Protocol Number: ASC-Man-P016

Official Title: A multicentre, open-label study to evaluate the safety and diagnostic efficacy of mangoral in patients with known or suspected focal liver lesions and severe renal impairment.

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Study Protocol

A Multicentre, Open-label Study to Evaluate the Safety and Diagnostic Efficacy of Mangoral in Patients with Known or Suspected Focal Liver Lesions and Severe Renal Impairment

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PROTOCOL SYNOPSIS

Title:	A multicentre, open-label study to evaluate the safety and diagnostic efficacy of mangoral in patients with known or suspected focal liver lesions and severe renal impairment.
Trial no.:	ASC-Man-P016
Trial name:	SPARKLE
EudraCT no.:	2019-001599-12
IND no.:	102,043
Clinical phase:	Phase III
Investigational medicinal product:	Manganese (II) chloride tetrahydrate ($MnCl_2 \bullet 4H_2O$; working name: mangoral) powder for oral administration.
Study objective(s):	The overall objective of this study is to evaluate the safety and diagnostic efficacy of mangoral (equiv. to 800 mg $MnCl_2 \bullet 4H_2O$) in patients with known or suspected focal liver lesions and severe renal impairment. *Primary objective:* • To assess the diagnostic efficacy of mangoral in liver MRI in terms of visualisation of detected focal liver lesions in combined MRI (CMRI: combined mangoral-enhanced and unenhanced MRI) compared to unenhanced MRI Lesion visualisation will be determined by qualitative assessments of lesion border delineation and lesion contrast compared to background liver on 4-point scales for up to 15 lesions per patient *Secondary objectives*: • To assess the diagnostic efficacy of mangoral in liver MRI in terms of: o number of lesions detected by each MRI method (unenhanced MRI, mangoral-enhanced MRI, and CMRI) o visualisation of detected focal liver lesions in mangoral-enhanced MRI compared to unenhanced MRI (determination of visualisation will be done in the same way as for the primary efficacy variable) o confidence in lesion detection and localisation separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI o lesion dimensions (independent off-site readers' assessment): longest diameter of the largest and smallest lesion o quantitative assessments by measuring percent signal intensity enhancement of liver, liver-to-lesion

contrast, signal-to-noise ratio, and contrast-to-noise ratio of up to 5 lesions per patient

- number of patients who had at least one new lesion identified on CMRI compared to unenhanced MRI alone

- To assess a proportion of patients having at least one malignant lesion identified on post-mangoral images that was not identified on pre-mangoral images.
- To evaluate the safety and tolerability of mangoral
- To evaluate the pharmacokinetics of manganese after a single dose of mangoral in a subgroup of patients (including a small number of dialysis patients)
- To evaluate the impact of diagnostic performance of CMRI and mangoral-enhanced MRI versus unenhanced MRI on the patients' management

Design:	Multicentre, open-label, pivotal Phase III study to evaluate the safety and diagnostic efficacy of mangoral.
Treatment:	Single oral dose of mangoral (800 mg manganese chloride [II] tetrahydrate, 500 mg L-alanine, and 800 IU vitamin D3).
Population:	Adult male and female patients with severe renal impairment (Chronic Kidney Disease [CKD] or Acute Kidney Injury [AKI]) and who are being evaluated for known or suspected focal liver lesions. Approximately 80% of enrolled patients should have liver metastasis or other common focal liver lesions. The proportion enrolled HCC patients will be monitored jointly by the CRO/sponsor in order to achieve approximately 20% HCC patients in the overall study population. The CRO/sponsor may instruct individual sites, from time to time, to specifically and exclusively enrol patients with either non-HCC or HCC. PK subgroup: Pharmacokinetic (PK) evaluations will be performed in a subgroup of patients included in the study. This PK subgroup will consist of 12 patients, including 4 patients currently on maintenance haemodialysis. These patients will be selected from few sites that are able to include these patients and follow PK requirements. *Inclusion criteria*: 1. Patients who have been fully informed and have personally signed and dated the informed consent 2. Male and female patients 18 years and older 3. Patients who have known or suspected focal liver lesions based on medical history and previous laboratory and/or imaging examinations 4. Patients with severe renal impairment; a. Chronic kidney disease (CKD) (estimated glomerular filtration rate [eGFR]

< 30 mL/min/1.73 m^2) based on medical history and previous laboratory examinations, at least once within the last 3 months prior to the Baseline Visit

OR

b. Acute kidney injury (AKI) with an increase in serum creatinine ≥ 0.3 mg/dL within 48 hours or ≥ 50% within 7 days, prior to the Baseline Visit

5. Female patients who are not of childbearing potential, or female patients who are of childbearing potential but are using highly effective contraception (including hormonal contraceptives, such as combined oral contraceptives, patch, vaginal ring, injectables, and implants, intrauterine device, intrauterine hormone-releasing system, vasectomised partner, and tubal ligation) or practising sexual abstinence (only accepted if this is the usual and preferred lifestyle of the patient), and have a negative urine or serum pregnancy test within 24 hours prior to the administration of contrast agent
6. Sexually active male patients (who are not vasectomised) must be willing to use condoms after administration of mangoral until the last follow-up visit
7. Patients who agree to comply with all study procedures as outlined within the study protocol and informed consent
8. Not applicable

Exclusion criteria:

1. Patients with simple liver cysts only
2. Patients who have received any investigational drug or were treated with an investigational device within 6 weeks prior to the Baseline Visit
3. Patients who have received any MRI contrast media within 6 weeks prior to the Baseline Visit or are scheduled to receive any other contrast medium other than mangoral before the last study visit
4. Patients who have previously been enrolled in the study and underwent mangoral-enhanced MRI
5. Patients who are clinically unstable or have an acute illness, as judged by the investigator; i.e. the underlying clinical condition is such that clinically relevant changes in condition can be reasonably anticipated during the study period
6. Patients with severe hepatic impairment (according to Child-Pugh score C)
7. Not applicable
8. Patients scheduled for surgery before the last study visit
9. Pregnant or lactating women
10. Patients with a history of severe allergies (e.g. anaphylaxis), as judged by the investigator

11. Patients with a known hypersensitivity to the IMP or any excipients in the IMP
12. Patients who are, in the investigator's opinion, unable to comply with the study requirements including follow-up procedures
13. Patients with known portosystemic shunts
14. Patients with encephalopathy, as judged by the investigator
15. Patients with neurodegenerative disorders
16. Patients with acute neurological disorders, as judged by the investigator
17. Patients with haemochromatosis
18. Patients receiving blood transfusion within 14 days prior to the Baseline Visit
19. Patients who cannot fast for 8 hours
20. Patients who cannot undergo MRI due to MRI incompatible pacemaker, metal implants, or other MRI contraindications
21. Patients with known inherited disorders of manganese metabolism
22. Patients with known pheochromocytoma
23. Patients with history of surgical/other interventional procedures or conditions which may interfere with absorption or excretion of IMP (e.g. small bowel obstruction, gastric bypass, gastric resection, biliary obstruction), as judged by the investigator
24. Patients who have been institutionalised by official or court order and therefore are not completely free in their decision-making process
25. Patients dependent on the sponsor, investigators, personnel of the trial site or any other individual, whose willingness to volunteer may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation.

Methodology: The study consists of the following periods/visits:

- Screening period (day -28 to -1; assessments can be performed anytime during the 4-week period): informed consent, inclusion/exclusion criteria, patient characteristics, serum pregnancy test (female patients of childbearing potential)
- Baseline period (day -1 to day 0, i.e. within 24 hours prior to the administration of mangoral): safety assessments, neurological assessments, unenhanced MRI examination of the liver (baseline MRI), pre-dose blood samples for manganese measurements, pregnancy test (female patients of childbearing potential)

- Day of MRI (day 0): intake of mangoral after a fast of at least 4 hours and after unenhanced MRI (note: fasting must be continued until after the mangoral-enhanced liver MRI examination has been performed (ie 4 [± 1] hours after mangoral administration)
- Follow-up visits following contrast administration (24 [± 4] hours, 48 [± 4] hours, and 5 [±2] days post-dose): safety assessments, neurological assessment, blood samples for manganese concentrations.

  Note: *In case a patient is not able to return for the follow-up visits to the study site, these follow-up assessments may be performed at an off-site location (e.g. at the patient's home or at an associated off-site clinic by a trained examiner): Patients with long travelling distances may stay at a hotel near the study site at the expense of the sponsor. If further adjustments are required, Visit 4 (at 24 [± 4] hours) and Visit 5 (48 [± 4] hours) can be performed as a remote visits, at the discretion of the investigator.*

- A subgroup of patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI may also undergo an optional brain MRI 7 (+ 2) days after mangoral-enhanced MRI of the liver

PK subgroup: additional blood samples (6 mL per time point) will be drawn for the determination of PK parameters on the day of the liver MRI examination (day 0) at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-dose.

Primary diagnostic efficacy in terms of visualisation of detected lesions will be evaluated centrally at an imaging core laboratory by three independent readers who are experienced in liver imaging. A fourth reader who is not involved in efficacy reads will track and match the detected lesions on unenhanced, mangoral-enhanced and CMRI to confirm lesion numbering and lesion location across the modalities. Lesion tracking will also confirm lesions that are identified on pre-mangoral, post-mangoral and CMRI images. Study MRIs will also be evaluated by the on-site radiologists for the assessment of secondary objectives and for clinical purposes. Both on-site and independent radiologists will be trained prospectively in a standardised way.

Efficacy variables:

Primary efficacy variable:

The primary efficacy endpoint will be the visualisation of detected focal liver lesions in combined MRI (CMRI, mangoral-enhanced MRI plus unenhanced MRI) as compared to unenhanced MRI.

Visualisation will be measured by two co-primary variables:

- lesion border delineation

- lesion contrast compared to liver background

Both parameters will be determined for each lesion (up to 15 lesions per patient) and by each of the three independent readers by qualitative assessment on 4-point scales with categories 'poor' (1), 'partial/moderate' (2), 'good' (3), and 'excellent' (4) lesion border delineation/lesion contrast.

Based on the scores on the 4-point scales, two sum scores will be calculated for each patient and separately for each MRI method: a lesion border delineation sum score and a lesion contrast sum score. Only lesions detected at baseline (unenhanced MRI) will be included in the assessment of diagnostic efficacy.

Secondary efficacy variables:

- Number of lesions detected by each MRI method: unenhanced MRI, mangoral-enhanced MRI, and CMRI (on-site and independent off-site readers' assessments)
- Visualisation of focal liver lesions in mangoral-enhanced MRI as compared to unenhanced MRI (independent off-site readers' assessment; visualisation will be assessed as for the primary efficacy endpoint)
- Confidence in lesion detection separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (3-point scale, on-site and independent off-site readers' assessments of up to 15 lesions per patient)
- Confidence in lesion localisation separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (3-point scale, on-site and independent off-site readers' assessments of up to 15 lesions per patient)
- Lesion dimensions (independent off-site readers' assessment): longest diameter of the largest and the smallest lesion
- Quantitative assessments will be performed for up to 5 detected lesions (independent off-site readers' assessment). These lesions will be the same for pre- and post-contrast assessments:
    - liver signal intensity (SI) enhancement (%)
    - liver-to-lesion contrast (LLC)
    - signal-to-noise ratio (SNR)
    - contrast-to-noise ratio (CNR)
- Change(s) in patients' management based on the diagnostic performance of CMRI or mangoral-enhanced MRI versus unenhanced MRI:
    - Any changes in patient management based on MRI findings (yes/no)
    - Next steps in patient management based on MRI findings (i.e. chemotherapy, surgery, local ablation procedure, combination therapy, or other [specify])

	Subgroup analyses: The primary efficacy endpoint will be evaluated in appropriate subgroups which will be defined in the statistical analysis plan (e.g. subgroups by MRI scanner's magnetic field strength, lesion type, lesion diameter, age, and eGFR).
Safety variables:	Vital signs, physical examination, neurological assessment, electrocardiogram (ECG), clinical safety laboratory testing (haematology, biochemistry, urinalysis), blood manganese concentrations, monitoring of adverse events, and evaluation of brain MRI in a subgroup of patients.
Pharmacokinetic variables	The following PK variables will be determined: AUC (area under the curve) for the interval 0-120 hours, C_{max} (maximum or peak concentration), t_{max} (time to achieve maximum concentration), $t_{1/2}$ (terminal half-life) and Cl_{app} (total apparent clearance).
Statistical methods:	The two co-primary variables (patients' lesion border delineation and patients' lesion contrast) will be analysed for the full analysis population (i.e. all patients who received mangoral and for whom all unenhanced/enhanced MRI are assessable). The superiority of CMRI over unenhanced MRI regarding both co-primary variables (using the mean score differences between the paired sum scores weighted by the number of lesions determined per patient) will be evaluated for each independent reader. The study will be considered successful if the reading results of at least two of three independent readers demonstrate the superiority of CMRI versus unenhanced MRI for both lesion border delineation and lesion contrast. Hypotheses will be tested using a one-sided paired t-test. A comparison of unenhanced MRI and mangoral-enhanced MRI alone will be evaluated in the same way as the primary analysis. The secondary efficacy variables will be analysed in an exploratory manner using descriptive statistics (including confidence intervals). Safety and PK data will be analysed descriptively.
Number of patients:	Approximately 197 patients will be enrolled to ensure a sample size of 167 patients for the primary analysis at an overall study power of 80%, assuming 85% of the image sets being completely evaluable. To adjust for testing the simultaneous success of two co-primary variables, the power of each individual test will be set to 90%. The simultaneousness does not influence the type I error. Thus, the tests will be performed at a global 1-sided level of significance of 0.025.
Number of sites:	Approximately 60 sites.

FLOW-CHART

Screening

V1 (day -28 to -1)

Total enrolled: N = 197; PK subgroup: N = 12 (including 4 dialysis patients)
Signing of informed consent; Screening of potential participants based on inclusion and exclusion criteria. Documentation / assessments:

- Demographic characteristics
- General medical and surgical history
- Clinical signs and symptoms related to the patient's planned evaluation
- Clinical evaluation for Child-Pugh status (blood tests, hepatic encephalopathy)
- Prior and concomitant therapies (within 14 days pre-dose)
- Serum pregnancy test (female patients of childbearing potential only)

Baseline period

V2 to V3 (day -1 to day 0)[1]

Baseline documentation / assessments:

- Verification of inclusion / exclusion criteria
- Prior and concomitant therapies (within 14 days pre-dose) / pre-dose events
- Vital signs, physical examination, ECG (within 3 hours prior to dosing)
- Neurological assessments
- Clinical safety laboratory tests
- Serum or urine pregnancy test (female patients of childbearing potential only)
- Manganese concentration: blood sample within 1 hour pre-dose
- Unenhanced MRI of the liver

Day of IMP dosing

V3 (day 0)

Documentation / assessments:

- Intake of a single dose of mangoral (equiv. to 800 mg $MnCl_2 \cdot 4H_2O$)
- Investigational medicinal product accountability
- Mangoral-enhanced MRI of the liver 4 (± 1) hours post-dose
- Vital signs, physical examination, ECG: 6 (± 1) hours post-dose
- Clinical safety laboratory tests: 2 (± 0.5) and 6 (± 1) hours post-dose
- Only in the PK subgroup: blood sample for manganese concentration
- Changes in concomitant therapies / adverse events

Follow-up period

V4 (day 1)[2]

Assessments

- Vital signs, physical examination, ECG
- Neurological assessments
- Clinical safety laboratory tests
- Manganese concentration (blood sample)
- Changes in concomitant therapies / adverse events

V5 (day 2)[2]

Assessments:

- Vital signs, physical examination
- Neurological assessments
- Clinical safety laboratory tests
- Manganese concentration (blood sample)
- Changes in concomitant therapies / adverse events

V6 (day 5)

Assessments

- Vital signs, physical examination
- Neurological assessments
- Clinical safety laboratory tests
- Manganese concentration (blood sample)
- Changes in concomitant therapies / adverse events

V7 (day 7)[3]

Assessments

- MRI of the brain
- Changes in concomitant therapies / adverse events

[1] Visit 2 and 3 must be performed sequentially, but can occur on the same day.

[2] If the patient is not part of the PK subgroup (see section 6.4.2), Visit 4 and Visit 5 can be performed as remote visits at the discretion of the investigator

[3] V7 will be performed only in a subgroup of patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI and for whom previous brain MRI images are available.

SERIOUS ADVERSE EVENT REPORTING CONTACT DETAILS

All SAEs must be reported immediately (within 24 hours from the investigator's awareness) to:
E-mail: [redacted]

Please refer to Section 6.3.7.4 for more details.

