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.

NCT Number: NCT04119843

Document Date: 02 June 2023

Statistical Analysis Plan (SAP)

Study 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.
Study Name:	SPARKLE
Protocol Version No. / Date:	ASC-Man-P016 version 7.0 / 12-Oct-2021
CRF Version No. / Date:	Version 5.0 / 19-Jul-2022
SAP Version No. / Date:	Final 4.0 / 02-Jun-2023

1.0 Approvals

Sponsor	
Sponsor Name:	Ascelia Pharma AB
Representative / Title:	[redacted]
Signature / Date:	
Representative / Title:	[redacted]
Signature / Date:	
PRA	
Biostatistician / Title:	[redacted]
Signature / Date:	

(NOTE: Electronic Signatures should only be used if all parties have the ability to eSign.)

2.0 Change History

Version / Date	Change Log
1.0 / 13-Nov-2020	Final (stable) version
1.1 / 15-Nov-2022	Updated due to protocol v6 and v7 respectively as well as sponsor comments Major changes: • Updated approvers section. • Updated based on CRF v5. • Updated changed from protocol section: definition of per protocol set and subgroup analyses planned. • Updated section on subgroup analyses. • Updated rules for handling of partial dates and times of medications and adverse events.
2.0 / 20-Dec-2022	Revised to sponsor comments. Major changes: • Additional ANOVA analyses on primary endpoint. • Removal of subgroup analyses by status of liver disease and additional subgroup analyses by sex instead. • Amended derivations for liver-to-lesion contrast and contrast to noise ratio. • Update to PK parameters to be derived. • Updated specification for intra-reader bland-altman plots.
3.0 / 14-Feb-2023	Sample size estimation updated. Missing reference added.
4.0/ 02-Jun-2023	Minor editorial changes and corrections to align with shells. Major changes • Removal of ANOVA analysis. • Laboratory shift tables is by CTCAE grade. • Concentration data listed and summarized for the safety analysis set instead of the full analysis set. • Clarification on patient management analysis. • Explanation on QTcB and QTcF re-derivations. • Updated wording of some analysis sets to be clearer.

3.0 Table of Contents

4.0 Abbreviations

AE	Adverse event
ATC	Anatomic therapeutic chemical classification
BD	Border delineation
CI	Confidence interval
CMRI	Combined mangoral-enhanced and unenhanced MRI
CNR	Contrast-to-noise ratio
CRF	Case report form
CRO	Clinical research organization
DWI	Diffusion-weighted imaging
GCP	Good clinical practice
FAS	Full analysis set
HCC	Hepatocellular carcinoma
ICH	International conference on harmonization
IMP	Investigational medicinal product
LC	Lesion contrast compared to liver background (qualitative)
LLC	Liver-to-lesion contrast (quantitative)
LLOQ	Lower limit of quantification
Max	Maximum
MedDRA	Medical dictionary for regulatory activities
MeMRI	Mangoral-enhanced MRI
Min	Minimum
MRI	Magnetic resonance imaging
N	Number of non-missing observations
Nmiss	Number of missing observations
PK	Pharmacokinetics
PPS	Per protocol set
PT	Preferred term

SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical analysis plan
SAS	Statistical analysis system
sBD	Sum of scores of border delineation
SD	Standard deviation
SI	Signal intensity
sLC	Sum of scores of lesion contrast compared to liver background (qualitative)
SOC	System organ class
SOP	Standard operating procedure
TFLs	Tables, Figures, Listings
UnMRI	Unenhanced MRI
V	Visit
WHO	World health organization (United Nations)
WHO-DD	World health organization drug dictionary

5.0 Purpose

The Statistical Analysis Plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Ascelia Pharma AB Protocol ASC-Man-P016 version 7.0 dated 12-Oct-2021.

6.0 Scope

The Statistical Analysis Plan outlines the following:

- Study Objectives
- Study Design
- Study Endpoints
- Applicable Study Definitions
- Statistical Methods.

7.0 Introduction

This Statistical Analysis Plan (SAP) is based on the relevant sections of study protocol. Tables, Figures and Listings (TFLs) Specifications are described in a separate document (TFL Shells). The purpose of the SAP is to describe in more detail how the analyses are to be performed and presented. It quotes the relevant statements directly from the protocol, but it does not give a full description of study design etc.

The TFL Shells specify the output to support the analysis. The TFL Shells are considered as a guideline for producing the output.

The SAP will be finalized prior to database lock or any unblinding of study team members.

The investigational medicinal product (IMP) is mangoral (previous candidate name: CMC-001) containing manganese (II) chloride tetrahydrate ($MnCl_2$ $4H_2O$) combined with L-alanine and vitamin D3. It will be referred to hereafter as mangoral in this document. Mangoral is for diagnostic use and for single dose administration only.

The aim 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.

7.1 Changes from Protocol

The planned analysis will be performed according to the study protocol, its amendments and this statistical analysis plan. If there are contradictions between the study protocol or its amendments and this statistical analysis plan, the analysis will be performed according to this analysis plan. Any deviation from the planned analysis according to the study protocol has to be described in the integrated report.

The protocol states that the per protocol set is all patients in the full analysis set without any important protocol deviations. The SAP has been revised to state that only a subset of important deviations will result in patients being excluded from the per protocol set. All important protocol deviations will be manually reviewed by ICON and Ascelia to agree which could have impact on the per protocol set.

