and the benefits and risks involved in study participation. Each patient will be given the opportunity to ask questions and will be informed about the right to withdraw from the study at any time without prejudice. Patients must be given adequate opportunity (at least 48 hours) to read the information and enquire about details of the study before consent is given. Patients will have to voluntary sign and date a written informed consent statement before participation (Appendix X). The informed consent statement will be signed and dated by the study physician, delegated with this responsibility by the PI. The patient will receive a copy of the signed consent statement. This consent statement will include use of the acquired data for regulatory approval and product information and obtaining medical information (such as concomitant medication, additional treatment) from the patient's (primary) physician(s) or pharmacy.

11.3 Benefits and risks assessment, group relatedness

11.3.1 Benefits

It is anticipated that treatment with radioactive microspheres will reduce tumour size and will improve quality of life as known from literature from yttrium-90 [39-41] and as was observed in the HEPAR I trial. It is anticipated that the gamma emission of ^{166}Ho will improve the safety of the procedure. In addition, the difference in specific activity of ^{166}Ho-PLLA-MS compared to the currently available yttrium-90 may theoretically improve tumour response and accordingly, liver specific progression-free survival.

Participation in this study may possibly produce useful scientific data for the future. Regular medical check-ups during the study can be seen as an additional benefit. The maximum

number of visits (*n*=12) (depending on time to tumour progression) is comparable to a standard chemotherapy protocol. However, the scheduling is different.
There are up to 7 extra visits compared to treatment with ^{90}Y-MS, which is a routinely performed treatment in the UMC Utrecht.

11.3.2 Risks

Apart from the angiographic procedures and device related toxicity as described in chapter 7, standard radiological and nuclear medicine procedures are also used that may have their inherent side effects. For the frequent blood sampling and/or pre- and posthydration, an indwelling cannula may be used and this may be accompanied by mild bruising and also, in rare cases, by transient phlebitis. After initial irritation, the presence of an indwelling cannula is usually painless and hardly noticeable. The same applies to single vein punctures for blood sampling. A urethral catheter, if used, may also cause infection.
The total amount of blood withdrawn during the study will be up to 110 ml (normal blood donation: 500 ml).

11.4 Confidentiality

All investigators and other persons involved in the study agree to keep confidential any information pertaining to the patient's identity which becomes known to them in the course of the study.

11.5 Financing

This academic study is investigator driven; the investigators are employed solely by the UMC Utrecht, a conflict of interest will not arise.

11.6 Compensation for injury

Injury directly related to participation in this study will be covered by the existing insurance of the UMC Utrecht. Investigators and appropriate staff will be indemnified by the UMC Utrecht for liability for study induced injury.

11.7 Incentives

The patients will receive standard compensation for travelling expenses (€ 0.19/km) and costs for parking. Cost for parking will be compensated by a lump sum, to be received during the visit 3 months after treatment. The lump sum consists of a 'VVV-bon' of €50.

12. ADMINISTRATIVE ASPECTS AND PUBLICATION

12.1 Electronic Case Report Forms

Electronic Case Report Forms (E-CRFs) will be provided by the UMC Utrecht. The E-CRF will be completed by the Clinical Research Coordinator, Research Nurse and PhD student.

12.1.1 Completing E-CRFs

It is the responsibility of each investigator to ensure that the E-CRFs are correct and complete. All relevant questions must be answered and no empty data blocks should exist. However, in a situation where it is unavoidable that data cannot be entered, this should be indicated in the E-CRF by entering the following in the relevant field:

ND - not done
NK - not known
NA - not available (i.e. test done but result not available) or not applicable

The electronic signature at the end of the E-CRF by the principal investigator, investigator or authorized co-investigator will serve as confirmation that the information recorded is complete, accurate and has not been falsified. The electronic signature consists of a form on which the signing investigator

12.1.2 Corrections to E-CRFs

Errors, changes and/or additions entered on the original E-CRF must be corrected by replacing the incorrect entry by the correct entry. A record of all changes made (including: account name of person(s) who entered the entries, date, time, overwritten entries) will be saved in the background of the E-CRFs. These data are accessible only by the data manager of the department's trial office (Cees Haaring) and may not be edited.

12.2 Source document verification

For the purpose of this study, the 'source documents' are defined as the patient's written and digital hospital medical records, clinician notes, laboratory print outs, digital and hard copies of imaging, memos, electronic data, etc. The Clinical Research Coordinator will require direct access to the source documents in order to verify E-CRF entries.

As an absolute minimum, the source documents must include:

- A record that the patient has participated in a clinical trial, by study title, protocol number, patient identification code and date of entry in the study (e.g. by signed Informed Consent Form)
- Evidence that the patient satisfied all inclusion and exclusion criteria
- A record of the doses and dates of administration of the investigational drugs
- A record of the safety parameters
- A record of the uptake and excretion data
- A record of concomitant medication
- Digital imaging stored in the Picture Archiving and Communication System (PACS)
- A record of all adverse events

Entries in the patient's source documents must be signed and dated according to usual hospital procedure. After completion of the study, the completed patient files will be stored in the hospital archives and maintained for a minimum of 20 years.

12.3 Monitoring plan

The monitoring plan is described in appendix XI.

12.4 Amendments

Amendments are changes made to the clinical investigation plan after a favourable opinion by the METC of the UMC Utrecht has been obtained. All amendments will be notified to the METC that gave the favourable opinion.

12.5 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the METC once a year. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.6 End of study report

The investigator will notify the METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the METC, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.7 Publication policy

Any publication of the study results will be considered as a collaborative effort between the investigators and appropriate personnel. Authorship shall be determined by mutual consent.

12.8 CE marking

The clinical data that are collected in this study will be used by the Division Imaging of UMC Utrecht and its partners (UMC Utrecht Holding Participations) as part of its submission to the Notified Body to support the CE marking application and/or other registration procedures for ^{166}Ho-RE or ^{166}Ho-MS.

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