Sponsor's Safety Responsible Person:

[redacted]
Email: [redacted]
Phone: [redacted]

ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse event
AKI	Acute Kidney Injury
ALAT	Alanine aminotransferase
ASAT	Aspartate aminotransferase
BD	Border delineation
BUN	(serum) Blood Urea Nitrogen
CKD	Chronic Kidney Disease
CMRI	Combined mangoral-enhanced and unenhanced MRI
CNR	Contrast-to-noise ratio
CRO	Contract research organisation
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DICOM	Digital Imaging and Communications in Medicine
DSMB	Data Safety Monitoring Board
DWI	Diffusion-weighted imaging
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
γ-GT	Gamma-glutamyltransferase
GBCA	Gadolinium-based contrast agent
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GRAS	'Generally Recognised As Safe'
HCC	Hepatocellular carcinoma
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
IDSMB	Independent Data Safety Monitoring Board
IEC	Independent ethics committee
IMP	Investigational medicinal product

INR	International normalised ratio
IRB	Institutional review board
IRC	Independent review charter
LC	Lesion contrast compared to liver background (qualitative)
LLC	Liver-to-lesion contrast (quantitative)
MDRD	Modification of Diet in Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
Mn	Manganese
$MnCl_2 \bullet 4H_2O$	Manganese (II) chloride tetrahydrate
MRI	Magnetic resonance imaging
N	Number of patients
NSF	Nephrogenic systemic fibrosis
PK	Pharmacokinetics
ROI	Region of interest
SAE	Serious adverse event
SD	Standard deviation
SI	Signal intensity
SNR	Signal-to-noise ratio
SOP	Standard operating procedure
SUN	Serum urea nitrogen
SUSAR	Suspected Unexpected Serious Adverse Reaction

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1 INTRODUCTION

1.1 Background

1.1.1 Focal liver lesions

The widespread use of imaging modalities has increased the detection rates of focal liver lesions, either incidentally in asymptomatic patients or in high-risk patients, such as patients with liver cirrhosis. These lesions can be either benign, including focal nodular hyperplasia, hepatocellular adenoma and hepatic cysts, or malignant.

Liver metastases are the most frequent type of malignant focal liver lesion. Although cancer can be hard to control when it has spread, some types of metastatic cancer can be cured, or the growth and burden of the metastases reduced or eliminated, leading to improved life expectancy and relieved symptoms if detected at an early stage and handled appropriately. This is especially relevant for liver metastases. The liver is the second most common organ for metastasis after the lymph nodes [1]. Many solid cancers originating e.g. from the lungs, breasts, colon, stomach or pancreas metastasise to the liver, and very often the liver is the first site of metastatic disease. If liver metastases from colorectal cancer are detected early and deemed eligible for surgical resection, the survival rate can be significantly improved, and sometimes full recovery is possible. The 5-year overall survival rate for patients undergoing resection for colorectal liver metastases has been reported to be 46% compared to only 6% for patients who were not subjected to surgical treatment of their liver metastases [2]. Surgical resection of liver metastases from non-colorectal primary tumours such as breast cancer has also been reported to lead to improved survival outcome [3, 4].

Hepatocellular carcinoma (HCC) is the most common primary malignant hepatic tumour and occurs mainly in patients with underlying chronic liver disease and cirrhosis. Time to recurrence is longer and 5-year survival rate is higher in patients with early HCC than in those with advanced stages [5]. Thus, early detection of HCC is crucial for successful treatment and reduction of mortality.

Factors affecting patient management include number, size and location of focal liver lesions. Cross-sectional imaging plays an essential role in both initial staging (i.e. determining the stage of the cancer), preoperative planning, monitoring of treatment effect and surveillance for recurrence of disease [6]. Contrast-enhanced computed tomography (CT) and contrast - enhanced magnetic resonance imaging (MRI) have shown higher sensitivity and specificity as compared to unenhanced CT / MRI for detecting and localizing focal liver lesions. However, in patients with severe renal impairment and focal liver lesions utilisation of both iodinated and gadolinium-based contrast agents is either restricted or contra-indicated. Oral mangoral is being developed to provide manganese-based contrast enhancement that has the potential to provide higher accuracy in lesion detection and visualisation as compared to unenhanced MRI.

1.1.2 Imaging of focal liver lesions

Several imaging modalities are available for the screening and evaluation of focal liver lesions, including liver metastases and HCC, such as CT, positron emission tomography combined with unenhanced CT, MRI, and ultrasound. Although the choice of imaging techniques may be based on the preference and expertise of the institution, contrast-enhanced CT and contrast-enhanced MRI are preferred techniques. Magnetic resonance is rapidly emerging as a preferred imaging option, especially for detection and characterisation of small liver lesions, due to its high accuracy resulting from optimal lesion-to-liver contrast and absent radiation exposure [7].

Contrast enhancement is an essential component of a liver MRI examination as it improves visualisation and delineation of focal liver lesions against normal liver parenchyma. Liver-specific imaging contrast agents offer greater lesion-to-liver contrast than conventional extracellular fluid space agents [8].

1.1.3 Manganese-based contrast agents for MRI

Manganese (Mn) is an essential trace element in humans, animals and plants and is found in nature in a wide variety of minerals, often in combination with iron. The manganese cation (Mn^{2+}) has a great potential for use in MRI contrast agents as it has powerful paramagnetic properties due to its 5 unpaired electrons. Similarly to gadolinium- and copper-containing contrast agents, manganese-based contrast agents increase the signal intensity of T1-weighted images [9, 10, 11]. Manganese is present in many intracellular organelles, especially in mitochondria, where it plays a role in protein synthesis. Thus, organs such as the liver consisting of cells with high mitochondrial content are predestinated targets for manganese-based contrast agents. Currently marketed liver MRI contrast agents are intravenous injections based on the lanthanide heavy metal gadolinium; however, there has been increasing safety concern with the use of gadolinium-based contrast agents (GBCAs), especially in patients with impaired renal function. Manganese based contrast agents offer an alternative to gadolinium [12]. A chelated form of manganese was used as the liver MRI contrast agent mangafodipir trisodium, which has been the only US Food and Drug Administration (FDA)-approved and marketed manganese-based contrast agent so far, but the agent was withdrawn from the US market in 2003 and from the European market in 2012. Mangafodipir trisodium was approved for intravenous injection. Following injection, the chelated manganese dissociates into the chelate and the manganese ion. The latter binds to serum proteins and is accumulated in several organs, including the liver, kidney and heart muscle. Adverse events (AEs) that were frequently reported in clinical studies of mangafodipir trisodium-included nausea, headache, pruritus, and sensations of heat and flushing; cardiovascular effects were also observed [9].

1.1.4 Mangoral

Mangoral is a manganese-based contrast agent for liver MRI developed for oral administration. It contains manganese (II) chloride tetrahydrate ($MnCl_2 \bullet 4H_2O$) as its active pharmaceutical ingredient. As manganese is poorly absorbed from the intestine, L-alanine and vitamin D3 are included in the formulation and function as absorption promoters to increase the uptake of manganese into hepatocytes in the liver [13]. As manganese accumulates in the normal hepatocytes but not in liver metastases, the hypointense lesions are clearly visible against the hyperintense liver parenchyma on MRI images.

The advantage of the oral route of administration of manganese is its portal venous transport to the liver and its fast uptake into hepatocytes resulting in a very low systemic availability of manganese. As mainly the enterohepatic circulation is involved, the risk for toxicities in the central nervous system, heart, or kidney is drastically reduced.

To date, mangoral has been studied in 6 completed clinical Phase I and II trials in healthy volunteers (N = 52, in which 2 received placebo) and patients with known liver metastases or suspected liver lesions (N = 75). An overview of these studies is given in Table 1. In addition, 77 patients received mangoral in a compassionate use program based on referral diagnoses that involved different types of focal liver lesions and diseases of the intestines (e.g. different types of primary cancers with liver metastases, hepatocellular carcinoma, hemangiomas,

jaundice, cholangitis, infections, cysts and other diseases) regardless of kidney function. All patients in the compassionate use program received 1600 mg mangoral.

Taken together, the diagnostic efficacy analyses of the conducted clinical studies showed improved diagnostic quality scores after administration of mangoral. Compared to unenhanced liver MRI, administration of mangoral resulted in increased relative signal intensity in the liver, and improved delineation and visualisation of liver lesions. Dose-dependency was observed for some of the efficacy variables assessed, with the 800 mg dose giving the best balance between safety and efficacy. In comparison to an intravenous gadobenate dimeglumine protocol, mangoral was comparable in the sensitivity to detect colorectal cancer liver metastases.

No safety concerns have been identified based on monitoring of AEs, clinical safety laboratory assessments, vital signs, electrocardiograms, and pharmacokinetic (PK) analyses in healthy volunteers and / or patients with liver metastases included in the clinical development program so far. Adverse drug reactions were mainly mild and transient gastrointestinal tract symptoms. In addition, blood concentrations of manganese were within the normal range at all four investigated doses. Thus, mangoral is expected to have a favourable safety profile compared to current intravenous and gadolinium-based MRI contrast media.

Table 1 Overview on previous Phase I and II studies on mangoral

Study	No. subjects	Study design	Mangoral ($MnCl_2 \bullet 4H_2O$ equiv.) dose	Key results
CMC-P001 [14]	Healthy subjects N=20 (2 received placebo)	Phase I, open-label, dose-rising	800 mg / 1600 mg	Data suggested that mangoral may be an effective MRI contrast agent
CMC-P002 [15]	Patients with liver metastases N=18	Phase II, open-label	1600 mg	Diagnostic quality scores improved after mangoral
CMC-P003 [16]	Patients with liver metastases N=20	Phase II, randomised, parallel group, open-label	800 mg / 1600 mg	Robust liver signal intensity enhancement; optimal diagnostic time window between 2 and 6 hours
CMC-P004 [17]	Patients with liver metastases N=20	Phase II, randomised, cross-over	1600 mg	Sensitivity to detect liver metastases was comparable between mangoral and gadobenate dimeglumine
CMC-P005 [not published]	Patients with liver metastases N=17	Phase II, randomised, parallel group, open-label	800 mg / 1600 mg	Improved delineation of focal liver lesions after mangoral
CMC-P010 [18]	Healthy subjects N=32	Phase II, randomised, double-blind, cross-over, dose-response	200 mg / 400 mg / 800 mg	Increase in liver-to-muscle signal intensity ratio and image quality superior after the 800 mg dose

Note: for a more detailed description of individual clinical study results and an overview on pre-clinical studies, refer to the current version of the Mangoral investigator's brochure [19], which will be provided to all investigators. Pharmacological and pharmaceutical aspects are also reviewed by Jørgensen et al [20].

1.2 Rationale of the study

Mangoral obtained Orphan Drug Designation by the FDA for use in diagnostic liver MRI in patients where the use of GBCAs may be medically inadvisable or where GBCAs cannot be administered. The currently used MRI contrast agents based on gadolinium have been associated with nephrogenic systemic fibrosis (NSF) in patients with severely impaired kidney function. Consequently, regulatory authorities have contraindicated or warned against the use of these contrast agents in patients with an estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2 [21]. Currently, there is no safe and effective contrast agent available for these patients to improve the detection of liver lesions in MRI examinations.

Due to the high first-pass of manganese in the liver upon oral administration, and the small fraction of manganese reaching the circulatory system, mangoral is expected to be a safe alternative to gadolinium-based MRI contrast agents or iodinated contrast agents used for computed tomography. Thus, mangoral may fill the unmet medical need for improved lesion detection in these patients.

This study is a Phase III study to investigate the diagnostic efficacy and safety of mangoral in patients with known or suspected focal liver lesions who also have severe renal insufficiency.

1.3 Risk-benefit assessment

Mangoral consists of manganese (II) chloride tetrahydrate, vitamin D3, and L-alanine. The components were evaluated by the FDA as GRAS ('Generally Recognised As Safe') substances (manganese: 21 CFR 184, 1446; vitamin D3: 21 CFR 184, 1950) or Direct Food Additive (L-alanine: 21 CFR 172, 320).

Reports of manganese intoxication have been associated with occupational exposure to airborne manganese, e.g. in miners, smelters, or welders, or environmental exposure through manganese-containing pesticides. Manganese toxicity mainly affects the central nervous system, resulting in symptoms similar to Parkinson's disease, but also cardiac, lung (upon inhalation of manganese), liver, reproductive and foetal toxicity have been observed [22].

A prerequisite for the potential exertion of toxic effects is the distribution of manganese via the systemic circulation and accumulation in tissue and organs. Serum manganese has been suggested as a reasonable biomarker for recent exposure to manganese [23].

Through the oral route of intake, the passage of manganese into the blood system is largely prevented, because manganese is very efficiently filtered out by the liver and subsequently excreted into the bile and finally the intestine [24]. This first-pass effect has been shown to be approximately 95%, leaving only trace amounts of manganese in the liver veins. Thus, approximately 5 % of the administered dose is absorbed by the small intestine. No correlations between manganese blood concentration and clinical chemistry including liver enzymes, after mangoral administration were observed [9].

The terminal plasma elimination of all manganese compounds in man is reported to be 5 to 11 hours [25]. The half-life for the elimination of injected Mn2+ ion is reported to be 10.1 ± 20.3 hours in healthy humans but 26.7 ± 19.0 hours in patients with hepatic impairment [26]. The biological half-life of manganese in the body is 37 days [27].

Pharmacokinetic results of previous clinical studies with mangoral showed that at the used oral doses of 800 mg and 1600 mg $MnCl_2 \bullet 4H_2O$ effects on blood manganese concentrations were relatively small (see investigator's brochure [19] for PK results of previous studies). No clinically relevant changes between pre- and post-contrast manganese blood concentrations or out-of-range values were observed [16, 18].

In these studies, both dose levels were found to be safe and not associated with serious adverse reactions. Overall, the frequency of AEs was higher with the higher dose level, but all events were of mild or moderate intensity. AEs that were assessed as being related to the administration of mangoral most frequently affected the gastrointestinal tract: the most common adverse reactions were diarrhoea (particularly at the higher dose level), nausea, and flatulence (refer to the investigator's brochure [19] for a summary of current safety data). Together with the evaluation of clinical safety laboratory parameters, vital signs, electrocardiogram (ECG) results, and PK analyses, these safety data support the conclusion that the administration of mangoral raises no safety concerns, especially at the dose level of 800 mg that will be used in this study. So far, clinical experience does not indicate potential hepatic, neurological or cardiovascular risks following the administration of the investigational medicinal product.

A risk for a hepatotoxic effect of mangoral is not foreseen, due to the fast elimination half-life, available preclinical data in dogs (section 4.2 of the IB [19]) and the absence of any clinical or laboratory data in humans suggesting such a risk. For further protection of the patients participating in this study, patients with severe hepatic impairment, i.e. those with a Child-Pugh score C (exclusion criterion 6) are excluded.

Efficacy results of the completed clinical studies demonstrate improved diagnostic quality of liver MRI after Mangoral enhancement and improved visualisation of liver and bowel. Individual patients eligible for inclusion into this study may benefit from their participation in this study in such a way that a more accurate diagnosis of their condition may have a positive impact on further treatment decisions regarding their cancer therapy. This research project may help to offer patients with severe renal impairment, who are in need for contrast-enhanced liver MRI, but cannot undergo gadolinium-enhanced MRI, a safe, efficacious, and convenient alternative to current contrast agents.

2 STUDY OBJECTIVES AND PURPOSE

The overall objective of this study is to evaluate the safety and diagnostic efficacy of mangoral in patients with known or suspected focal liver lesions and severe renal impairment.

2.1.1 Primary objective

- To assess the diagnostic efficacy of mangoral in liver MRI in terms of visualisation of detected focal liver lesions in combined MRI (CMRI: combined mangoral-enhanced and unenhanced MRI) compared to unenhanced MRI.

  Lesion visualisation will be determined by qualitative assessments of lesion border delineation and lesion contrast compared to background liver on 4-point scales for up to 15 lesions per patient.

2.1.2 Secondary objectives

- To assess the diagnostic efficacy of mangoral in liver MRI in terms of:
  - number of lesions detected by each MRI method (unenhanced MRI, mangoral-enhanced MRI, and CMRI)
  - visualisation of detected focal liver lesions in mangoral-enhanced MRI compared to unenhanced MRI (determination of visualisation will be done in the same way as for the primary efficacy variable)
  - confidence in lesion detection and localisation separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI
  - lesion dimensions (independent off-site readers' assessment): longest diameter of the largest and the smallest lesion
  - quantitative assessments by measuring percent signal intensity enhancement of liver, liver-to-lesion contrast, signal-to-noise ratio, and contrast-to-noise ratio of up to 5 lesions per patient
  - number of patients who had at least one new lesion identified on CMRI compared to unenhanced MRI alone
- To assess a proportion of patients having at least one malignant lesion identified on post-mangoral images that was not identified on pre-mangoral images.
- To evaluate the safety and tolerability of mangoral
- To evaluate the pharmacokinetics of manganese after a single dose of mangoral in a subgroup of patients (including a small number of dialysis patients)
- To evaluate the impact of diagnostic performance of CMRI and mangoral-enhanced MRI versus unenhanced MRI on the patients' management.