The protocol mentions the requirement for adverse drug reactions (defined per protocol as a causal relationship between a medicinal product and an AE to be at least a reasonable possibility) to be collected

and summarized. However, in this study adverse events are only assessed as related or not related so this will be used for the outputs.

The wording and definition of the PK dialysis subgroup population is amended.

The protocol Section 8.1.5 mentions subgroup analysis to be performed on the co-primary variables to be done by status of liver disease, on the subgroup of patients with PK data and also subgroup of patients with brain MRI, this is not included in the SAP as the patient groups are considered to be too small for a meaningful comparison. Subgroup analysis on the co-primary variables by sex will be added. Additionally subgroup analyses is mentioned in the protocol for by lesion diameter on the primary endpoint, this is not planned but different subgroup analyses is planned as described in Section **13.6.3**.

The derivations for liver-to-lesion contrast and contrast to noise ratio are amended slightly as described in Section **11.2.5**.

QTcB interval (msec) and QTcF interval (msec) were derived in the database but issues were found with these calculations, for this reason these parameters were re-derived in the datasets per the derivations in Section **11.2.1**.

8.0 Study Objectives

8.1 Primary Objective

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

8.2 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);
  - visualization of detected focal liver lesions in mangoral-enhanced MRI compared to unenhanced MRI (determination of visualization will be done in the same way as for the primary efficacy variable);
  - confidence in lesion detection and localization separately in unenhanced MRI, mangoral-enhanced MRI and CMRI;
  - lesion dimensions (independent off-site readers' assessment): longest diameter of largest and 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.

9.0 Study Design

9.1 Overview

This is a multicenter, open-label, pivotal phase III study to evaluate safety and diagnostic efficacy of mangoral in patients with known or suspected focal liver lesions and concurrent severe renal impairment.

IMP is intended for diagnostic use and for single dose administration only.

Primary diagnostic efficacy in terms of visualization of detected focal liver lesions will be evaluated centrally at a core imaging laboratory by 3 independent readers (off-site readers). In each patient, 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.

As all readings of MRIs are performed in each patient (in Parts I, II and III), no randomization procedure is used. Nevertheless, all independent central readers will be blinded to clinical data, site and country information.

Secondary diagnostic endpoints will be evaluated by off-site readers and on-site readers independently, as described in Table 5 of the protocol (see also Table 1 below).

Table 1 Overview of efficacy variables

Assessment	**Off-site (central)**			**On-site**
Reader(s)	Three (3) independent readers			Investigator (on-site radiologist)
Reading session[a]	Part I	Part II	Part III	
Primary variable: Visualization of detected focal liver lesions in combined MRI (CMRI, mangoral-enhanced MRI plus unenhanced MRI) as compared to unenhanced MRI.	X	X		
Secondary variables[b]**:**				
- Number of lesions detected by each MRI method: unenhanced MRI, mangoral-enhanced MRI and CMRI.	X	X	X	X
- Visualization of focal liver lesions in mangoral-enhanced MRI as compared to unenhanced MRI.	X		X	
- Confidence in lesion detection separately in unenhanced MRI, mangoral-enhanced MRI and CMRI.	X	X	X	X
- Confidence in lesion localization separately in unenhanced MRI, mangoral-enhanced MRI and CMRI.	X	X	X	X

Assessment	Off-site (central)			On-site
Reader(s)	Three (3) independent readers			Investigator (on-site radiologist)
Reading session[a]	Part I	Part II	Part III	
- Lesion dimensions.	X		X	
- Quantitative assessments.		X		
- Change(s) in patients' management based on diagnostic performance of CMRI or mangoral-enhanced MRI vs. unenhanced MRI.	X	X	X	X

[a]Part I: unenhanced MRI; part II: paired reading (CMRI); part III: mangoral-enhanced MRI
[b]Any simple liver cysts are excluded from primary and secondary variable assessments.

Up to 197 patients with severe renal impairment who are being evaluated for known or suspected focal liver lesions were originally planned to be enrolled at about 60 investigational sites in Europe, Asia, USA and South America. Following a revised sample size estimation (see Section **9.3**), 80 patients were considered sufficient for the analysis of the primary endpoint.

Individual sites are allowed to include study patients with known or suspected HCC; the majority of enrolled patients are considered to have liver metastasis or other common focal liver lesions. Overall, HCC patients are expected to constitute approximately 20% of the total study population.

The schedule of visits and study-related procedures at each visit are presented in the protocol Table 9. Scheduled follow-up visits (FU1, FU2, FU3 and FU4, if required) may be performed as outpatient visits. 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, for whom previous brain MRI images are available and who have separately consented to this optional brain MRI, shall return to the site for the FU4 visit 7 (+ 2) days after the mangoral-enhanced liver MRI for MRI of the brain.

For the schedule of assessments refer to the protocol Table 9.

9.2 Parameters

All parameters through which the study objectives will be carried out, will be listed. Exact computations are declared in Section **11.2**.

Primary and secondary efficacy assessments will be performed centrally by independent readers who will be blinded with regard to patient identity and all clinical information of a patient.

Independent readers and on-site radiologists will assess focal liver lesions on images from unenhanced, combined and mangoral-enhanced MRI described in detail in Section 6.2.1 of the protocol.

9.2.1 Visualization of lesions

In every patient, up to 15 lesions will be assessed by each MRI method: unenhanced MRI, mangoral-enhanced MRI and CMRI (on-site and independent off-site readers' assessments).

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 MRI images for inclusion in the analysis of the co-primary variables.

9.2.2 Primary Efficacy Parameter

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

The visualization will be assessed using two co-primary variables:

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

Both parameters will be determined for each lesion (up to 15 lesions per patient) and by each of the 3 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 (defined in the Section 6.2.3.1 of the study protocol). 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.

For primary efficacy endpoint analysis, only lesions that are seen on both pre- and post-contrast (Part I and Part II) will be considered and results of individual readers will be reported separately. The matcher will match lesions for unenhanced, enhanced and combined MRI. It is possible, that lesions are not seen on an unenhanced MRI scan, but become visible on the combined MRI and/or enhanced MRI scan. Since the primary endpoint refers to the visualization of the lesion, in this event, these lesions will not be considered for the primary efficacy analysis.

9.2.3 Secondary Efficacy Parameters

The secondary efficacy variables will be based on the evaluations by independent readers (off-site readers’ assessment) and selected endpoints (number of lesions per MRI method, change in patients’ management and confidence in lesion detection and localization and change in patients’ management) also by the on-site radiologist (on-site readers’ assessment) as specified in Table 1. The secondary efficacy endpoints assessed off-site will be evaluated using the same number of lesions as the primary endpoint, if not specified otherwise.

9.2.3.1 Number of lesions detected by each MRI method and proportions of patients

Number of lesions detected by each MRI method (unenhanced MRI, mangoral-enhanced MRI and CMRI) per patient will be presented using descriptive statistics.

In addition, the following proportions of patients will be calculated for assessments performed by independent off-site readers:

- The number of patients having at least one lesion identified on post-mangoral images that was not identified on pre-mangoral images.
- The number of patients having at least one malignant lesion identified on post-mangoral images that was not identified on pre-mangoral images based on patients with at least one malignant lesion at baseline. For patients with malignant lesions at baseline any new lesions identified are also assumed to be malignant.
- The number of patients who had improvement in at least one co-primary variable for at least one lesion on post-mangoral images compared to pre-mangoral images.

9.2.3.2 Visualization in mangoral-enhanced MRI vs. unenhanced MRI

Visualization of focal liver lesions in mangoral-enhanced MRI as compared to unenhanced MRI will be assessed in the same way as for the primary efficacy endpoint (by the off-site readers only).

9.2.3.3 Confidence in lesion detection and localization

Confidence in lesion detection is defined in Section **11.2.3** and will be assessed 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 localization is defined in Section **11.2.4** and will be assessed 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).

The lesions will be listed in ordinal sequence from the smallest to the largest with specification of the liver segment in which they have been identified. For assessments provided by on-site readers, all lesions detected per MRI method (regardless if they are matched compared to unenhanced MRI) will be included in descriptive statistics.

9.2.3.4 Lesion dimensions

Lesion dimension measurements (diameter measured in mm) will be done by each of the 3 independent readers on mangoral-enhanced MRI and unenhanced MRI images for all lesions. The longest diameter of the largest and of the smallest visualized lesions, respectively, will be summarized.

9.2.3.5 Quantitative assessments

Quantitative assessments are specified in Section **11.2.5** and 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).

9.2.3.6 Change in patients' management

Change(s) in patients' management based on 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).

A patient can have more than one recommended management per MRI method, for this reason all results will be included in the listings and the tables.

9.2.4 Safety Parameters

This section defines the general principles for the analysis of categorical and continuous parameters, unless specified otherwise. In general, the analysis of all parameters will be done by visit/time point and treatment group and overall.

Categorical parameters will be descriptively summarized per visit/time point and overall post-baseline. For an overall post-baseline analysis, the worst value during the on-treatment period, including unscheduled visits, will be derived. If applicable, the number of not exact values will be displayed in the respective table. Continuous parameters will be descriptively summarized. Changes from baseline will be presented for all post-baseline visits/time points. Baseline values are defined as described in Section **11.2.1**.

Where applicable, continuous laboratory parameters will be classified as *low*, *normal*, or *high* based on reference (normal) ranges as provided by the central laboratories.

A listing of patients with values outside the reference ranges will be provided. All parameter values will be presented in a patient data listing including respective flagging with respect to reference ranges.

Unscheduled measurements of parameters will be presented in the patient data listing. In general, unscheduled measurements will not be included in the analysis.

9.2.4.1 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].

The investigator will report any worsening from baseline in vital signs as AE (see Section 6.3.7 of the protocol).

9.2.4.2 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 abnormalities must be specified in the eCRF and recorded as an AE (see Section 6.3.7 of the protocol).

9.2.4.3 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.

Results of the assessments will be recorded in the eCRF. All new (i.e. no pre-existing condition) abnormal findings observed after mangoral administration must be recorded and reported as AEs (see Section 6.3.7 of the protocol).

9.2.4.4 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.

ECG parameters will be recorded and assessed: overall interpretation, heart rate (beats/min), QT interval (msec), RR interval (msec), PR interval (msec), QRS duration (msec), QTcB interval (msec) and QTcF interval (msec).

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 specified in the eCRF and if observed after administration of mangoral, the abnormality must also be recorded as an AE.