3 OVERALL STUDY DESIGN AND PLAN DESCRIPTION

This will be a multicentre, open-label, pivotal Phase III study to evaluate the safety and diagnostic efficacy of mangoral in patients with known or suspected focal liver lesions and concurrent severe renal impairment.

Approximately 197 patients will be enrolled at about 60 investigational sites in Europe, Asia, USA and South America. Patients must fulfil all of the inclusion criteria and none of the exclusion criteria (Section 4 for inclusion and exclusion criteria).

An overview on the schedule of assessments is provided in Section 7.1. After signing the informed consent, patients will undergo a screening examination within 4 weeks prior to the investigational medicinal product administration to verify the patients' eligibility, to document patient characteristics, including medical and surgical history, and to determine the Child-Pugh score to assess the patients' hepatic impairment status. A serum pregnancy test will be performed in women of childbearing potential at the local laboratory at screening (Section 6.3.5.4).

Within 24 hours prior to the administration of mangoral, patients will undergo safety assessments, including clinical safety laboratory tests, electrocardiogram (ECG), vital signs, and physical examination, and neurological assessments. A urine or serum pregnancy test will be performed in women of childbearing potential at the local laboratory (Section 6.3.5.4). Prior and concomitant therapies (taken within 14 days pre-dose) will be recorded in the electronic case report form (eCRF) (Section 5.2).

On the day of contrast administration (day 0), patients will undergo an unenhanced MRI examination of the liver prior to the intake of mangoral (baseline MRI; unenhanced MRI can also be done on day -1, i.e. within 24 hours prior to contrast administration). Blood samples for measurement of blood manganese concentrations will be drawn immediately prior to dosing. Each enrolled patient will receive a single dose of orally administered mangoral after a fast of at least 4 hours (Section 5.1). A mangoral-enhanced liver MRI examination will be performed 4 (± 1) hours after the mangoral administration. Patients must continue to fast until after the mangoral-enhanced liver MRI examination has been performed.

Follow-up evaluations (FU 1, FU 2, FU 3) will be performed on 3 visits following contrast administration, at 24 (± 4) hours, 48 (± 4) hours, and 5 (± 2) days post-dose, for safety assessments including clinical safety laboratory tests, measurement of blood manganese, ECG, vital signs, physical examination, including neurological assessments. Changes in concomitant therapies will be recorded. Follow-up evaluations at 24 (± 4) hours and 48 (± 4) hours can at the discretion of the investigator be performed as remote visits when required to ease the burden for the patient. Participants in the PK subgroup are required to have all assessments including blood samples as scheduled.

An additional follow-up visit (FU 4) for the optional brain MRI may be performed 7 (+ 2) days after mangoral-enhanced MRI of the liver only in those patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI and for whom previous brain MRI images are available for comparison (Section 6.2.4.1.2).

In a subgroup of patients, additional blood samples (6 mL per time point) will be drawn for the determination of pharmacokinetic parameters on the day of the liver MRI examination (day 0) at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-dose (Section 6.4). This PK subgroup will consist of 12 patients, including 4 patients currently on maintenance haemodialysis.

Each unenhanced and each mangoral-enhanced liver MRI examination will consist of axial T1- and T2-weighted image sequences and a diffusion-weighted imaging (DWI) sequence. Details of MRI acquisition parameters are described in Section 6.2.4.1.1.

Primary diagnostic efficacy in terms of visualisation of detected focal liver lesions will be evaluated centrally at an imaging core laboratory by 3 independent readers (Section 6.2.5). Reading will be performed in 3 parts (Part I: unenhanced MRI alone; Part II: combined MRI, i.e. paired reading of both unenhanced and mangoral-enhanced images; Part III: mangoral-enhanced MRI alone). There will be a minimum 2-weeks gap between the reads to reduce recall bias. All independent central readers will be blinded to clinical data, site, and country information.

The site investigators will also evaluate unenhanced and mangoral-enhanced MRI images (on-site read). Their assessments will be used for the analysis of secondary variables.

Both on-site and independent central (off-site) readers will be prospectively trained utilizing standardised methodology. The details of site training, image collection, anonymisation, read methodology and read platform will be described in an imaging manual and/or independent review charter (IRC) developed by a centralised imaging core laboratory.

3.1 Discussion of study design

Mangoral is currently being developed for visualisation of focal liver lesions, including liver metastases, in patients where the use of GBCAs may be medically inadvisable or cannot be administered. Patients with severe renal impairment are at risk of experiencing serious adverse effects if they are given any of the intravenously administered contrast agents currently available on the market for liver MRI enhancement. NSF is a rare, but potentially fatal, complication of GBCAs that has been reported in patients with renal failure [21].

Previous clinical Phase I and II studies included healthy volunteers and patients with liver metastases. This Phase III study will be performed to assess the safety and diagnostic efficacy of mangoral in a patient cohort with severe renal impairment (eGFR < 30 mL/min/1.73 m^2, or increase in serum creatinine ≥ 0.3 mg/dL within 48 hours or ≥ 50% within 7 days) and who are being evaluated for known or suspected focal liver lesions. In addition, as patients with severe renal impairment are likely to need dialysis, patients on haemodialysis or peritoneal dialysis will be included in this study to obtain information on safety, pharmacokinetics, and diagnostic efficacy of mangoral in such patients.

A multicentre and multinational design is used to increase representativeness and to reduce bias.

Results of a previous clinical Phase II study demonstrated that signal intensity enhancement increased in liver parenchyma until 3 hours post-dose and there was no significant difference in the liver-to-lesion contrast between 3 and 6 hours after mangoral administration [16]. Thus, the primary time point for mangoral-enhanced liver MRI chosen in this study is 4 hours after intake with a time window of ± 1 hour.

4 SELECTION OF STUDY POPULATION

Approximately 197 patients with severe renal impairment (Chronic Kidney Disease [CKD] or Acute Kidney Injury [AKI]) who are being evaluated for known or suspected focal liver lesions will be enrolled. Patients will be recruited among in- and outpatients at approximately 60 radiological sites.

The proportion enrolled HCC patients will be monitored jointly by the CRO/sponsor in order to achieve approximately 20% HCC patients in the overall study population. The CRO/sponsor may instruct individual sites, from time to time, to specifically and exclusively enrol patients with either non-HCC or HCC.

It is intended that potential patients for this study will be identified from a group of patients currently attending, or being referred to the study site for the diagnosis and treatment of known or suspected focal liver lesions. Potentially eligible patients will be approached by the investigator to ascertain whether they would be interested in participating in the study.

Competitive patient recruitment between countries and sites will be used during the entire recruitment period to ensure that the required number of patients are enrolled within the planned recruitment period.

4.1 Inclusion criteria

For inclusion in this study, the patients must fulfil all of the following criteria:

1. Patients who have been fully informed and have personally signed and dated the informed consent
2. Male and female patients 18 years and older
3. Patients who have known or suspected focal liver lesions based on medical history and previous laboratory and/or imaging examinations
4. Patients with severe renal impairment presented as:
   a) Chronic kidney disease [CKD] (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m^2) based on medical history and previous laboratory examinations, at least once, within the last 3 months prior to the Baseline Visit

      OR

   b) Acute kidney injury [AKI]) with an increase in serum creatinine ≥ 0.3 mg/dL within 48 hours or ≥ 50% within 7 days, prior to the Baseline Visit
5. Female patients who are not of childbearing potential, or female patients who are of childbearing potential but are using highly effective contraception (including hormonal contraceptives, such as combined oral contraceptives, patch, vaginal ring, injectables, and implants, intrauterine device, intrauterine hormone-releasing system, vasectomised partner, and tubal ligation) or practising sexual abstinence (only accepted if this is the usual and preferred lifestyle of the patient), and have a negative urine or serum pregnancy test within 24 hours prior to administration of contrast agent
6. Sexually active male patients (who are not vasectomised) must be willing to use condoms after administration of mangoral until the last follow-up visit
7. Patients who agree to comply with all study procedures as outlined within the study protocol and informed consent

8. Not applicable

4.2 Exclusion criteria

Patients will not be included in the study if any of the following criteria are met:

1. Patients with simple liver cysts only
2. Patients who have received any investigational drug or were treated with an investigational device within 6 weeks prior to the Baseline Visit
3. Patients who have received any MRI contrast media within 6 weeks prior to the Baseline Visit or are scheduled to receive any other contrast medium other than mangoral before last study visit
4. Patients who have previously been enrolled in the study and underwent mangoral-enhanced MRI
5. Patients who are clinically unstable or have an acute illness, as judged by the investigator; i.e. the underlying clinical condition is such that clinically relevant changes in condition can be reasonably anticipated during the study period
6. Patients with severe hepatic impairment (according to Child-Pugh score C)
7. Not applicable
8. Patients scheduled for surgery before the last study visit
9. Pregnant or lactating women
10. Patients with a history of severe allergies (e.g. anaphylaxis), as judged by the investigator.
11. Patients with a known hypersensitivity to the IMP or any excipients in the IMP
12. Patients who are, in the investigator's opinion, unable to comply with the study requirements including follow-up procedures
13. Patients with known portosystemic shunts
14. Patients with encephalopathy, as judged by the investigator
15. Patients with neurodegenerative disorders
16. Patients with acute neurological disorders, as judged by the investigator
17. Patients with haemochromatosis
18. Patients receiving blood transfusion within 14 days prior to the Baseline Visit
19. Patients who cannot fast for 8 hours
20. Patients who cannot undergo MRI due to MRI incompatible pacemaker, metal implants, or other MRI contraindications
21. Patients with known inherited disorders of manganese metabolism
22. Patients with known pheochromocytoma
23. Patients with a history of surgical/other interventional procedures or conditions which may interfere with the absorption or excretion of the IMP (e.g. small bowel obstruction, gastric bypass, gastric resection, biliary obstruction) as judged by the investigator

24. Patients who have been institutionalised by official or court order and therefore are not completely free in their decision-making process
25. Patients dependent on the sponsor, investigators, personnel of the trial site or any other individual, whose willingness to volunteer may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation

4.3 Withdrawal criteria

Premature study termination for an individual patient should be considered under the following circumstances:

- The patient has an AE that may jeopardise the patient's safety (e.g. allergic reaction to IMP)
- Non-compliance (e.g. break of required fast before the examination)
- Lost to follow-up (the patient did not show up for the examination and study personnel were unable to contact the patient)

Decisions concerning the premature study termination of individual patients will be made in agreement with the sponsor.

The trial must be terminated for an individual patient if the patient withdraws his / her consent. Patients may decide to withdraw their consent to participate at any time and for any reason without prejudice to their further medical care. The investigator should make a reasonable effort to ascertain the reason(s) for the patient's withdrawal while fully respecting the patient's rights.

The reason(s) for premature termination are to be recorded on the eCRF. For all patients who are prematurely withdrawn, all observations and test results available at the time of withdrawal are to be recorded in the eCRF.

Patients who terminate the study prematurely will not be replaced.

For criteria concerning the termination of the whole study please refer to Section 7.4.2.

5 TRIAL TREATMENTS

5.1 Investigational medicinal product(s)

5.1.1 Administration

The Investigational medicinal product is mangoral combined with L-alanine and vitamin D3. Mangoral is intended for diagnostic use and single-dose administration only.

All patients will receive a single dose of IMP (equivalent to 800 mg $MnCl_2 \bullet 4H_2O$) orally after a fast of at least 4 hours. Patients must continue to fast until after the mangoral-enhanced liver MRI examination has been performed (i.e. 4 [± 1] hours after IMP administration). Patients are allowed to drink water during the fasting period.

Note: please inform and consult with the sponsor or designee in case administered IMP has not been fully absorbed by the patient (i.e. if the patient vomited after IMP intake and prior to the MRI examination).

The IMP is dispensed in two stick packs, one containing the active pharmaceutical ingredient and the other containing the two absorption promoters. The contents in both stick packs (Section 5.1.2) should be dissolved together in 200 mL of water, preferably cold, and ingested as an oral solution within 2 hours after dissolution. The patient should drink the solution slowly within 10 minutes.

5.1.2 Identity of the investigational medicinal product

[redacted]

[redacted] physio-chemical properties of the active ingredient $MnCl_2 \bullet 4H_2O$ are summarised in [redacted] Table 3[redacted].

Table 3 Physio-chemical properties of manganese (II) chloride tetrahydrate

Solubility:	Soluble in water (1980 g/L [20°C]) and alcohol
pH:	A 5% solution has a pH between 3.5 and 6.0
Melting point:	+58°C
Density:	2.01 g/cm^3
Hygroscopicity:	Hygroscopic

5.1.3 Method of assigning patients to treatment groups

Not applicable; all patients will receive the same dose the IMP.

5.1.4 Selection of doses in the study

Results from a previous Phase I study have shown that relative signal intensity (SI) of the liver on T1-weighted images was significantly increased at both dose levels of 800 mg and 1600 mg $MnCl_2 \bullet 4H_2O$ [14]. Similar relative SI enhancement after administration of the 800 mg or 1600 mg dose was seen in a Phase II study in patients with liver metastases [16]. Another Phase II study showed that a dose of 800 mg $MnCl_2 \bullet 4H_2O$ resulted in a significantly higher increase in liver-to-muscle SI and a superior overall image quality compared to lower doses of 400 mg and 200 mg $MnCl_2 \bullet 4H_2O$, respectively [18].

Safety experiences in previous clinical Phase I and Phase II studies showed that the number of AEs, especially of gastrointestinal tract reactions, tended to be higher in the 1600 mg dose group than in the 800 mg dose group. Thus, based on the results of the dose comparisons and the observed better tolerability of 800 mg versus 1600 mg $MnCl_2 \bullet 4H_2O$, the 800 mg dose was chosen for this pivotal Phase III study.

5.1.5 Selection and timing of dose for each patient

The IMP will be administered 4 (± 1) hours prior to mangoral-enhanced MRI of the liver. The timing of dose was chosen based on the results of a previous Phase II study showing that the optimal diagnostic time window with mangoral is between 2 and 6 hours [16].

Patients must have been fasting for at least 4 hours prior to the administration of IMP.

5.1.6 Supply, manufacturing, packaging and labelling

The IMP stick packs for oral administration will be provided under the responsibility of the sponsor.

Each study site will be supplied with two stick packs for each patient (one stick pack 1 and one stick pack 2) for use within this study to match the expected number of patients to be enrolled at a given site.

The IMP will be manufactured, packed, labelled, and released (by a Qualified Person) and distributed under the responsibility of the sponsor in accordance with the principle of Good Manufacturing Practice (GMP) and the applicable national and/or local regulatory requirements. The labels will be available in local languages.

5.1.7 Storage

The IMP (stick pack 1 and stick pack 2) is to be kept at the study site at room temperature (15 – 25°C) in a place with authorised access only.

5.1.8 Shipment

The IMP will be shipped according to local regulations. The responsible investigator / responsible study personnel at the study site will confirm correct receipt of the IMP in writing and ensure it is stored safely and correctly. The investigator will document the distribution date and the amount used on the forms provided for this purpose.

5.1.9 Blinding and randomisation

Not applicable as this is an open-label study with regard to the administration of IMP.

5.1.10 Investigational medicinal product compliance and accountability

The oral solution of the IMP, containing the content of both stick packs, will be prepared by the investigator or by study personnel assigned by the investigator at the study site and handed to the patient. Date and time of IMP administration will be recorded in the eCRF.

The investigator will ensure that the IMP provided will be used only within the framework of this study and as directed in the study protocol.

IMP accountability must be recorded at each site and will be checked by a study monitor. Upon the completion of the study, all IMPs will be reconciled and destroyed locally or returned to sponsor or designee according to local regulations and site standard operating procedures. Receipt, distribution and destruction of the IMP must be recorded according to the study-specific instructions provided separately.

5.1.11 Management of investigational medicinal product overdose

Accidental overdosing of IMP is unlikely as the oral solution will be prepared by qualified personnel at the study sites. In case of accidental overdosing, the patient should be closely

monitored and treated, if necessary, at the discretion of the investigator. A dose of 1600 mg $MnCl_2 \bullet 4H_2O$, which is double the dose used in this trial, has been well-tolerated in previous clinical Phase I and II studies. Cases of overdose must be reported as adverse events (Section 6.3.7). If an overdose is associated with a serious adverse reaction, this reaction is subject to expedited reporting, i.e. within 24 hours (Section 6.3.7.4).

5.2 Prior and concomitant therapy

Any medication taken within 14 days prior to the administration of IMP will be recorded in the eCRF, regardless of whether the treatment will be continued during the study. Any changes in concomitant medication or the addition of a new treatment during the study will be recorded. This also includes any dietary supplements and hormonal contraception. The following details must be given: nature of the disease (indication), name of the medicine (including active ingredients) or specification of measures, dosage, unit, route of administration, and the start and end dates of treatment.