Note: As specified in Section 6.3.7 of the protocol, if electrocardiographic abnormalities observed in a pre-existing condition are preserved also after the administration of mangoral, these should not be considered as AEs. Only if abnormalities are worsening of pre-existing ones, they are considered as AEs.

9.2.4.5 Laboratory variables

Details of laboratory parameters are specified in Section 6.3.5 of the protocol. In summary, Table 2 provides an overview of the screening laboratory tests and Table 3 provides an overview of other laboratory parameters measured at baseline and post-dose visits.

The investigator has to 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.

Table 2 Screening laboratory parameters

Child-Pugh: *(local lab)*	Serum bilirubin Serum albumin International normalized ratio (INR)	eGFR: (*local lab*)	Estimated Glomerular Filtration Rate (eGFR) Serum creatinine Serum albumin Serum urea nitrogen (SUN/BUN)

Table 3 Clinical safety laboratory parameters (baseline and post-dose)

Hematology: *(central lab)*	Hemoglobin Hematocrit Erythrocytes Leukocytes Platelets Neutrophils Eosinophils, Basophils Lymphocytes Monocytes INR	Biochemistry: (*central lab*)	Alkaline phosphatase Aspartate aminotransferase (AST) Alanine aminotransferase (ALT) Gamma-glutamyl transferase (GGT) Creatinine Glucose Urea Total Bilirubin Albumin Sodium Potassium Calcium Magnesium Chloride
Urinalysis: *(local lab)*	pH Leucocytes Erythrocytes Protein Glucose Urobilinogen Ketones Bilirubin Nitrite Specific gravity		

All clinically significant abnormal laboratory values observed at 5 (± 2) days post-dose are to be followed up until they have normalized, or until in the investigator's opinion there is no medical necessity for further blood tests.

9.2.4.6 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.

For patients in the PK subgroup (selected from a few sites) additional blood samples for PK assessments will be taken on the day of mangoral administration. Blood samples will be taken on the day of mangoral administration immediately (1 hour pre-dose) and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 120 hours post-dose.

9.2.4.7 Pregnancy test

A serum pregnancy test will be performed in women of childbearing potential at the local laboratory at 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. In the case of pregnancy, the patient is not allowed to enter the study or will be withdrawn from further study participation. The event is to be captured on dedicated CRF page.

9.2.5 Pharmacokinetic parameters

The aim is to evaluate the pharmacokinetics of manganese in a subgroup of patients for whom blood samples were taken after mangoral administration according to a specific sampling schedule. These patients will be selected from a few sites that are able to include these patients and follow PK requirements.

The pharmacokinetic evaluation of manganese will be performed using non-compartmental pharmacokinetic analysis by or under the supervision of the sponsor. Based on the individual blood concentration-time profiles (using the actual sampling times), the pharmacokinetic parameters given in Table 4 will be derived.

Table 4 Pharmacokinetic parameters

Parameter	Description
AUC	Area under the concentration-time curve from zero up to infinity with extrapolation of the terminal phase.
AUC_{0-120}	Area under the concentration-time curve from zero until time (120 hours). This parameter may contain an extrapolated portion if *t* is replaced by a definite time.
AUC_{0-24}	Area under the concentration-time curve from zero until time (24 hours). This parameter may contain an extrapolated portion if *t* is replaced by a definite time.
C_{max}	Maximum observed concentration after administration.
λ_z	First order rate constant associated with the terminal elimination phase estimated by linear regression of the logarithmic transformed concentration-time plot.
R^2_{adj}	Adjusted coefficient of determination of the terminal regression line.
$t_{1/2,z}$	The half-life associated with the terminal elimination phase.
t_{max}	Time to attain C_{max}.
Cl_{app}	Total apparent clearance calculated as dose/AUC

LLOQ = lower limit of quantification.

This analysis will also be repeated considering minimum concentration adjusted results (by patient) as below, the new pharmacokinetic parameters will be referred as “adjusted by C_{min}“:

Minimum concentration adjusted = Concentration at timepoint - Minimum concentration

Further the percentages of:

($AUC_{0-24\ h}$ adjusted by C_{min} / $AUC_{0-24\ h}$ unadjusted)*100

(C_{max} adjusted by C_{min} / C_{max} unadjusted)*100

9.3 Sample Size Considerations

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 studies, 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. When designing the ASC-Man-P016 study, a conservative approach was applied when calculating the sample size due to uncertainties regarding the assumed treatment effect overall and in patients with HCC lesions in particular, and the impact of using diffusion-weighted imaging (DWI).

The original sample size determination was based on a study investigating a manganese-based contrast agent with T1 / T2-weighted imaging and DWI. Efficacy was evaluated by comparison of unenhanced versus CMRI qualitative score results for "likelihood that a lesion represented a metastasis" on a scale from 1 to 5 **[1]**. These results were used to derive estimates for mean and standard deviation (SD) and scaled down for the 1 to 4 scale used in this study. Calculating a mean score per patient and averaging readers after rescaling, lead 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) was assumed, which resulted in SD of differences of 0.95.

Conservatively assuming no effect of the subpopulation of the HCC patients (20% of the study population), the difference in the full population was estimated to 0.3 x (1-0.2) = 0.24.

This resulted in a need of 167 patients for the analysis to reach 90% power for each test. With an assumption that only 85% of the image sets would be evaluable (all mangoral-enhanced and unenhanced MRI images) and that no patients would drop out (since the MRI procedures are completed within a few hours for each patient), it was concluded that 197 enrolled patients were required as per the original sample size estimation.

However, since the original sample size estimation, new information relevant to the underlaying assumptions of the effect size has become available. This includes a recent re-analysis of the early phase CMC-P004 study **[2]**. In Study CMC-P004, the MRI images were assessed by 2 investigating radiologists in consensus, who were not blinded to the treatment or the clinical information. A re-read of images from Study CMC-P004 has been performed by utilizing the 3-reader paradigm that is recommended in the guidance for developing medical imaging drugs **[3]** and is to be used in the present study (Study ASC-Man-P016). The primary objective of the re-read (P004A) was to estimate the diagnostic efficacy of mangoral MRI (combined mangoral-enhanced and unenhanced MRI) and MultiHance® MRI (combined MultiHance®-enhanced and unenhanced MRI) for visualization of focal liver lesions assessed by 3 independent radiologists. The MRI images from Study CMC-P004 were re-assessed according to the procedures described in Section **9.2.2**. The preliminary analysis showed that the median effect size (CMRI vs. unenhanced) was 0.74 (data on file).

Study CMC-P004 used a dose of 1600 mg manganese chloride tetrahydrate, which is conservatively assumed to give a 20% higher effect than the 800 mg dose that is used in the ASC-Man-P016 study resulting in an effect size of 0.74 / 1.2 = 0.62. Further, the effect in the subpopulation with HCC lesions is assumed to be half the effect seen in the population with metastases and potentially constituting up to 30% of the total patient population resulting in the further reduction of effect size to 0.52. The impact of DWI has conservatively been assumed to reduce the contrast effect by further 25%. Taken together, an effect size of 0.52/1.25=0.42 is considered relevant and has been used for a new estimation of the sample size.

Sample size

With the effect size of 0.42, 59 evaluable patients are required to achieve 81% power in a single reader test. With the success rule of 2 out of 3, this corresponds to 90% power to meet the co-primary efficacy endpoint. With this smaller sample size, a somewhat larger margin to account for non-evaluable patients

has been applied. In the original calculation 85% evaluable patients of the total population was assumed, but now ~75% of the total population is assumed to be evaluable. With this assumption, at least 80 patients with both unenhanced and mangoral-enhanced MRI will be needed for the primary analysis.

9.4 Randomization

No randomization procedure is used, this study is open-label and only has 1 treatment for all patients. However, central read data of the liver from the imaging vendor will be masked to ICON statistics/programming and Ascelia prior to database lock (ICON data management will be unblinded for reconciliation purposes).

10.0 General Statistical Considerations

In general, all continuous measures will be summarized descriptively, including number of available values, minimum, 1st quartile, median, mean, standard deviation, 3rd quartile and maximum. Categorical data will be presented by frequency and percentage. Ordinal ratings may be handled as continuous data.

10.1 Descriptive Statistics

The following descriptive statistics will be calculated for continuous data and for ordered categorical data (ordinal data):

Summary statistics are displayed with the following digits:

Description	Characteristic	Number of decimal places
Count	n	0
Count corresponding to the number of patient for a treatment group	N	0
Mean	Mean	As in source + 1
Standard Deviation	SD	As in source + 2
Minimum, Maximum	Min , Max	As in source
Median	Median	As in source + 1
1st Quartile / 3rd Quartile	Q1/Q3	As in source + 1
Percentage relative to N	%	1
Coefficient of Variation (%)	CV (%)	1

+ Number of decimal places will not be displayed where the count is zero, also if percentage is 100% no decimal places will be shown,

For ordered categorical data and nominal data, absolute and relative frequencies (in %) will be calculated:

- Display percentage information only for number of events bigger than zero.
- Percentages will be displayed to 1 decimal place except if it is 100%.

All data will be presented in the patient data listings.

If an output does not include any observations, then the following placeholder will be used: “NO DATA CONTRIBUTED TO THIS TABLE / LISTING / FIGURE”.

10.2 Inferential Statistics

Inferential methods will be based on a significance level of 0.05 for 2-sided tests and 0.025 for 1-sided tests. All statistical tests besides the primary efficacy analysis will be interpreted in an exploratory manner.

The testing of efficacy will be done separately for each of the 3 independent readers and will be based on mean scores of BD and LC. For each independent reader, the hypotheses for the superiority of CMRI versus unenhanced MRI will be tested using a 1-sided paired t-test comparing mean score differences between treatment groups, assuming that the differences are normally distributed. As a sensitivity analysis, the non-parametric Wilcoxon signed-rank test will be also performed.

In all statistical tables, p-values will be reported as specified by the statistical program used, at least up to three decimal places. The p-values less than 0.001 will be reported as provided by SAS (e.g. '<0.001').

10.3 Analysis Populations

All patients enrolled in the study (fulfil all inclusion criteria, but none of the exclusion criteria and have been included in the clinical study at Visit 2) will be included in the safety analysis (safety population). The efficacy analysis will be performed in the full analysis set of patients (i.e. who have taken a single dose of the investigational medicinal product) and on a ‘per protocol’ basis, see definitions below (Table 5).