5.2.1 Prohibited or prohibited with restrictions

Several dietary components, such as iron [28], magnesium, calcium, vitamin C, and vitamin D3 [20], and medications, such as tetracycline or tetracycline derivatives [20], may negatively or positively influence the absorption of manganese from the intestine. Therefore, the use of the following substances is prohibited during this study from 6 hours prior to IMP administration until after the mangoral-enhanced MRI:

- Dietary iron supplementation, including multivitamin that contains iron, may interfere with manganese absorption through competition for the same transporters
- Supplemental magnesium and/or supplemental calcium may reduce manganese absorption
- Supplemental vitamin C or vitamin D3, which promote manganese absorption
- Magnesium-containing antacids and laxatives
- Quinolone and tetracycline antibiotics

5.2.2 Permitted oral intake

The use of following is allowed:

- Glucose or juice intake (e.g. orange juice) for the purpose to prevent or treat hypoglycaemia in patients with diabetes mellitus type I or II

6 VARIABLES AND METHODS

6.1 Population Characteristics

6.1.1 Demographics

The following demographic characteristics will be recorded in the eCRF:

- Year of birth
- Sex

- Ethnicity and race (only if the collection of such data is permitted by local regulations)
- Weight and height

6.1.2 Medical history

6.1.2.1 General medical history

General medical history findings considered relevant to the study by the investigator will be recorded in the eCRF together with the respective dates and periods (whether the condition is a past disease/illness or still ongoing at study entry). Relevant findings include, but may not be limited to, major surgeries, heart diseases, respiratory diseases, central nervous system and neurological diseases, psychiatric disorders, blood disorders, hepatorenal disorders, genitourinary disorders, and known allergies.

In addition, it will be recorded whether or not the patient underwent recent brain MRI. If yes, the date and the clinical reason for the examination will be recorded. In case that at the day of the mangoral-enhanced liver MRI the date of the brain MRI lays within the last 6 months and previous brain MRI images are available, the patient may consent to undergo a brain MRI 7 (+ 2) days after the mangoral-enhanced liver MRI.

6.1.2.2 Referral diagnosis

Details of the underlying condition related to the evaluation of known or suspected focal liver lesions will be recorded in the eCRF, including:

- Diagnosis
- Diagnosis of underlying primary malignancy, if applicable
- Date of first diagnosis
- Presence of metastases (body region)
- Clinical signs and symptoms

6.1.2.3 Child-Pugh score

The Child-Pugh score [29, 30, 31, 32] will be determined during the screening period to assess hepatic impairment of the patient.

The score comprises 5 measures of liver disease, which will each be scored 1, 2, or 3, depending on the severity as shown in Table 4. The sum of the scores provides the Child-Pugh score (class A, B, or C).

Table 4 Child-Pugh scoring system

Measure	Points scored for observed findings		
	1	2	3
Encephalopathy grade[a]	0	1 or 2	3 or 4
Ascites	Absent	Slight	Moderate
Serum bilirubin (mg/dL)	< 2.0	2.0 to 3.0	> 3.0
Serum albumin (g/dL)	> 3.5	2.8 to 3.5	< 2.8
International normalised ratio (INR)	< 1.7	1.7 to 2.3	> 2.3

[a]Grade 0: normal consciousness, personality, neurological examination, electroencephalogram
Grade 1: restless, sleep disturbed, irritable/agitated, tremor, impaired handwriting, 5 cps waves
Grade 2: lethargic, time-disoriented, inappropriate, asterixis, ataxia, slow triphasic waves
Grade 3: somnolent, stuporous, place-disoriented, hyperactive reflexes, rigidity, slower waves
Grade 4: unrousable coma, no personality/behaviour, decerebrate, slow 2-3 cps delta activity

Interpretation of the Child-Pugh score (cirrhotic liver disease):

5 to 6 points: Class A, indicating a well-functioning liver

7 to 9 points: Class B, indicating significant functional compromise

10 to 15 points: Class C, indicating decompensation of the liver

Note: patients who fall into Class C must be excluded from study participation; also, patients who fall into Class A or B but with encephalopathy grade 1 or higher must be excluded (exclusion criteria no. 6 and 14, Section 4.2).

6.1.2.4 Estimated glomerular filtration rate (eGFR)

For the verification of inclusion criterion no. 4, serum creatinine measurement will be used for the estimation of the GFR. eGFR will be determined according to the Modification of Diet in Renal Disease (MDRD) formula with 6 variables as detailed below [33].

$$eGFR = 170 \times (Scr)^{-0.999} \times (Age)^{-0.176} \times (GNF) \times (ETF) \times (SUN)^{-0.170} \times (Alb)^{0.318}$$

where:

- eGFR = estimated glomerular filtration rate (mL/min/1.73 m^2)
- Scr = standardised serum creatinine (mg/dL)
- Age = patient age (years)
- GNF = gender factor (male = 1.0; female = 0.762)
- ETF = ethnicity factor (white, non-black = 1.0; black race = 1.18)
- SUN (BUN) = serum urea nitrogen concentration (mg/dL)
- ALB = serum albumin (g/dL)

6.2 Diagnostic efficacy

6.2.1 Definition of liver segments

Independent readers and on-site radiologists will assess focal liver lesions on images from unenhanced and mangoral-enhanced MRI in 8 anatomical segments of the liver according to the Couinaud classification [34, 35], which divides the liver into the following 8 segments (Figure 1):

Segment I: caudate lobe, situated posteriorly around the inferior vena cava

Segment II: left lateral superior segment, situated to the left of the left hepatic vein and falciform ligament superior to the portal plane

Segment III: left lateral inferior segment, situated to the left of the left hepatic vein and falciform ligament inferior to the portal plane

Segment IV: left medial segment, situated between the left and middle hepatic veins, subdivided into IVa (superior) and IVb (inferior) subsegments

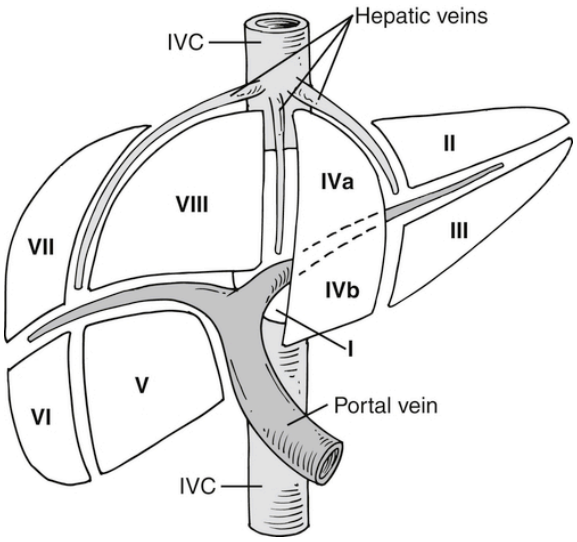
Segment V: right anterior inferior segment, situated below the portal plane between the middle and right hepatic veins

Segment VI: right posterior inferior segment, situated below the portal plane to the right of the right hepatic vein

Segment VII: right posterior superior segment, situated above the portal plane to the right of the right hepatic vein

Segment VIII: right anterior superior segment, situated above the portal plane between the middle and right hepatic veins

Figure 1 Schematic representation of liver segments according to Couinaud
IVC = inferior vena cava (image taken from: https://clinicalgate.com/liver-3/)



6.2.2 Primary and secondary efficacy variables

6.2.2.1 Primary efficacy variable

The primary efficacy endpoint will be the visualisation of detected focal liver lesions in combined MRI (CMRI, mangoral-enhanced MRI plus unenhanced MRI) as compared to unenhanced MRI.

Visualisation will be measured by two co-primary variables:

- lesion border delineation
- lesion contrast compared to liver background

Both parameters will be determined for each lesion (up to 15 lesions per patient) and by each of the three independent readers by qualitative assessment on 4-point scales with categories 'poor' (1), 'partial/moderate' (2), 'good' (3), and 'excellent' (4) lesion border delineation/lesion contrast (Section 6.2.3.1). Based on the scores on the 4-point scales, two sum scores will be calculated for each patient and separately for each MRI method: a lesion border delineation sum score and a lesion contrast sum score. Both scores will be weighted by the number of lesions per patient. Only lesions detected at baseline (unenhanced MRI) will be included in the assessment of diagnostic efficacy.

Superiority of CMRI over unenhanced MRI regarding visualisation of focal liver lesions will be analysed by testing the superiority of each of the two co-primary variables separately for each of the three independent readers. Reader success will be achieved if the reading results of a reader demonstrate superiority of CMRI versus unenhanced MRI for both lesion border delineation and lesion contrast. For the success of the trial, two of three readers must achieve reader success.

6.2.2.2 Secondary efficacy variables

The secondary efficacy variables will be based on the evaluations by independent readers (off-site readers' assessment) and by the on-site radiologist (on-site readers' assessment):

- Number of lesions detected by each MRI method: unenhanced MRI, mangoral-enhanced MRI, and CMRI (on-site and independent off-site readers' assessments)
- Visualisation of focal liver lesions in mangoral-enhanced MRI as compared to unenhanced MRI (independent off-site readers' assessment; visualisation will be assessed as for the primary efficacy endpoint)
- Confidence in lesion detection separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (3-point scale, on-site and independent off-site readers' assessments of up to 15 lesions per patient)
- Confidence in lesion localisation separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (3-point scale, independent on-site and off-site readers' assessments of up to 15 lesions per patient)
- Lesion dimensions (independent off-site readers' assessment): longest diameter of the largest and the smallest lesion
- Quantitative assessments will be performed for up to 5 detected lesions (independent off-site readers' assessment). These lesions will be the same for pre- and post-contrast assessments:
    - liver signal intensity (SI) enhancement (%)

- liver-to-lesion contrast (LLC)
- signal-to-noise ratio (SNR)
- contrast-to-noise ratio (CNR)

- Change(s) in patients' management based on the diagnostic performance of CMRI or mangoral-enhanced MRI versus unenhanced MRI (on-site and independent off-site readers' assessments):
  - Any changes in patient management based on MRI findings (yes/no);
  - Next steps in patient management based on MRI findings (i.e. chemotherapy, surgery, local ablation procedure, combination therapy, or other [specify]).

An overview of the primary and secondary efficacy variables by off- and/or on-site assessment of MRI images) is provided in Table 5. For details on deriving the efficacy variables Section 6.2.3.

Table 5 Overview of efficacy variables

Assessment	**Off-site (central)**			**On-site**
Reader(s)	Three (3) independent readers			Investigator (on-site radiologist)
Reading session (Section 6.2.5)[a]	Part I	Part II	Part III	
Primary variable[b]: Visualisation of detected focal liver lesions in combined MRI (CMRI, mangoral-enhanced MRI plus unenhanced MRI) as compared to unenhanced MRI (Section 6.2.3.1).	X	X		
Secondary variables[b]:				
- Number of lesions detected by each MRI method: unenhanced MRI, mangoral-enhanced MRI, and CMRI.	X	X	X	X
- Visualisation of focal liver lesions in mangoral-enhanced MRI as compared to unenhanced MRI (Section 6.2.3.1).	X		X	
- Confidence in lesion detection separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (Section 6.2.3.2).	X	X	X	X
- Confidence in lesion localisation separately in unenhanced MRI, mangoral-enhanced MRI, and CMRI (Section 6.2.3.3).	X	X	X	X
- Lesion dimensions (Section 6.2.3.4).	X		X	
- Quantitative assessments (Section 6.2.3.5).		X		
- Change(s) in patients' management based on the diagnostic performance of CMRI or mangoral-enhanced MRI vs. unenhanced MRI.	X	X	X	X

[a]Part I: unenhanced MRI; part II: combined CMRI; part III: mangoral-enhanced MRI

[b] Any simple liver cysts are excluded from primary and secondary variable assessments.

6.2.3 Derivation of efficacy variables

6.2.3.1 Visualisation of focal liver lesions

Visualisation of focal liver lesions will be measured by the two variables 'lesion border delineation' and 'lesion contrast compared to liver background'. Both variables will be determined by qualitative assessment on the following 4-point scales for up to 15 lesions per patient (best-visualised lesions, including the smallest and the largest detected lesions):

Lesion border delineation:

1 =	Poor:	lesion border is poorly distinct, the lesion cannot be separated from surrounding tissues or structures
2 =	Partial:	delineation of the lesion border is fair, but not complete; the lesion cannot be clearly separated from surrounding tissues or structures
3 =	Good:	delineation of the lesion border is complete; the lesion is adequately separated from surrounding tissues or structures
4 =	Excellent:	lesion border is sharply and clearly distinct, the lesion is sharply separated from surrounding tissues or structures

Lesion contrast:

1 =	Poor:	the difference in signal intensity between the lesion and the surrounding normal liver tissue is poor; the lesion can barely be identified and it is not possible to evaluate and measure the size (maximum diameter) of the lesion
2 =	Partial:	the difference in signal intensity between the lesion and the surrounding normal liver tissue is fair; the lesion can be identified, but it is not possible to evaluate and measure the size (maximum diameter) of the lesion
3 =	Good:	the difference in signal intensity between the lesion and the surrounding normal liver tissue is adequate; the lesion can be identified and its size (maximum diameter) can be evaluated and measured
4 =	Excellent:	the difference in signal intensity between the lesion and the surrounding liver is marked; the lesion can be optimally identified and its size (maximum diameter) can be easily measured

Assessments will be done by the three independent readers during central reading sessions (Section 6.2.5): part I (unenhanced MRI alone) and part II (paired reading of both unenhanced and mangoral-enhanced images) and part III (mangoral-enhanced images alone).

For this study, a specific MRI manual will be prepared for training the investigators on MRI acquisition parameters, and local reading procedures and rules. An Independent Review Charter will be prepared for the independent reading process. The independent readers will be trained on MRI assessments and the training will be documented.

The handling of MRI scans with more than 15 lesions will be described in these documents. Briefly, the readers will identify the smallest lesion and the largest lesion. If there are more than 15 lesions, the reader will start counting starting from the smallest lesion to the largest lesion and will stop at 15 lesions.

6.2.3.2 Confidence in lesion detection

Confidence in lesion detection will be evaluated for each lesion during the central reading sessions (Section 6.2.5), i.e. part I (unenhanced MRI alone), part II (CMRI), and part III (mangoral-enhanced MRI alone), by the three independent readers, and on mangoral-enhanced MRI and unenhanced MRI images by the on-site radiologists. Up to 15 lesions per patient will be evaluated on the following 3-point scale:

1 = The lesion is detected with low confidence
2 = The lesion is detected with moderate confidence
3 = The lesion is detected with high confidence

6.2.3.3 Confidence in lesion localisation

Confidence in lesion localisation will be assessed for each lesion during the central reading sessions (Section 6.2.5), i.e. part I (unenhanced MRI alone), part II (CMRI), and part III (mangoral-enhanced MRI alone), by the three independent readers, and on mangoral-enhanced MRI and unenhanced MRI images by the on-site radiologists using the Couinaud liver segment classification system [34]. Up to 15 lesions per patient will be evaluated on the following 3-point scale:

1 = The lesion is localised to a liver segment with low confidence
2 = The lesion is localised to a liver segment with moderate confidence
3 = The lesion is localised to a liver segment with high confidence

Note: If a lesion is large enough to be present in more than one segment it shall be considered to be in the segment in which its site lies.

6.2.3.4 Lesion dimensions

Lesion dimension measurements will be done by each of the three independent readers on mangoral-enhanced MRI and unenhanced MRI images. The longest diameter of the largest and of the smallest visualised lesions, respectively, will be measured.

6.2.3.5 Quantitative assessments

For the quantitative analysis, the signal intensities (SI) of liver parenchyma and liver lesion, as well as the standard deviation (SD) of the background noise will be determined by each of the three independent readers during central reading session part II (paired reading of both unenhanced and mangoral-enhanced images; Section 6.2.5). The assessment will be done only during reading session part II as this allows the readers to assess the same lesions on unenhanced and enhanced images in the absence of formal lesion matching. Up to 5 lesions per patient of ≥ 2 cm in diameter will be evaluated and these lesions will be the same on pre-and post-contrast images.

Quantitative SI will be measured by positioning circular regions of interest (ROIs) in a homogenous area in the liver and the assessed liver lesion on the same image. The ROI placed in the lesion should at least encompass half of the lesion. ROIs of a constant size will be placed in the same locations on all pre- and post-contrast images. SD of the background noise will be measured using the largest possible rectangular ROI vertical to the patient's abdomen in the direction of the phase-encoding gradient.

The following quantitative measures will be determined:

Signal intensity (SI) enhancement:

- Liver SI enhancement (%) =
  $([SI_{liv}$ post contrast $- SI_{liv}$ pre contrast$]/[SI_{liv}$ pre contrast$]) \times 100$

Liver-to-lesion contrast (LLC):

- $LLC = (SI_{liv} - SI_{les})/(SI_{liv} + SI_{les})$

Signal-to-noise ratio (SNR):

- $SNR = SI_{liv}/SD_{noise}$

Contrast-to-noise ratio (CNR):

- $CNR = (SI_{liv} – SI_{les})/SD_{noise}$

with SI_{liv} = signal intensity of the liver, SI_{les} = signal intensity of the lesion, SD_{noise} = standard deviation of the background noise.