Table 5 Definition of analysis populations

Population	Description
Screened Patients	All patients with date of informed consent not missing.
Safety population (SAF) and enrolled patients	All patients enrolled in the study (fulfil all inclusion criteria, but none of the exclusion criteria and have been included in the clinical study at Visit 2).
Dosed Patients	All patients of the safety population who received the IMP.
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 important protocol deviations that could affect the study objectives. All important protocol deviations will be manually reviewed by ICON and Ascelia to agree which to exclude from PPS.
PK population (PKS)	Defines the subset of patients in the FAS who have a blood sample taken at a PK timepoint (0.5, 1, 2, 3, 4, 6, 8 or 12 hours post-dose). These patients will be selected from a few sites that are able to include these patients and follow PK requirements.
Dialysis subgroup population (DSP)	All in the FAS currently on maintenance hemodialysis.
Brain MRI subgroup population (BMSP)	All patients in the FAS 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.

Demographic data and baseline characteristics will be displayed for the SAF, FAS and PPS populations. Efficacy data will be displayed for the FAS and the PPS populations. Safety data will be displayed for the SAF population. Manganese blood concentration data will be displayed for the FAS population, but PK parameters data will only displayed for the PKS population.

10.4 Protocol Deviation

The relevant protocol deviations have to be defined by a systematic data review prior to database closure and unblinding. For this purpose, protocol deviations that occurred during the study such as deviations of inclusion/exclusion criteria or forbidden concomitant medications or patient non-compliance will be assessed as 'important' depending on their potential to interfere with the primary endpoint and the safety endpoints of the study. Listings will be prepared to show the eligibility of all patients. Comprehensive justification for the classification of a protocol deviation as "important" will be given in the integrated clinical study report.

Important protocol deviations and the assessment of analysis sets will be defined during last data review before database closure. All definitions given in the Minutes of the Final Data Review will be taken into account in the analysis.

The list of protocol deviations will be reviewed by the Medical Director, discussed with and approved by sponsor and finalized before locking the database. The sponsor will identify important protocol deviations which will lead to the exclusion of patients from the per protocol set (PPS). Important protocol deviations related to study objectives for patients excluded from PPS will be flagged in the protocol deviation listing.

11.0 Conventions and Derivations

11.1 Data Handling

11.1.1 Imputation of missing data

There will be no imputation of missing data. All data will be analyzed as they appear in the database. Missing data will be displayed in patient data listings and will be declared in tables as appropriate.

11.1.2 Handling of dates and times

- Adverse events with unknown onset date/time will be counted as treatment emergent AEs.
- Adverse events with unknown end date/time will be counted as an ongoing AEs, in addition to those marked as ongoing in the CRF.
- Adverse events with unknown relationship to IMP will be counted as related in AE summary tables.
- Any adverse events with missing seriousness will be counted as serious in AE summary tables.
- Adverse events with partial onset/end date/time will be treated as specified in Section **15.1.4.1**.
- For medications with partial end dates see Section **15.1.3.2**.

11.1.3 Representation of Missing Data

Times should be printed in the format "HR:MI". "HR" represents the 2--digit hour portion of the time of 24h-format. "MI" represents the 2-digit minute portion of the time. Both hour and minute portions of time are zero padded integer values. Missing time portions should be represented on patient listings as dashes ("10:--" and/or "--:--").

11.2 Definitions and Derived Variables

11.2.1 Definitions

For the statistical analysis, the different MRI methods will be denoted according to the following table.

Table 6 Labeling of MRI methods

MRI Method	Short label (for tables etc.)
unenhanced MRI	UnMRI
mangoral-enhanced MRI	MeMRI
combined MRI (mangoral-enhanced MRI plus unenhanced MRI)	CMRI

In the following table, the definitions and calculation of derived variables are summarized.

Table 7 Further definitions

Variable / Term	Definition / Way of calculation
Baseline	The last assessment made before the administration of IMP will be used as baseline.

Variable / Term	Definition / Way of calculation
Change from baseline at visit	The difference between a value at time point and the value at baseline
Study day	The day of IMP is defined as study Day 1. Calculate the study day according to the following rules: If date < date of IMP, then study day = date of IMP - Date. If date ≥ date of IMP, then study day = Date – date of IMP + 1.
Age [years]	Year of informed consent – Year of birth
Age categories	>= 18 - < 65 years >= 65 - < 75 years >= 75 years
Body Mass Index (BMI)	BMI [kg/m²] $= \frac{\text{Weight [kg]}}{\text{Height } [m]^2}$
Study duration [days]	Date of completion/discontinuation – date of informed consent + 1
Prior medication	Any medication taken and stopped before the date of administration of mangoral. If the end date of prior medication is missing or partial see Section **15.1.3.2**.
Concomitant medication	Any medication taken prior to the administration of mangoral and continuing after treatment or started after the date of administration of mangoral. If the end date of concomitant medication is missing or partial see Section **15.1.3.2**.
Adverse events	All untoward medical occurrences after signing the informed consent. These can be adverse events either prior to treatment or after treatment as collected in the CRF.
Time to onset of adverse events	Time to onset of adverse events will be calculated based on the administration start date time of IMP based on the imputed value for adverse event start date/time.
Duration of adverse events	Duration of adverse events will be calculated based on the imputed values for adverse event start date/time and stop date/time. If the duration of the adverse event could not be calculated due to unknown date information, the following assessment to categories will be used: • If the adverse event is marked as “ongoing” in the CRF or the end date is missing, the duration will be categorized as “ongoing”. • Otherwise, the duration category will be set to “missing”.
Flagging of laboratory parameters	Continuous parameters will be classified as *low*, *normal*, or *high* based on reference (normal) ranges. In addition, laboratory parameters are flagged also according to CTCAE grading (G) as G1, G2, G3, or G4
Reproducibility	A consistency of results obtained when the same imaging is performed at short intervals on the same patients using different methods and by different readers.
QTcB interval (msec)	$\text{QTcB interval (msec)} = \frac{\text{QT interval (msec)}}{(\text{RR interval (sec)})^{1/2}}$

Variable / Term	Definition / Way of calculation
QTcF interval (msec)	$\text{QTcF interval (msec)} = \frac{\text{QT interval (msec)}}{(\text{RR interval (sec)})^{1/3}}$

The raw datasets will be in the Study Data Tabulation Model (SDTM) and the analysis datasets will be in the Analysis Data Model (ADaM), where ADaM datasets are derived from SDTM datasets. Throughout the entire study only one CDISC version will be used, there will be no change even if a higher version is available.

11.2.2 Visualization of focal liver lesions

Visualization of focal liver lesions will be measured by the two variables 'lesion border delineation' (BD) and 'lesion contrast compared to liver background' (LC). Both variables will be determined by qualitative assessment on the following 4-point scales for up to 15 lesions per patient as defined in Section 6.2.3.1 of the protocol: 1='poor', 2='partial / moderate', 3='good', 4='excellent'.

For each reader, the two sum scores (sBD and sLC) are determined for each patient by summarizing the individual scores over all lesions detected by treatment groups UnMRI (unenhanced MRI), MeMRI (mangoral-enhanced MRI) and CMRI (combined mangoral-enhanced MRI plus unenhanced MRI). Both scores are weighted by the number of lesions per patient. Mean difference (sum score weighted by number of matched lesions) will also be derived for matched lesions: Unenhanced MRI vs. Combined MRI and Unenhanced MRI vs. Enhanced MRI.

The handling of MRI scans with more than 15 lesions is described in specific documents (MRI manual for training the investigators on MRI reading procedures and rules and Independent Review Charter) prepared for the independent reading process. 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.

11.2.3 Confidence in lesion detection

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

11.2.4 Confidence in lesion localization

Confidence in lesion localization will be assessed for each lesion during the central reading sessions (part I, part II and part III) by 3 independent readers and by on-site radiologists. Up to 15 lesions per patient will be evaluated on 3-point scale:

1 = The lesion is localized to a liver segment with low confidence

2 = The lesion is localized to a liver segment with moderate confidence

3 = The lesion is localized to a liver segment with high confidence.

Note: If a lesion is large enough to be present in more than 1 segment it shall be considered to be in the segment in which its center lies.

11.2.5 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). The assessment will be done only during reading session part II as this allows the off-site readers to assess the same lesions on unenhanced and enhanced images. 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. If in a patient, all lesions are < 2 cm in diameter, only normal liver SI and background noise will be measured.

Quantitative SI will be measured by positioning circular regions of interest (ROIs) in a homogenous area in the liver and in 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):

- $LLC = (SI_{liv} - \text{Mean of } SI_{les})/(SI_{liv} + \text{Mean of } SI_{les})$

Signal-to-noise ratio (SNR):

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

Contrast-to-noise ratio (CNR):

- $CNR = (SI_{liv} - \text{Mean of } SI_{les})/SD_{noise}$

with SI_{liv} = signal intensity of the liver, SI_{les} = signal intensity of the lesion, SD_{noise} = standard deviation of the background noise, Mean of SI_{les} is the mean of all lesion signal intensities for a patient.

11.3 Statistical Software

All statistical analyses will be performed with SAS®, Version 9.4 or later. Working instructions are printed in the highlighted italic text below the shells.

12.0 Interim Analyses

No interim analysis is planned.

13.0 Statistical Methods

13.1 Patient Disposition

Patient disposition will be tabulated with number and percentages of patients enrolled, dosed, completed according to the protocol (completion of Visit 7 if part of the brain MRI subgroup population, otherwise completion of Visit 6) or discontinued prematurely. The patients who prematurely discontinued the study and the reasons for their discontinuation will be presented. The number and percentage of patients screened, not enrolled and reasons for non-enrollment will also be presented. The number and percentage of patients in each analysis population will also be presented as part of the disposition output.

13.2 Protocol Deviations

Protocol deviations will be summarized based on the assessment of major (important) or minor (non-important) by type for the SAF, FAS and PPS populations. In addition, for the subset of patients with major (important) or minor (non-important) deviations we will present the number and percentage who completed the study and those who discontinued the study.

All protocol deviations will be listed.

13.3 Demographic Data and Baseline Characteristics

The demographic data and baseline characteristics will be analyzed using descriptive statistics in summary or frequency tables for the following parameters for the SAF, FAS and PPS populations:

- Sex
- Age
- Age group (>= 18 - < 65 years, >= 65 - < 75 years and >= 75 years)
- Ethnicity
- Race
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)

The screening laboratory results will also be summarized for the SAF, FAS and PPS populations showing the below:

- Laboratory results related to GFR (eGFR categorization, eGFR value). Serum creatinine, serum albumin and serum urea nitrogen values are recorded in the source documents at site for assessing GFR but will not be summarized.