6.2.4 Imaging procedures

6.2.4.1 Image acquisition

Details of imaging procedures and acquisition will be provided to each study site in a study-specific imaging manual (imaging site manual).

6.2.4.1.1 MRI examination of the liver

Unenhanced MRI of the liver will be performed during the baseline period, i.e. either on the day prior to the mangoral-enhanced MRI or pre-dose on the same day as the mangoral-enhanced MRI. Mangoral-enhanced MRI of the liver will be performed 4 (± 1) hours after mangoral administration.

MRI examinations will be done using a 1.5 Tesla or 3.0 Tesla MRI scanner. An overview of image sequences utilised for both pre- and post-contrast examinations and MRI parameters is given in Table 6. All relevant details of MRI methodology will also be given in the imaging site manual.

6.2.4.1.2 MRI examination of the brain

Unenhanced MRI of the brain will be performed 7 (+ 2) days after mangoral-enhanced MRI of the liver in a subgroup of patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI and for whom previous brain MRI images are available for comparison.

MRI examination should be done with the same parameters used in the brain MRI images performed before participating in this study. If parameters from previous brain MRI are unknown, standard settings can be used.

MRI scans will be evaluated by the off-site imaging core laboratory.

Table 6 MRI parameters

General Instructions										
Scanner type	1.5 Tesla or 3.0 Tesla									
Patient Orientation	Supine									
Breathing instructions	Breath-hold technique or ultra-fast acquisition									
Sequences (both pre- and post-contrast)	• T1-gradient echo (T1-GRE), • T1-gradient echo with fat suppression (T1-GRE-Fat Sat); • T2-fast spin echo without fat suppression (T2-SS-FSE, HASTE), • Optional: T2- fast spin echo with fat suppression (T2-RTR-FSE) • Diffusion-weighted imaging (DWI).									
Sequence parameters										
	T1-GRE		**T1-GRE Fat Sat**		**T2-SS-FSE, HASTE**		**T2-RTR-FSE (optional)**		**DWI**	
Scanner (Tesla)	**1.5**	**3.0**	**1.5**	**3.0**	**1.5**	**3.0**	**1.5**	**3.0**	**1.5**	**3.0**
Plain	Axial	Axial	Axial	Axial	Axial	Axial	Axial	Axial	Axial	Axial
Dimension	2D or 3D	2D or 3D	2D or 3D	2D or 3D	2D	2D	2D	2D	2D	2D
TR (msec)	2D: 100-200 3D: 3-10	2D: 100-200 3D:3 -10	2D: 100-200 3D: 3-10	2D: 100-200 3D: 3-10	800-2500	800-2500	R-R inter-val	R-R inter-val	1000-5400 (min.)	1000-5400 (min.)
TE (msec)	1.5-3.0 & 4.0-6.0	1.0-1.5 & 2.0-3.0	1.5-3.0 & 4.0-6.0	1.0-1.5 & 2.0-3.0	80-200	80-200	70-200	70-200	Minimum	Minimum
Flip angle (°)	2D: 60-80 3D: 10-15	2D: 60-80 3D: 10-15	2D: 60-80 3D: 10-15	2D: 60-80 3D: 10-15	90	90	90	90	90	90
NEX	0.6-1	0.6-1	1	1	0.5-1	0.5-1	2-4	2-4	2-6	2-6
Matrix (frequency)	250-350	250-350	250-350	250-350	250-350	250-350	250-350	250-350	80-192	80-192
Matrix (phase)	180-256	180-256	180-256	180-256	180-256	180-256	180-256	180-256	80-192	80-192
Phase direction	AP	AP	AP	AP	AP	AP	AP	AP	AP	AP
Slice thickness (mm)	2D: 4-6 (true) 3D: 3-5 (true)	2D: 4-6 (true) 3D: 3-5 (true)	2D: 4-6 (true) 3D: 3-5 (true)	2D: 4-6 (true) 3D: 3-5 (true)	4-8	4-8	4-8	4-8	4-8	4-8
Slice gap (mm)	2D: 0-2 3D: 0	2D: 0-2 3D: 0	2D: 0-2 3D: 0	2D: 0-2 3D: 0	0-3	0-3	0-3	0-3	0-3	0-3
B-values	-	-	-	-	-	-	-	-	0-100, 500-800	0-100, 500-800

AP = anterior to posterior, min. = minimum, NEX = number of excitations, TE = echo time, TR = repetition time

6.2.4.2 Image handling and processing

Independent off-site assessments will be performed at a centralised imaging core laboratory (lab) in a digital fashion using a validated software. The independent readers will be blinded with regard to patient identity and all clinical information of a patient.

Each site will transfer all image data acquired to the imaging core laboratory. All study-related image data have to be submitted in DICOM (Digital Imaging and Communications in Medicine) format. In accordance with Good Clinical Practice (GCP) guidelines, investigational sites are required to maintain the original images as source documentation of the study.

The imaging core laboratory will check and document image quality and completeness of image sets prior to the independent reading sessions. All independent read operational details will be described in the IRC developed by the core lab.

6.2.5 Efficacy assessments

6.2.5.1 Off-site (central) assessments

All unenhanced and mangoral-enhanced MRI image sets will be evaluated centrally at the core imaging laboratory by 3 independent readers.

The central evaluation of all MRI images will consist of 3 parts:

- Part I: unenhanced MRI alone
- Part II: combined MRI, i.e. paired reading of both unenhanced and mangoral-enhanced images
- Part III: mangoral-enhanced MRI alone

Each of the three independent readers will assess all images of all patients during each reading part. There will be a 2-week gap between reading parts to reduce recall bias.

The analysis of the primary efficacy variable (Section 6.2.2.1) will be based on the qualitative assessments performed centrally.

Lesion tracking and lesion matching

At the time of efficacy reads, the three independent readers will identify the location of the detected lesions in liver segments. A fourth reader who is not involved in efficacy reads will track and match the detected lesions on unenhanced, mangoral-enhanced and combined (unenhanced plus mangoral-enhanced MRI) to confirm lesion numbering and lesion location across the modalities. Lesion tracking will also confirm lesions that are identified on pre-mangoral, post-mangoral and combined (unenhanced plus mangoral-enhanced) MRI images.

6.2.5.2 On-site assessment

On-site evaluation will be done by each site investigator (or on-site appointed radiologist) within 5 working days after the MRI examination (Table 5).

6.3 Safety

Section 7.1, study schedule provides details on assessments per visit, if a visit is conducted as a remote visit (see Sections 7.2.4 and 7.2.5 as applicable).

6.3.1 Vital signs

Vital signs will be measured at baseline within 3 hours prior to contrast administration, and after contrast administration at 6 (± 1) hours, 24 (± 4) hours, 48 (± 4), and 5 (± 2) days post-dose:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Pulse rate (bpm)
- Respiratory rate (breaths per minute)
- Body temperature (°C)

Patients must be sitting, and measurements must be started after at least 4 minutes of rest. The investigator will evaluate any worsening in vital signs. Any new or worsening events (irrespective of clinical significance) observed after mangoral administration compared to previous pre-dose assessments must be reported as an AE.

6.3.2 Physical examination

A physical examination will be performed at the following times: baseline (within 3 hours prior to contrast administration), and at 6 (± 1) hours, 24 (± 4) hours, 48 (± 4) hours, and 5 (± 2) days post-dose. Any new or worsening observed changes (irrespective of clinical significance) after administration of mangoral compared to previous pre-dose assessments must be reported as an AE (Section 6.3.7).

6.3.3 Neurological assessments

Neurological assessments to monitor for any potential neurotoxic effects will be performed pre-dose during the baseline period and 24 (± 4) hours, 48 (± 4) hours, and 5 (± 2) days hours after the administration of mangoral. At each site, a trained examiner (e.g. nurse practitioner, physician) will be appointed for the neurological assessments after receiving appropriate training (as applicable).

Any new or worsening observed changes (irrespective of clinical significance) after mangoral administration compared to previous pre-dose assessments must be reported as an AE (Section 6.3.7).

6.3.4 Electrocardiogram (ECG)

A 12-lead ECG will be performed after 5 minutes supine rest at baseline (within 3 hours prior to contrast administration) and after administration of mangoral at 6 (± 1) and 24 (± 4) hours post-dose.

Standard ECG parameters will be recorded and assessed (including corrected QT interval, RR interval, PR interval, and QRS interval).

The investigator will record on the eCRF whether the results are normal, abnormal (not clinically significant or clinically significant). If recorded as abnormal and clinically significant, the abnormality must be reported in the eCRF as an AE (Section 6.3.7).

6.3.5 Laboratory variables

6.3.5.1 Screening laboratory tests

Table 7 provides an overview of the laboratory parameters to be measured at the local laboratory during the screening period for the determination of the Child-Pugh score.

Table 7 Screening laboratory parameters

Child-Pugh: *(local lab)*	Serum bilirubin Serum albumin International normalised ratio (INR)	eGFR: (*local lab*)	Serum creatinine Serum albumin Serum urea nitrogen (SUN/BUN)

Note: eGFR measurement/assessment is only required if not available within the last 3 months prior to the Baseline Visit.

6.3.5.2 Clinical safety laboratory tests

Blood samples for haematology and biochemistry tests as well as a urine sample for urinalysis for safety monitoring will be collected at baseline (within 24 hours prior to contrast administration), and 2 (± 0.5) hours, 6 (± 1) hours, 24 (± 4) hours, 48 (± 4) hours, and 5 (± 2) days after administration of mangoral. A blood volume of approximately 12 mL will be drawn per time point. An overview of measured parameters is given in Table 8.

Table 8 Clinical safety laboratory parameters (baseline and post-dose)

Haematology: *(central lab)*	Haemoglobin Haematocrit Erythrocytes Leucocytes Thrombocytes Neutrophils Eosinophils, Basophils Lymphocytes Monocytes INR	Biochemistry: (*central lab*)	Alkaline phosphatase Aspartate aminotransferase (ASAT) Alanine aminotransferase (ALAT) Gamma-glutamyl transferase (γ-GT) Creatinine Glucose Urea Total Bilirubin Albumin Sodium Potassium Calcium Magnesium Chloride
Urinalysis: *(local lab)*	pH Leucocytes Blood Protein Glucose Urobilinogen Ketones Bilirubin Nitrite Specific density		

All blood tests (haematology, biochemistry) will be analysed at a central laboratory. Special tubes, labels, packaging, and sample handling instructions will be provided by the central laboratory. Blood sampling and handling procedures are described in the study-specific laboratory manual.

Urinalysis will be performed by using sites' standard of care dipsticks or using dipsticks provided by the central laboratory.

The investigator must document the clinical significance of abnormal laboratory values in the eCRF. If the clinically significant abnormal value is observed after administration of mangoral, the abnormality must also be recorded as an AE (Section 6.3.7). All clinically significant abnormal laboratory values observed at 5 (± 2) days post-dose will be followed upon until they have normalised, or until there is no medical necessity for further blood tests in the investigator's opinion (Section 6.3.7.3).

After completion of all analyses, any leftover blood or blood samples will be destroyed immediately after the termination of the study.

6.3.5.3 Blood manganese concentration

Blood samples for manganese measurements will be taken on the day of mangoral administration immediately (within 1 hour) prior to dosing, and at 24 (± 4), 48 (± 4), and 5 (± 2) days post-dose in all patients. A blood volume of 6 mL will be drawn per time point. Additional

blood samples for PK assessments will be taken on the day of mangoral administration only in the PK subgroup (Section 6.4.2).

Blood samples for determination of manganese concentrations will be handled as described in Section 6.4.2.

6.3.5.4 Pregnancy test

A serum pregnancy test will be performed in women of childbearing potential at the local laboratory at the screening. A urine or serum pregnancy test will be performed in women of childbearing potential at the local laboratory at baseline, i.e. within 24 hours prior to the administration of mangoral. Pregnancy test dipsticks will be provided by the central laboratory or sites will be using their standard of care dipsticks.

If local regulations require serum pregnancy tests, a blood sample will be taken and a serum beta-hCG test will be performed at the local laboratory. In case of pregnancy, the patient is not allowed to enter the study or will be withdrawn from further study participation.

All pregnancies diagnosed after mangoral intake will be reported to the sponsor/ designee (page 10 for contact details) on a paper pregnancy reporting form and will be monitored by the investigator up to the final outcome. In addition, the event should be captured on dedicated CRF pages as timelines detailed in the eCRF- Completion Guidelines. The outcome, including premature termination, must be reported to the sponsor as a follow-up to the initial report.

6.3.6 Additional safety assessments

Manganese is required for normal brain function as it plays a role as co-factor or regulator of brain enzymes, in particular of glutamine synthetase. However, it has been shown that overexposure to manganese, when absorbed in large amounts exceeding the homeostatic range, may have neurotoxic effects resulting in neuropsychological alterations, psychiatric symptoms and Parkinsonian symptoms with a significant decrement of performance in motor functions related to coordination and tremor [22, 36].

Manganese accumulation in the brain occurs mainly in the globus pallidus and the striatum of the basal ganglia which can be detected by MRI imaging as high signal intensity in these regions on T1-weighted images [37, 38]

6.3.6.1 MRI of the brain – Optional

An unenhanced MRI examination of the brain (Section 6.2.4.1.2) will be done 7 (+ 2) days after the administration of mangoral only in those patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI and for whom previous brain MRI images are available for comparison. Note that the brain MRI procedure is optional and requires a consent from the patient if to be performed. Post-dose images will be evaluated for any signs of manganese accumulation in the brain and will be compared by the independent off-site radiologist to the earlier brain MRI results.

6.3.7 Adverse events

Any untoward medical occurrence that is experienced by the patient from the time of signing the informed consent to the time immediately prior to the administration of IMP will be recorded in the eCRF as a pre-dose event. A pre-dose event cannot be causally related to the IMP; however, it may be causally related to study-related procedures (e.g. blood sampling during

the screening period). Thus, for each pre-dose event, relationship to study-related procedures and seriousness will be assessed and recorded.

All untoward medical occurrences which are noticed after the administration of IMP up until the end of the follow-up period (end of day 7 [+2] in the brain MRI subgroup, end of day 5 [±2] in all other patients) will be reported as adverse events (AEs). The relationship to the IMP and also to study procedures will be recorded for all AEs that occur after administration of IMP.

6.3.7.1 Definition of adverse events

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not related to the IMP.

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

Laboratory values outside the normal laboratory-specific reference ranges that make intervention necessary and / or are considered clinically significant by the investigator must be reported as AEs. If the abnormal laboratory value is a sign of a disease, only this diagnosis should be reported as an AE.

An AE is considered an adverse drug reaction (ADR) if a causal relationship between a medicinal product and an AE is at least a reasonable possibility.

6.3.7.2 Assessment of adverse events

The investigator will identify the occurrence of pre-dose events and / or AEs through non-leading questioning and examination of the patient.

For each AE, the investigator will document the following:

- **Signs and symptoms** of the event (if a specific disease can be diagnosed, this disease should be the reported AE; if only signs and symptoms can be evaluated, each sign or symptom should be reported as a separate AE)
- **Onset date and time** (if a change from pre-dose in a laboratory test is reported as an AE, the start date is the date of collection of the first laboratory sample that shows the change)
- **End date and time** (if a change from pre-dose in a laboratory test is reported as an AE, the end date is the date of collection of the first laboratory sample that shows a return to pre-dose level)
- **Measure taken** (none, drug treatment required, hospitalisation or prolonged hospitalisation, study discontinuation, other measures [specification])
- **Outcome** (recovered/resolved, recovering/resolving, recovered/resolved with sequelae, not recovered/not resolved, fatal, unknown)

In addition, each AE will be rated by the investigator according to the following categories (the sponsor must carry out a separate assessment for causal relationship, seriousness, and expectedness):

Relationship to the investigational medicinal product

The causal relationship between an AE and the IMP will be evaluated and classified as follows:

Related:	After careful medical consideration, there is enough evidence (e.g. reasonable time sequence, known response pattern, not attributable to concomitant medications or concurrent diseases) to assume a causal relationship between the AE and the investigational medicinal product or a causal relationship the AE and the investigational medicinal product cannot be ruled out with certainty
Not related:	After careful medical consideration, the AE is clearly and incontrovertibly due to causes other than the investigational medicinal product (e.g. documented pre-existing condition)

Intensity

Regardless of the classification of an AE as serious or non-serious (see below), its intensity must be assessed according to medical criteria alone using the Common Terminology Criteria for Adverse Events (CTCAE) classification (v5.0):

Grade 1 (mild):	asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 (moderate):	minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (e.g. preparing meals, shopping, managing money
Grade 3 (severe):	severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living (e.g. bathing, dressing, undressing, feeding self, using the toilet, taking medications
Grade 4 (life-threatening):	life-threatening consequences; urgent intervention indicated
Grade 5 (death:)	death related to AE

It should be noted that a severe AE does not necessarily have to be serious in nature and that a serious AE does not need to be of severe intensity.

Seriousness

A serious adverse event (SAE) is an AE that at any dose:

- Results in death
- Is life-threatening (i.e. the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability/incapacity (defined as a permanent or substantial disruption of the patient's ability to carry out normal life activities)
- Is a congenital anomaly or birth defect

or

- Is an important medical event that does not have to be immediately life-threatening or result in death or hospitalisation, but may, based upon appropriate medical judgment, jeopardise

the patient or may require medical or surgical intervention to prevent any of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, and blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse. Note: events of seizure, stroke, cerebral venous thrombosis, QTcF or QTcB greater than 480 msec or QTcF or QTcF increase of 60 msec over baseline must be reported as serious.

All AEs that do not fall into any of the above categories are defined as non-serious.

Note: planned hospitalisation or prolongation of hospitalisation for a pre-existing condition or a procedure required by this study protocol, without a serious deterioration in health, is not considered an SAE.

Expectedness (only evaluated by the sponsor):

Expected: An ADR is considered expected if its description agrees in nature, severity, frequency and specificity with the description of the up-to-date reference safety information included in the investigator's brochure

Unexpected: An unexpected ADR is an adverse reaction for which the nature or severity is not consistent with the applicable product information (e.g., investigator's brochure)

A Suspected Unexpected Serious Adverse Drug Reaction (SUSAR) is defined as an untoward and unintended response to an IMP that is not listed in the applicable product information and meets one of the above mentioned serious criteria. The sponsor will ensure that all SUSARs associated with the IMP will be reported to the applicable regulatory authority/competent authorities and the relevant independent ethics committees (IECs)/institutional review boards (IRBs) in accordance with the applicable national/local regulations (usually within 7 calendar days for life-threatening and fatal SUSARs or within 15 calendar days for all other SUSARs). The sponsor/CRO will also promptly inform all investigators about any SUSAR.

6.3.7.3 Follow-up of adverse events

Each unresolved AE at the patients' individual study termination must be followed up until it subsides or until the cause is known or an adequate final assessment can be given. The maximum follow-up period will be 4 weeks after the patient's study termination. If after 4 weeks no final outcome can be acquired, the outcome of the AE is 'unresolved'.

For all patients with clinically significant abnormal laboratory findings at 5 (± 2) days post-dose compared to the baseline evaluation (within 24 hours prior to the administration of IMP), the laboratory test will be repeated to ensure the validity of the abnormal result. Confirmed abnormal values will be followed up until normalisation to baseline level or until there is no medical necessity for further blood tests in the investigator's opinion. Therefore, the patient might have to come to the site for one or more unscheduled follow-up visits.

6.3.7.4 Documentation and reporting of adverse events

Any AE must be recorded in the eCRF with the specifications detailed in Section 6.3.7.2.

All SAEs must be reported by the investigator immediately (within 24 hours) to the sponsor/ designee (minimum information required: patient number, site identifier, investigational medicinal product, event term, and causality assessment) on a dedicated paper SAE form.

A study-specific SAE form for immediate reporting will be provided to the investigators as part of investigator site file. In case of an SAE, this form should be completed and submitted immediately (no later than 24 hours after the investigator's awareness of the event) to the sponsor/ designee (page 10 for contact details). Secure email of the SAE form is the preferred method to transmit this information. In addition, the event should be captured on the dedicated (Serious) Adverse Event page of the eCRF within 3 days.

In order to perform an independent medical assessment of the reported case, the Sponsor or a designee may follow-up by telephone, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g. hospital discharge summaries, consultant reports, autopsy reports).

Follow-up reports will be provided by the investigator each time new information on the SAE becomes available (until the case is resolved or the patient has recovered). Every effort should be made to follow all SAEs considered to be related to the IMP or trial-related procedures until a final outcome can be reported. For information, a similar procedure as for reporting of initial SAEs will apply.

Investigators are not obliged to actively seek AE or SAE after the end of the AE reporting period (defined as 5 [± 2] days after IMP intake). However, if the investigator learns of any SAE, at any time after the end of AE reporting period, and he/she considers the event to be reasonably related to the IMP or study participation, the investigator must promptly notify the sponsor.

Notification of the IECs/IRBs and competent authorities about all relevant events will be performed by the sponsor (or designated CRO) and / or by the investigator (as applicable) according to all applicable national/local regulations.

6.4 Pharmacokinetics

6.4.1 Pharmacokinetic variables

Pharmacokinetics will be evaluated only in the PK subgroup (N = 12) at selected study sites. Blood concentrations of manganese will be corrected for baseline (pre-dose) concentration of manganese.

The following PK variables will be determined:

- AUC (area under the curve): for the interval 0-120 hours
- C_{max} (maximum or peak concentration): determined from tabulated observed blood level data
- t_{max} (time to achieve maximum concentration)
- $t_{1/2}$ (terminal half-life): determined as $0.693/K_{el}$, where K_{el} is a first-order elimination rate constant determined from the slope of the terminal phase of semilog plot of concentration vs. time data
- Cl_{app} (total apparent clearance): calculated as dose/AUC

6.4.2 Blood sampling time points, handling and labelling of blood samples

Patients, who are part of the PK subgroup shall have V4 and V5 at the study site.

Blood samples for PK evaluation will be collected by venipuncture (or by using an indwelling catheter) during the screening period, immediately prior to the administration of the IMP (within

1 hour pre-dose), and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 120 hours post-dose. A blood volume of 6 mL will be drawn per time point.

Handling and labelling of blood samples for PK analysis will be performed according to the instructions provided by the central laboratory. Special tubes, labels, packaging, and instructions for storage and shipment will also be provided by the central laboratory. Blood samples should be shipped for analysis on the day of collection.

Blood manganese concentrations will be determined from all collected samples using a validated sensitive analytical procedure (e.g. inductively coupled plasma mass spectrometry) in accordance with the EMA *Guideline on Bioanalytical Method Validation* and the FDA *Guidance for Industry* at a specialised central analytical laboratory.

After completion of all analyses, any leftover blood or blood samples will be destroyed immediately after the termination of the study.

7 STUDY CONDUCT

7.1 Study schedule

The schedule of visits and study-related procedures at each visit are presented in Table 9.

Patients who are interested in participating in the study will sign an informed consent form after having been thoroughly informed about the purpose and procedures of the study and before any screening examination is performed. Screening will be performed within 4 weeks (between days -28 and -1) prior to the day of the mangoral-enhanced liver MRI.

Depending on the clinical condition of the patient and the routine at the study site, hospitalisations for up to 2 days for the baseline evaluations and the performance of unenhanced/enhanced MRI, as well as other study-related procedures on the day of the MRI, may be necessary. Patients in the PK subgroup will be hospitalised at baseline for at least 2 days because these patients will undergo additional blood sampling for PK assessments within 1 hour pre-dose and between 0.25 and 12 hours post mangoral administration.

Scheduled follow-up visits (FU1, FU2, FU3, and FU4, if required) may be performed as outpatient visits. In case a patient is not able to return to the study site for the follow-up visits, the follow-up assessments may be performed by a trained examiner at an off-site location, e.g. at the patient's home or at an associated off-site clinic. For patients with long travelling distances, it may be possible that a hotel room will be booked for the patient and an accompanying person (caregiver) by the study site coordinator in agreement with the sponsor. Hotel costs will be at the expense of the sponsor. Costs for travelling to the study sites may also be reimbursed upon request and submission of tickets and/or receipts.

In the event, a patient is not part of the PK subgroup, FU1 (V4 at 24 [± 4] hours) and FU 2 (V5 at 48 [± 4] hours) can be performed as remote visits at the discretion of the investigator, if further adjustments are required to ease the burden for the patient. If an on-site visit is replaced by a remote visit, information on possible adverse events and changes in concomitant therapies shall be collected timely.

Only a subgroup of patients, i.e. patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI and for whom previous brain MRI images are available, will return to the site for the FU4 visit. At this visit, which will take place 7 (+ 2) days after the mangoral-enhanced liver MRI, an MRI of the brain will be performed.

Table 9 Study schedule

Visit	Screening period (day -28 to -1) V1[a]	Baseline Visit (day -1 to day 0) V2[b]	Baseline Visit Day of IMP dosing (day 0) V3[b]	FU1 (day 1) V4[c,d]	FU2 (day 2) V5[c,d]	FU3 (day 5) V6[d]	[FU4] (day 7) V7[e]
Time relative to mangoral administration	Within 28 days pre-dose	Within 24 hours pre-dose	0 - 12 hours post-dose	24 ± 4 hours post-dose	48 ± 4 hours post-dose	5 ± 2 days post-dose	[7 + 2 days post-dose]
Procedures and Assessments							
Informed consent[f]	X						
Inclusion / exclusion	X	X					
Demographics	X						
Medical history	X						
Clinical signs & diagnosis	X						
Prior and concomitant therapies[g]	X	X	X	X	X	X	[X]
IMP accountability			X				
Child-Pugh evaluations[h]	X						
Child-Pugh score	X						
Vital signs			X[i]	X	X	X	
Physical examination			X[i]	X	X	X	
Neurological assessment[j]		X		X	X	X	
ECG			X[i]	X			
[eGFR][k]	[X]						
Unenhanced MRI of liver			X				
Mangoral administration			X				
Mangoral-enhanced MRI of liver			X[l]				
Unenhanced MRI of brain							[X]
Pregnancy test[m]	X	X					
Clinical safety laboratory tests		X	X[n]	X	X	X	
Blood sampling for manganese concentration / PK (subgroup)			X[o]	X	X	X	
Adverse events[p]	X	X	X	X	X	X	[X]

[a] During the Screening period (day -28 to -1; assessments can be performed anytime during this period.

[b] Visit 2 and Visit 3 (i.e. Baseline period visits) must be performed sequentially, but can occur on the same day (at Visit 3, i.e. Day of dosing, Day 0).

[c] If the patient is not part of the PK subgroup (see Section 6.4.2), Visit 4 and Visit 5 can be performed as remote visits at the discretion of the investigator.

[d] Patients who withdraw from the study prior to any Follow-up visits will be asked to complete the last scheduled assessments, if at all possible. Information will only be collected in relation to the scheduled Follow-up Visits (Section 7.2.4, 7.2.5, 7.2.6) and information needed for the follow-up of adverse events (Section 6.3.7).

[e] [FU4] will be performed only in the brain MRI subgroup, i.e. only in patients who underwent brain MRI due to any clinical reason within the last 6 months prior to mangoral-enhanced MRI

[f] Informed consent must be signed by the patient prior to any study-specific procedures.

[g] All prior and concomitant therapies/medications taken within 14 days prior to the administration of mangoral and up to 5 (± 2) days post-dose are to be recorded.

[h] Child-Pugh evaluations: laboratory tests (serum bilirubin, serum albumin, international normalised ratio [INR]; Section 6.3.5.1), hepatic encephalopathy.

[i] Vital signs (Section 6.3.1), physical examination (Section 6.3.2), and ECG (Section 6.3.4): within 3 hours before administration of mangoral and at 6 (± 1) hours post-dose.

[j] Site-specific neurological examination procedure.

[k] eGFR measurement/assessment is only required if not available within the last 3 months prior to the Baseline Visit.

[l] Mangoral-enhanced MRI of the liver will be performed 4 (± 1) hours after mangoral administration yet fasting.

[m] Only in female patients of childbearing potential. A serum pregnancy test will be performed in women of childbearing potential at the local laboratory at the screening visit. An additional pregnancy test (urine or serum) will be performed within 24 hours prior to mangoral administration.

[n] Clinical safety laboratory tests (Section 6.3.5.2) to be performed within 24 hours prior to mangoral administration (either on the day before or on the day of the MRI examination) and after 2 (± 0.5) and 6 (± 1) hours post-dose on the day of the MRI examination. All clinically significant abnormal laboratory values observed at 5 (± 2) days post-dose will be followed up until they have normalised, or until there is no medical necessity for further blood tests in the investigator's opinion.

[o] Blood samples for manganese measurements will be taken on the day of mangoral administration immediately prior to dosing (within 1 hour pre-dose; Section 6.3.5.3) in all patients; additional blood samples will be taken at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-dose only in the PK subgroup (Section 6.4.2).

[p] AE reporting starts from the time of signing the informed consent; an event that occurs prior to the administration of mangoral will be recorded as 'pre-dose event' in the eCRF. Any unresolved AE at the patients' individual study termination must be followed up until it subsides or until the cause is known or an adequate final assessment can be given for a maximum follow-up period of 4 weeks, Section 6.3.7.3).

7.2 Observations by visit

7.2.1 Screening visit: V1 (day -28 to -1)

A Screening Visit will be performed within 4 weeks and up to 1 day prior to the planned mangoral-enhanced MRI examination. Patients who are interested in participating in the study will be informed verbally and in writing about the purpose, nature, risks, and benefits of the study.

The following will be carried out and / or recorded at the Screening Visit:

- Patient signs informed consent form as a part of the written patient information including the agreement concerning protection of personal data and direct access to source documents
- Demographic data (incl. year of birth, age, sex, height, weight)
- Relevant general medical history (Section 6.1.2.1)
- Clinical signs and symptoms (including diagnosis) of the underlying primary malignancy (Section 6.1.2.2)
- Pregnancy test (serum test for female patients of childbearing potential) (Section 6.3.5.4)
- Review of inclusion and exclusion criteria (Sections 4.1 and 4.2)
- Child-Pugh evaluations/determination of Child-Pugh score (Sections 6.1.2.3 and 6.3.5.1)
- Concomitant and prior medications taken within 14 days pre-dose (Section 5.2)

7.2.1.1 Screening

Potential participants will be screened based on inclusion and exclusion criteria.

7.2.1.2 Re-screening and re-consenting

Re-screening is allowed if the patient has not undergone the IMP-enhanced MRI, earliest 5 days post dosing attempt.

A new screening number will be assigned in the eCRF.

7.2.2 Baseline period: V2 (day -1) to V3 (day 0), within 24 hours pre-dose

The following will be performed and/or recorded during the baseline period, i.e. within 24 hours prior to mangoral administration (note: some of the baseline assessments may be done/recorded on the day/evening before the MRI, or all baseline assessments may be done/recorded on the day of the MRI):

- Concomitant and prior medications taken within 14 days pre-dose (Section 5.2)
- Vital signs within 3 hours prior to mangoral administration (Section 6.3.1)
- Physical examination within 3 hours prior to mangoral administration (Section 6.3.2)
- ECG within 3 hours prior to mangoral administration (Section 6.3.4)
- Neurological assessments within 24 hours prior to mangoral administration (Section 6.3.3)
- Verification of inclusion/exclusion criteria (eligibility check), as applicable
- Pregnancy test (urine or serum test for female patients of childbearing potential) within 24 hours prior to the administration of mangoral (Section 6.3.5.4)
- Blood sampling for clinical safety laboratory tests (haematology, biochemistry) within 24 hours prior to mangoral administration (Section 6.3.5.2)

- Urine collection for urinalysis within 24 hours prior to mangoral administration (Section 6.3.5.2)
- Unenhanced MRI of the liver either on day -1 or on day 0 prior to mangoral administration (Section 6.2.4.1.1)
- Blood sampling for determination of manganese concentration immediately (within 1 hour) before administration of mangoral (Section 6.3.5.3)
- Pre-dose events (for definition Section 6.3.7)

7.2.3 Day of Dosing: V3 (day 0), dosing, mangoral-enhanced MRI and post-dose assessments

After the performance/documentation of all baseline assessments (Section 7.2.2), the following examinations will be carried out and/or recorded on the day of MRI:

- Administration of IMP (to be taken after fasting for at least 4 hours; Section 5.1)
- Mangoral-enhanced MRI of the liver at 4 (± 1) hours after mangoral administration (Section 6.2.4.1.1)
- Any changes in concomitant therapies (including medications taken only once and any non-prescription medicine)
- Vital signs at 6 (± 1) hours post-dose (Section 6.3.1)
- Physical examination at 6 (± 1) hours post-dose (Section 6.3.2)
- ECG at 6 (± 1) hours post-dose (Section 6.3.4)
- Blood sampling for clinical safety laboratory tests (haematology, biochemistry) at 2 (± 0.5) and 6 (± 1) hours post-dose (Section 6.3.5.2)
- Urine collection for urinalysis at 2 (± 0.5) and 6 (± 1) hours post-dose (Section 6.3.5.2)
- Only in the PK subgroup: blood sampling for determination of manganese concentration at 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hours post-dose (PK; Section 6.4.2)
- Adverse events (Section 6.3.7)

7.2.4 Follow-up visit V4 (day 1)

The following examinations will be carried out and / or recorded on the first day following the day of mangoral dosing and the mangoral-enhanced MRI (24 [± 4] hours post-dose):

- Any changes in concomitant therapies (including medications taken only once and any non-prescription medicine)
- Vital signs at 24 hours post-dose (Section 6.3.1)
- Physical examination at 24 hours post-dose (Section 6.3.2)
- Neurological assessments at 24 hours post-dose (Section 6.3.3)
- ECG at 24 hours post-dose (Section 6.3.4)
- Blood sampling for clinical safety laboratory tests (haematology, biochemistry) at 24 hours post-dose (Section 6.3.5.2)
- Urine collection for urinalysis at 24 hours post-dose (Section 6.3.5.2)
- Blood sampling for determination of manganese concentration at 24 hours post-dose (Section 6.3.5.3)
- Adverse events (Section 6.3.7)

If Visit 4 is conducted as a remote visit, the reason for the change of the visit type shall be recorded in the medical records. As a minimum, information on possible adverse events and changes in concomitant medication must be collected.

7.2.5 Follow-up visit V5 (day 2)

The following examinations will be carried out and / or recorded on the second day following the day of mangoral dosing the mangoral-enhanced MRI examination (48 [± 4] hours post-dose):

- Any changes in concomitant therapies (including medications taken only once and any non-prescription medicine)
- Vital signs at 48 hours post-dose (Section 6.3.1)
- Physical examination at 48 hours post-dose (Section 6.3.2)
- Neurological assessments at 48 hours post-dose (Section 6.3.3)
- Blood sampling for clinical safety laboratory tests (haematology, biochemistry) at 48 hours post-dose (Section 6.3.5.2)
- Urine collection for urinalysis at 48 hours post-dose (Section 6.3.5.2)
- Blood sampling for determination of manganese concentration at 48 hours post-dose (Section 6.3.5.3)
- Adverse events (Section 6.3.7)

If Visit 5 is conducted as a remote visit, the reason for the change of the visit type shall be recorded in the medical records. As a minimum, information on possible adverse events and changes in concomitant medication must be collected.

7.2.6 Follow-up visit V6 (day 5)

The following examinations will be carried out and/or recorded on the third day following the day of the day of IMP dosing and mangoral-enhanced MRI examination (5 [± 2] days post-dose):

- Any changes in concomitant therapies (including medications taken only once and any non-prescription medicine)
- Vital signs at 5 (± 2) days post-dose (Section 6.3.1)
- Physical examination at 5 (± 2) days post-dose (Section 6.3.2)
- Neurological assessments at 5 (± 2) days post-dose (Section 6.3.3)
- Blood sampling for clinical safety laboratory tests (haematology, biochemistry) at 5 (± 2) days post-dose (Section 6.3.5.2)
- Urine collection for urinalysis at 5 (± 2) days post-dose (Section 6.3.5.2)
- Blood sampling for determination of manganese concentration at 5 (± 2) days post-dose (Section 6.3.5.3)
- Adverse events (Section 6.3.7)

7.2.7 Follow-up visit V7 (day 7) in optional brain MRI subgroup

The following examinations will be carried out and/or recorded on the seventh day following the day of the MRI:

- Any changes in concomitant therapies (including medications taken only once and any non-prescription medicine)

- Unenhanced MRI of the brain (Section 6.2.4.1.2)
- Adverse events (Section 6.3.7)

7.2.8 Withdrawal Visit

Patients who withdraw from treatment or the study prior to the last Follow-Up Visit will be asked to attend a Withdrawal Visit, if at all possible. The visit must be scheduled as soon as possible after withdrawal.

No new information will be collected from patients who withdraw from the study, except information collected in relation to the scheduled Follow-up Visits (Section 7.2.4, 7.2.5, 7.2.6) and information needed for the follow-up of AEs (Section 6.3.7). The reason for withdrawal must be recorded in the eCRF.

7.3 Independent Data Safety Monitoring Board (IDSMB)

An Independent Data Safety Monitoring Board (IDSMB) will review all safety data available once 30 patients have completed the third follow-up visit (5 days after the administration of mangoral), and make recommendations to the Sponsor to either continue, modify or discontinue the study.

After consideration, the Sponsor will inform the IDSMB of any action that will be taken in response to the IDSMB's recommendations.

The role and responsibilities of the IDSMB, their operational procedures, and methods of communication with the Sponsor, are described in a separate IDSMB Charter. The IDSMB will consist of three expert members who are independent of the Sponsor. The IDSMB will be composed by:

- A radiologist, who participated in the previous Phase II study or Phase I studies
- A neurologist and
- A biostatistician experienced in DSMB evaluation

All IDSMB members will have experience in the conduct of clinical studies. Members will not be investigators in the trial, nor will they have any conflict of interest with the Sponsor. Members will not have a bias with regard to the use of mangoral for imaging diagnostic. Sponsor representatives are not eligible for membership on the IDSMB

7.4 Duration of the study

7.4.1 Planned duration for the individual patient

After a screening visit within 4 weeks prior to the administration of mangoral, the core study phase will last 4-5 days for each individual participant and comprises a baseline period of up to 24 hours prior to mangoral administration, the day of mangoral administration and 3 follow-up visits on 24 (± 4) hours, 48 (± 4) hours and 5 (± 2) days following the day of mangoral administration. In a subgroup of patients (i.e. patients who underwent brain MRI for any clinical reasons within 6 months prior to the mangoral-enhanced liver MRI and for whom previous brain MRI images are available), a fourth follow-up visit will be performed 7 (+ 2) days after contrast administration for an unenhanced brain MRI examination.

In case of clinically significant abnormal laboratory findings, the patient will be asked to return to the study site for follow-up blood tests until normalisation of the value(s) to baseline level is observed or until further blood sampling is not medically necessary in the investigator's opinion.

7.4.2 Premature termination

Study

The study as a whole may be terminated prematurely by the sponsor at any time if:

- New toxicological or pharmacological findings or SAEs invalidate the earlier positive benefit–risk assessment
- The development of the IMP is discontinued, a marketing authorisation is no longer intended or the study does not meet the expected goal; or
- Other important reasons, not named above

Site

A participating study site can be excluded from participation by the sponsor if:

- The site fails to comply with the requirements of the protocol
- The site fails to comply with GCP standards; or
- The first patient is not recruited within a reasonable period after initiation of the site

Patient

Individual patients are to be withdrawn from the trial according to the criteria specified in Section 4.3.

8 STATISTICS

8.1 Statistical and analytical plans

All statistical analyses will be outlined in detail in a statistical analysis plan which will be prepared and signed prior to any study-specific analyses. In general, all continuous measures will be summarised descriptively, including the number of available values, minimum, 1st quartile, median, mean, standard deviation, 3rd quartile, maximum, if appropriate. Categorical data will be presented by frequency and percentage. Ordinal ratings may be handled as continuous data, if appropriate.

Inferential methods will be based on a significance level of 0.05 for two-sided tests and 0.025 for one-sided tests. All statistical significance tests besides the primary efficacy analysis should be interpreted in an exploratory manner.

8.1.1 Analysis populations

All patients who have taken a single dose of the IMP will be included in the safety analysis (safety population). The efficacy analysis will be performed in the full analysis set of patients and on a per-protocol basis, see definitions below (Table 10). Patients with major protocol violations will be excluded from the latter. For this purpose, protocol violations that could interfere with the objectives of the study, e.g. incorrect concomitant medications or violation of the inclusion/exclusion criteria will be assessed as minor or major in collaboration with the sponsor. The criteria for this assessment will be defined before analysis. Listings will be prepared to show the eligibility of all patients.

The primary analysis will be done according to the intention-to-treat principle for the full analysis set. In addition, a per-protocol analysis will be prepared as an assessment of sensitivity.

Table 10 Definition of analysis populations

Population	Description
Safety population	All patients enrolled in the study (fulfil all inclusion criteria, but none of the exclusion criteria and have been included in the clinical trial at Visit 2).
Full analysis set (FAS)	All patients of the safety population who received the IMP and for whom the primary efficacy variable is assessable, i.e. all unenhanced/enhanced liver MRI images are assessable.
Per-protocol set (PPS)	The per-protocol set defines the subset of the patients in the FAS without major protocol violations, including violations of inclusion/exclusion criteria.
PK population	All patients in the PK subgroup with PK blood samples taken after mangoral administration.
PK dialysis subgroup population (DSP)	All patients in the PK subgroup, currently on maintenance haemodialysis.
Brain MRI subgroup population (BMSP)	All patients with brain MRI performed within 6 months prior to V3 who underwent the optional brain MRI 7 (+ 2) days after mangoral-enhanced MRI of the liver.

8.1.2 Adaptive study design

The study design is not adaptive since no interim analysis is planned.

8.1.3 Primary efficacy analysis

The primary population for this analysis will be the full analysis set (FAS). Efficacy will be assessed by testing the superiority of each of the 2 co-primary variables:

- lesion border delineation (BD), and
- lesion contrast compared to liver background (LC).

The testing will be done separately for each of the three independent readers and will be based on the mean scores of BD and LC. As described in Section 6.2.5, each of the three independent readers will assess all unenhanced and enhanced liver MRI images of all patients distributed over 3 reading parts (part I: unenhanced alone, part II: paired reading, part III: enhanced alone). Each reading part will yield a qualitative assessment of BD and LC on 4-point scales (score 1 to 4; Section 6.2.2.1) per lesion.

The two sum scores (sBD and sLC) are determined for each patient by summarising the individual scores over all lesions detected at baseline for part I (unenhanced MRI) and part II (CMRI). Then, the mean score differences between the paired sum scores weighted by the number of lesions are determined per patient:

$$\text{Diff(BD)} = \frac{\text{sBDcombined} - \text{sBDunenhanced}}{\text{Number of lesions}}.$$

$$\text{Diff(LC)} = \frac{\text{sLCcombined} - \text{sLCunenhanced}}{\text{Number of lesions}}.$$

For each independent reader, the hypotheses for the superiority of CMRI versus unenhanced MRI will be tested using a 1-sided paired t-test using the means of the paired score differences:

$$H_{0;BD}: \mu_{Diff(BD)} \leq 0 \quad \text{versus} \quad H_{1;BD}: \mu_{Diff(BD)} > 0$$

and

$$H_{0;LC}: \mu_{Diff(LC)} \leq 0 \quad \text{versus} \quad H_{1;LC}: \mu_{Diff(LC)} > 0$$

If both tests indicate the superiority of CMRI at the one-sided significance level of 0.025, reader success is achieved. The corresponding two-sided 95% confidence intervals will be calculated as well. These results will be presented in a table together with a summary of the mean scores per reader. For the success of the trial, two of three readers must achieve reader success.

As a sensitivity analysis, the analogous 1-sided Wilcoxon signed-rank test will be performed additionally.

The primary variable will be analysed for the full analysis population and additionally in an exploratory manner for the per-protocol population using the above-described methodology.

8.1.4 Secondary efficacy analyses

A comparison of part I (unenhanced MRI alone) and part III (mangoral-enhanced MRI alone) will be analysed in the same way as the primary endpoint.

The other secondary efficacy variables (Section 6.2.2.2) will be analysed in an exploratory manner using descriptive statistics (including two-sided 95% confidence intervals) for the full analysis population and the per-protocol population. Exploratory statistical tests may be

defined if deemed beneficial in addition to the confidence intervals. Details will be defined in a statistical analysis plan, which will be signed prior to any study-specific analyses.

Inter-reader and intra-reader agreement:

Additionally, assuming repeated assessments per subject by three independent readers, an intra-reader and inter-reader reproducibility will be evaluated separately for MRI readings in Parts I, II and III using linear mixed-effects models fitted to individual mean scores of border delineation (BD) and lesion contrast (LC), considering patients as a random effect. Details of the statistical model will be specified in the SAP.

8.1.5 Subgroup analyses

The primary efficacy endpoint will be evaluated in appropriate subgroups which will be defined in the statistical analysis plan. These subgroups may include but are not limited to:

- Subgroups by MRI scanner's magnetic field strength (1.5 Tesla or 3 Tesla)
- Subgroup by lesion type (e.g. liver metastasis, primary liver tumour)
- Subgroups by lesion diameter (average of the measurements by the three independent readers):
    - small lesion (diameter < 1 cm)
    - medium lesion (diameter ≥ 1 to ≤ 3 cm)
    - large lesion (diameter > 3 cm)
- Subgroups by age (≤ 65 years, > 65 years)
- Subgroups by eGFR (eGFR < 15, ≥ 15 to ≤ 30, > 30 mL/min/1.73m^2)
- Subgroups by status of liver disease (no liver disease, Child-Pugh A [e.g. mild fibrosis])
- Subgroup of patients with PK data
- Subgroup of patients with brain MRI

8.1.6 Safety analyses

AEs will be encoded using the MedDRA thesaurus in the most recent version available at the beginning of the study. This version will be used throughout the entire study. Updates of the MedDRA thesaurus will not be applied afterwards. The same will apply to the Anatomic Therapeutic Chemical Classification System developed by the World Health Organisation, which will be used for the coding of previous and concomitant medications. Frequency tables for coded previous and concomitant medication will be compiled

Frequency tables by system organ class and preferred term will be prepared based on patients experiencing an AE and based on the number of AEs. These frequency tables will also be prepared for AEs assessed as related to the investigational medicinal product. A frequency table will be created to give an overview of numbers of AE and patients with an AE in relation to investigational product, severity and seriousness (if an AE is recurring with the same term, the highest reported severity will be used for categorisation).

Results of vital sign measurements, physical examinations, neurological assessments, brain MRI assessment and ECG will be presented using descriptive statistics.

Laboratory data will be presented using descriptive statistics as well as using shift-tables with respect to normal ranges.

Concomitant diseases and medical history will be presented using frequency tables.

8.1.7 Pharmacokinetic analyses

The analysis of PK variables (Section 6.4) will be based on the PK population (for definition see Table 10). PK variables will be summarized descriptively. A plot will show the manganese concentration over time for each patient (spaghetti plots).

8.1.8 Missing data

Adverse events with unknown onset times will be counted as post mangoral administration AEs. AEs with missing relationship to mangoral will be counted as possibly related AEs.

Missing efficacy data will not be replaced. Technical errors, e.g. laboratory values that have to be regarded as technical errors from a medical point of view, will not be included in the statistical analysis and will only be reported in the patient data listings. Exclusion of medically implausible data will be decided during data cleaning or a data review meeting before database lock.

8.1.9 Multicentre trial

This trial will be performed at multiple sites. The assessments used for the primary analysis will be performed by three independent readers for the pool of all MRI images of all sites. Therefore, the efficacy analysis will be performed for each reader separately and not stratified by site. Each of the study sites is expected to include only a small number of patients; therefore, also the analysis of secondary efficacy parameters based on on-site reads will not be stratified by site.

8.1.10 Patient data listings

All recorded data will be presented in the patient data listings.

8.1.11 Deviations from the planned statistical analysis

Any deviations from the planned statistical analysis have to be discussed in the final study report and should be defined in the statistical analysis plan. If the deviation has any impact on the confirmatory analysis, the deviation must be specified in an amendment to the study protocol.

8.1.12 Interim analysis

No interim analysis is planned.

8.1.13 Software used for statistical analysis

The SAS software version 9.4 or higher will be used for the statistical analysis and for the reporting of this trial.

8.1.14 Determination of sample size

The primary endpoint analysis consists of two co-primary one-sided t-tests at the conventional one-sided significance level of 0.025. Following the FDA's draft guidance on multiple endpoints in clinical trials, adjustment of the type-I-error is not necessary since both primary variables

need to show superiority simultaneously. Nonetheless, this affects the type II error. To counteract the inflation of the type II error, each test's power is set to 0.9 to achieve a study power of at least 0.9 x 0.9 = 0.81.

Effect size

The primary efficacy is based on qualitative parameters, but no exact estimates are available from previous studies due to the use of DWI in this study. A study investigating a manganese-based contrast agent with T1 / T2 and DWI comparing also unenhanced versus CMRI [39] reported qualitative score results on a scale from 1 to 5. These results are used to derive estimates for mean and standard deviation (SD) and scaled down for the 1 to 4 scale used in this study. Calculating an average score per patient and averaging readers after rescaling, leads to averages of 2.3 for unenhanced versus 2.6 for CMRI. This is a difference of 0.3 with each sample's standard deviation of ~1.9, or ~1.5 after rescaling. For estimation of the SD of the samples' differences, a high correlation (0.8) is assumed, which results in 0.95.

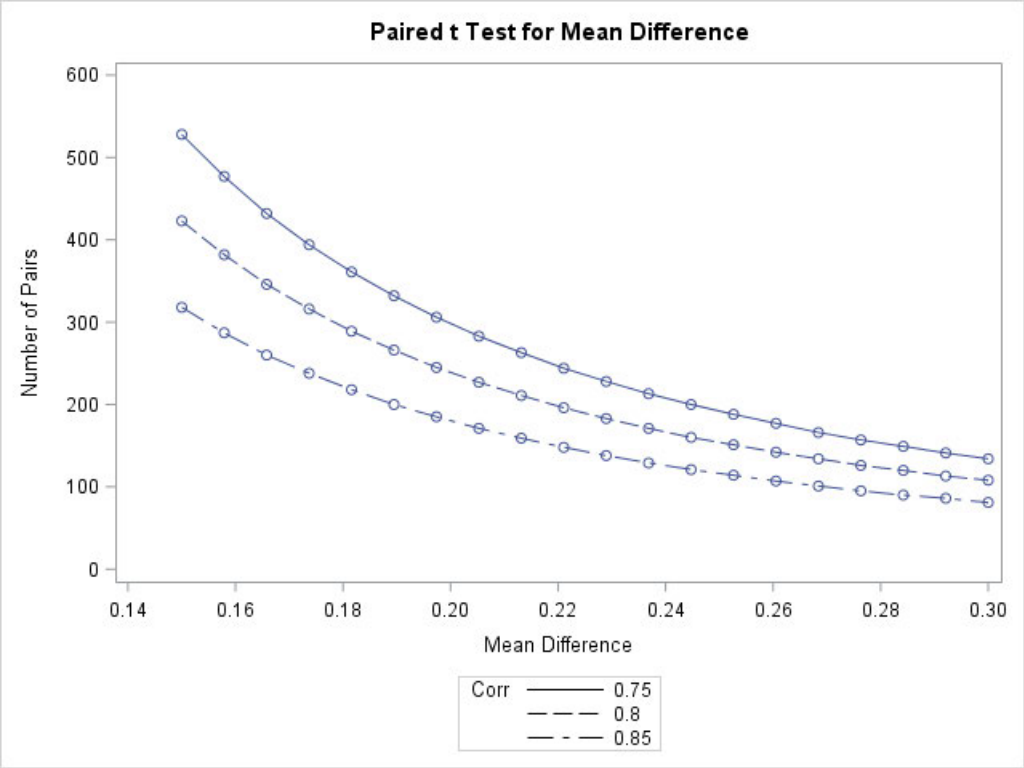
Conservatively assuming no effect of the subpopulation of the HCC patients (20% of the study population), the effect is 0.3 x (1-0.2) = 0.24.

Sample size calculation was performed using SAS® version 9.4 resulting in 167 patients needed for analysis (Table 11, Figure 2). Assuming, that only 85% of the image sets will be completely evaluable (all enhanced and unenhanced liver MRI images) and that no patients will drop out (since the efficacy procedure is completed within a few hours for each patient), 197 patients need to be included in the study.

Table 11 Sample sizes

Computed N Pairs				
SD paired	**Mean Difference**	**Correlation**	**Actual Power**	**N Pairs**
1.5, 1.5	0.2	0.75	0.9	298
1.5, 1.5	0.2	0.8	0.9	239
1.5, 1.5	0.2	0.85	0.9	180
1.5, 1.5	0.22	0.75	0.9	247
1.5, 1.5	0.22	0.8	0.9	198
1.5, 1.5	0.22	0.85	0.9	149
1.5, 1.5	0.24	0.75	0.9	208
1.5, 1.5	**0.24**	**0.8**	**0.9**	**167**
1.5, 1.5	0.24	0.85	0.9	126
1.5, 1.5	0.26	0.75	0.9	177
1.5, 1.5	0.26	0.8	0.9	142
1.5, 1.5	0.26	0.85	0.9	107
1.5, 1.5	0.3	0.75	0.9	134
1.5, 1.5	0.3	0.8	0.9	108
1.5, 1.5	0.3	0.85	0.9	81

Figure 2 Sample sizes depending on correlation and mean differences



9 DATA HANDLING AND DATA QUALITY ASSURANCE

9.1 Data management

Data will be captured using the eCRF provided by the designated CRO. The eCRF is specifically designed to meet the data recording requirements of the clinical study protocol. Only the investigator and his/her authorised staff (as listed on the form 'Task Delegation and Signature Form' provided by the CRO) who are trained appropriately on the eCRF system are allowed to fill-in the eCRFs or to make corrections, and will have access to the data or may change data (however without the possibility to delete the original entries). After completion, each eCRF will be electronically signed and dated by the investigator. Central laboratory test results will be imported automatically into the eCRF. For all laboratory and central imaging data, the units or any transformation of units must be clearly defined (if not otherwise agreed).

The overall procedures for quality assurance of clinical trial data are described in the respective standard operating procedures (SOPs) of the CRO. A document describing the functional specification will be prepared by the CRO in cooperation with the eCRF provider and be approved by the sponsor prior to the start of the trial. This functional specification will describe in detail the structure and functionality of the eCRF. In addition, the CRO will prepare a data management plan to describe the procedures and processes of data collection and data coding.

Electronic validation of the data entered in the eCRFs will take place (e.g. check of ranges, consistency, and plausibility). All plausibility checks will be defined in the data validation plan to be approved by the sponsor. Additionally, the eCRFs will be reviewed by qualified data management personnel and medically qualified personnel for completeness, consistency, and plausibility. Any data anomalies will be communicated to the site(s) for clarification/resolution (data query). Procedures for data entry and correction will be tracked and appropriately recorded in the audit trail of the eCRF.

9.2 Documentation

9.2.1 Screening log and patient identification list

A screening log will be kept by the investigator, listing all patients screened for this study. Patients will be identified by initials and date of birth. The status of the patient, i.e. whether enrolled in this study or not, will be entered on the log.

All patients who have given informed consent to study participation – regardless whether the patient has received any IMP or not – have to be entered on the patient identification list by the investigator, giving initials, date of birth, and patient identification code.

The screening log and patient identification list will be kept in the investigator's file.

9.2.2 Source data and patient records

In this study, all collected data in the patient file are considered source data, including MRI images and interpretations. Prior to including patients in the study, the investigator must sign a source data agreement that identifies the source documents (original documents, data, and records) at the site. The document will also list which data may be recorded directly on the eCRFs or any electronic device (as applicable).

Definition of source data: All information in original records and certified copies of original records of clinical findings, including observations or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

9.2.3 Electronic case report form (eCRF)

An appropriate eCRF in English will be developed by the CRO. An eCRF completion manual for the use of the electronic data capture system will be provided prior to study start and investigators will be trained on how to enter all study-specific data into the eCRF. Investigators must enter the information required by the protocol into the eCRF and should complete the eCRF as soon as possible, but latest on the third working day after a patient visit (or in case of on-site assessment results: within 3 working days after the on-site assessment). No questions must be left unanswered. Note: all SAEs must be reported by the investigator immediately (within 24 hours) via the SAE paper form and additionally in the eCRF in timelines specified in CRF completion manual (Section 6.3.7.4).

For all countries, the eCRFs have to be completed in English. Monitors will review the eCRFs for completeness and accuracy, and instruct site personnel to make any required corrections or additions. The eCRFs are saved immediately after entering the data by the investigator. All eCRF data are stored in a central database at the eCRF provider while the trial is ongoing and at the responsible CRO for final analysis after trial completion.

After trial completion, the trial site will receive a digital media with a compilation of the eCRF trial data per site (electronic PDF files) for filing with its trial documents. The sponsor will receive electronic, bookmarked PDF files of individual eCRFs (including audit trail). The sponsor will also receive a list of queries and SAE forms after database closure.

9.3 Direct access to source data/documents

In accordance with ICH GCP E6(R2), the investigator/study site will permit direct access to source data/documents for trial-related monitoring, audits, IECs/IRBs review, and regulatory inspection(s). Direct access includes permission to examine and verify any records that are important for the evaluation of the study. Patients will consent in writing to the direct access to their original medical record for trial-related monitoring (source data verification), audits and regulatory inspections.

9.4 Monitoring

Monitoring will be done in accordance with the stipulations of Section 5.18 of the ICH GCP E6(R2) guideline and sponsor's / CRO's SOPs by designated representatives of the sponsor or CRO (study monitor). The sponsor / CRO will develop a separate clinical monitoring plan tailored to the specific patient protection and data integrity risks of the study.

The study monitor will visit the study site prior to starting the first study-related procedure at this site (initiation visit) to ensure the eligibility of the study site, qualification of staff, and appropriateness of technical equipment, to discuss the protocol, study-related procedures, and responsibilities, and to ensure that the investigator receives all documents (including an up-to-date version of the mangoral investigator's brochure [19]) needed to conduct the study properly. The investigator will allow the study monitor to make regular visits during the course of the study to:

- Verify that written informed consent was obtained prior to each patient's participation in the study
- Discuss any emergent problems
- Check the eCRFs for completeness and plausibility
- Review accountability, correct storage, and handling of the investigational medicinal product
- Ensure that the study is being conducted according to pertinent regulatory requirements, ICH GCP E6(R2), and the protocol

eCRF entries will be verified against source documentation in the patient's medical record or in the location stated in the source data agreement. Study monitors will maintain confidentiality of the patients' personal data.

A close-out visit will be performed upon conclusion of the study site's participation in the study.

9.5 Quality assurance

Risk-based quality management will be implemented in this study based on the requirements of the Integrated Addendum of the Good Clinical Practice guideline ICH E6(R2). Aspects of risk-based quality management will be detailed in a risk management plan.

A member of the sponsor's (or designated CRO's) quality assurance unit may conduct an audit at the study site to ensure compliance with the protocol, GCP and applicable regulatory requirements.

In addition, inspections by regulatory health authority representatives and IEC(s) / IRB(s) are possible. The investigator or his / her designee should notify the sponsor immediately of any such inspection.

The investigator is expected to cooperate with such inspections and to discuss any findings with the auditor. The observations and findings of the auditor will be recorded and the investigator will be informed of the audit outcome.

10 ETHICAL, LEGAL AND ADMINISTRATIVE ASPECTS

The study will be carried out in conformity with the ethical principles enunciated in the Declaration of Helsinki [40]. The currently valid version according to applicable national regulatory and legal requirements will be considered.

The study will also be carried out in accordance with the Integrated Addendum of the ICH guideline on Good Clinical Practice E6(R2), the Regulation EU No. 536/2014, which will replace the EU Clinical Trial Directive 2001/20/EC, the Commission Directive 2005/28/EC (April 8, 2005), and all local laws, regulatory requirements, and guidelines applicable in the participating countries.

By signing the Investigator's Declaration (Appendix I), the investigator agrees to conduct the study as set out in this protocol and in accordance with the moral, ethical and scientific principles governing clinical research.

The study personnel and vendors (i.e. key study personnel) is provided in Appendix II.

The signatures of sponsor and coordinating investigator can be found in Section 12.

10.1 Approval procedures

Before the start of the study, the study protocol, patient information / informed consent form, and other relevant documents will be submitted by the investigator or designated CRO to the relevant IEC/IRB for all participating sites/countries in accordance with national laws, guidelines, and GCP provisions. Written IEC approval must be obtained prior to patient enrolment.

The relevant local authorities will be notified/asked for approval of the intended study in accordance with local legal requirements.

10.2 Protocol amendments

After initiation of the study, any change in this protocol will require a formal amendment. The amendment must be signed by all of the signatories to the original protocol. Once the study has started, amendments will be made only in exceptional cases.

If ethically relevant aspects are concerned, the IEC/IRB will be informed of amendments and approval will be sought. All protocol amendments will be submitted to the regulatory authorities as regulated by national law.

Changes to the protocol may only be implemented after all appropriate requirements listed above (ethics, regulatory approval of all responsible personnel) have been fulfilled.

10.3 Informed consent

The procedure of informed consent will be carried out in accordance with all applicable laws and regulations. The purpose and nature of the study, possible risks and benefits of IMP administration, planned procedures and potential discomforts, and usage, transfer, and disclosure of personal data and study-related health data will be explained in writing in a patient information sheet, which will be handed to the patient by the investigator, or an authorised designee. In addition, the investigator, or an authorised designee, will verbally provide all relevant information to the patient. Sufficient time will be allowed to discuss any questions raised. The patient will be made aware that participation in this study is voluntary and that he/she can withdraw their consent to participate at any time without giving a reason and without having to fear any disadvantageous effects on his/her current or future medical care. The

investigator will inform the patient that in providing informed consent, he / she is giving permission for representatives of the sponsor, ethic committees, or competent authorities to inspect their medical records for data verification. Patients will be informed that all personal information made available for inspection will be handled in the strictest confidence and in accordance with all applicable data protection laws and regulations.

Only after written and verbal information has been provided, can consent for participation be given by the patient by personally signing and dating the approved informed consent form. The signed documents must be retained by the investigator as part of the study records. A copy of the signed informed consent form will be given to the patient.

The investigator will not undertake any procedures required for this study until informed consent has been obtained. The date of obtaining informed consent will be entered in the eCRF.

After releasing an amendment to the protocol, that might influence the patient's decision for participation, the patient information sheet and the informed consent form must be amended accordingly. Revised informed consent sheets must be submitted to the relevant IECs/IRBs and the relevant authority as required by local law. In addition, any relevant new information about the IMP that becomes available during the study will be passed on to the investigators and the patients. Depending on the nature of the amendment, it might be necessary for patients who are already enrolled to confirm their informed consent on the basis of the new information.

10.4 Confidentiality and data protection

All relevant provisions of the European General Data Protection Regulation (GDPR [EU] 2016/679, 27 April 2016) and all local legal requirements regarding the protection of personal data will be adhered to.

The anonymity of trial patients will be maintained. Patients' names will not be supplied to the sponsor or designated CRO. Patients will be identified on all eCRFs, image media and other documents by a specific patient identification (screening) number. Documents that identify the patient (e.g. the signed informed consent) must be maintained in confidence by the investigator. Sponsor, CRO, central laboratory, and off-site readers will only be given pseudonymised data, laboratory samples, or images.

The patients will be informed about all media used in this study for documentation, storing, and transferring the patients' study findings and will be assured that all data will be handled in the strictest confidence.

This study protocol, any other unpublished documentation (e.g. eCRF), any information regarding the IMP (e.g. investigator's brochure), and any results derived from the study will be regarded as confidential. The investigators and study site members will not be allowed to disclose such information without prior written approval from the sponsor.

10.5 Liability, insurance and finances

The sponsor has taken out appropriate third-party liability insurance coverage in accordance with all local legal requirements in the respective country in which the study is performed. It covers the eventuality that personal injury may be caused by using the IMP or by any trial-specific procedure carried out according to this protocol.

The general insurance conditions will be kept in the investigator's file and will be made available for patients at any time.

The financial agreements for each site (e.g. Clinical Trial Agreement) are addressed in one or more documents. The parties must sign the agreement before each site is initiated. All investigators and other relevant site staff participating in this study must complete a Financial Disclosure Form.

10.6 Clinical study report, publication and use of study results

An integrated study report according to the standards of the ICH E3 guideline, covering clinical and biometrical aspects, will be prepared by the sponsor or its designee within 1 year of the end of the study (last protocol-defined contact with any enrolled patient). The report will be reviewed and approved by the coordinating investigator.

A publication policy will be prospectively defined before the start of the study. Any results derived from this study may be published in a scientific journal or presented at a scientific meeting with the sponsor's consent. The sponsor will be provided with a copy of the manuscript for review and approval prior to any such submission.

The sponsor will make the protocol and results of this study, publicly available on the internet at www.clinicaltrials.gov and in the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database. Upon completion of the study and when the study results are available, the patient has the right to be informed by the investigator about the overall study results.

10.7 Archiving of study documents

Essential documents as listed in ICH GCP E6(R2), Section 8, shall be archived safely and securely in such a way that they are readily available upon authorities' request.

Copies of the protocol, patient information sheet / informed consent form, patient identification list, a printout of eCRF (or e.g. digital media with the eCRF), the patient record with all original data and original images, and all other documents pertaining to this study will be retained at the study site for a minimum of 25 years after the completion and approval of the integrated study report or for a period that is in accordance with national regulatory requirements, whichever is longest. . The investigator site file is not to be destroyed without the sponsor's approval. Ascelia Pharma or a designee will remind the investigator in writing of this obligation when the clinical study report synopsis is distributed to the site.

The final integrated study report must be retained by the sponsor for 5 years beyond the lifetime of the IMP.

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12 CLINICAL STUDY PROTOCOL - SIGNATURE PAGES

SIGNATURES

The signatories are obliged to comply in all respects with

- this clinical study protocol;
- the standards of Good Clinical Practice as defined in the Note for Good Clinical Practice (CPMP/ICH 135/95) and related guidelines;
- the Declaration of Helsinki current valid version according to applicable national regulatory and legal requirements;
- all applicable regulatory requirements including national drug and data protection law.

I hereby confirm that I am of the opinion that the ethical and scientific basis of this study is sound.

Signatures of Sponsor Representatives:

Ascelia Pharma

[redacted]

Place / date Signature

[redacted]

Signature of Study Co-ordinating Investigator:

The signatories are obliged to comply in all respects with

- this clinical study protocol;
- the standards of Good Clinical Practice as defined in the Note for Good Clinical Practice (CPMP/ICH 135/95) and related guidelines;
- the Declaration of Helsinki current valid version according to applicable national regulatory and legal requirements;
- all applicable regulatory requirements including national drug and data protection law.

[redacted]

Place / date Signature

13 APPENDICES

I Declaration by the Investigator

Kept separate

II Study personnel and vendors (i.e. key study personnel)

Kept separate