- Laboratory results related to Child-Pugh scoring (Child-Pugh score, encephalopathy grade, ascites, serum bilirubin, serum albumin and INR) by liver disease based on medical history records.

Disease history of focal liver lesions will be summarized and also listed.

13.4 Medical History and Signs and Symptoms

All general medical history conditions, surgical history and signs and symptoms will be coded in MedDRA version 25.0 or higher. General medical history conditions and signs and symptoms will be summarized by system organ class and preferred term for the SAF, FAS and PPS populations. The version of the utilized dictionary will be presented as part of the provided tables and listings.

All medical history, surgical history and signs and symptoms will be listed. Any medical history marked as ongoing on the CRF or with a missing end date will be considered as “ongoing”.

13.5 Prior and Concomitant Medications

Prior and concomitant medications will be coded according to the World Health Organization (WHO) Drug Dictionary (2022MAR GLOBAL B3) or later and summarized frequencies and percentages according to ATC level 2 and preferred term for the SAF, FAS and PPS populations. Patients taking the same medication multiple times will be counted once per medication. The version of the utilized dictionary will be presented as part of the provided tables and listings.

13.6 Investigational Medicinal Product Compliance and Accountability

IMP administration will be summarized for the SAF, FAS, PPS and PKS. Frequencies and percentages and/or descriptive statistics will be provided for each category. In addition, the number and percentage of patients receiving 200 mL of IMP as planned per protocol will be provided.

13.7 Efficacy Analysis

13.7.1 Primary Efficacy Analysis

The primary population for this analysis will be the full analysis set (FAS). The two sum scores (sBD and sLC) are determined for each patient (both combined and unenhanced) by summarizing the individual scores over matched lesions. Then, the mean score differences between the paired sum scores weighted by the number of matched 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 paired score differences:

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

and

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

If both tests indicate superiority of CMRI at the 1-sided significance level of 0.025, reader success is achieved. The corresponding 2-sided 95% confidence intervals will be also calculated. These results will be presented in a table together with a summary of the mean scores per reader.

Additionally, as a sensitivity analysis, the analogous 1-sided Wilcoxon signed rank test will be performed. The primary analysis will be also repeated in an exploratory manner for the per protocol set using the above-described methodology.

Superiority of CMRI over unenhanced MRI regarding visualization of focal liver lesions will be analyzed 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. The acceptance by two out of three readers will be considered to be a successful demonstration of efficacy in the study.

13.7.2 Secondary Efficacy Analysis

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

All other efficacy variables specified in Section **9.2.3** (and listed in Table 1) will be analyzed using descriptive statistics (including 2-sided 95% confidence intervals) for the full analysis population (FAS) and the per protocol set (PPS).

For each reader, the percentage of patients who had improvement in at least one co-primary variable for at least one lesion on part II (CMRI) and part III (mangoral-enhanced MRI alone) compared to part I (unenhanced MRI) will be calculated. In addition, the percentage of patients who had improvement in both BD and LC for at least one lesion on part II (CMRI) and part III (mangoral-enhanced MRI alone) compared to part I (unenhanced MRI) will be calculated. Similarly, there will be also calculated percentage of patients who had at least one new lesion identified on part II (CMRI) and part III (mangoral-enhanced MRI alone) compared to part I (unenhanced MRI).

Additionally, assuming repeated assessments per patient (for approximately 20 patients) by three independent readers, an intra-reader reproducibility will be evaluated separately for readings of MRIs in parts I, II and III. Based on a patient-level approach, the intra-reader and inter-reader variability will be analyzed using linear mixed-effects models fitted to individual mean scores of border delineation (BD) and lesion contrast (LC). Basically, the model will include reader, patient and replicate as a random effects and divide the total variability into an intra- and inter-reader component, in case this model does not converge we will have patient as a fixed effect instead.

Considering reader as a random effect, this model is used to express the observed value as the true value plus the intra-reader error with inter-reader variance $\sigma_B^2 = var(\mu_i)$ and intra-reader variance $\sigma_W^2 = var(e_{ik})$. As a relative measure of reproducibility, the intra-reader coefficient of variation (*wCV*) defined as $wCV = \sigma_W / \mu$ where $\mu = E(\mu_i)$ is taken as the mean of true value. The Intraclass Correlation Coefficient (ICC) will also be derived as the inter-reader variability divided by the total variability.

Bland–Altman plots **[4]** (showing differences versus mean, with mean difference and 95% limits of agreement) will be used for graphical presentations of intra- and inter-reader agreements for part I (unenhanced MRI) versus part II (CMRI) and part I (unenhanced MRI) versus part III (mangoral-enhanced MRI alone) as well.

For inter-reader plots, separate plots will be shown for BD and LC. For each MRI type, plots will be done for each pairwise comparison: reader 2 vs. reader 1, reader 3 vs. reader 1 and reader 3 vs. reader 2.

For a number of patients BD and LC will be assessed twice by each reader and MRI type. For intra-reader plots, separate plots will be shown for BL and LC, for each reader and MRI type plots will be done against first vs. second read (for those matched lesions).

13.7.3 Subgroup Analyses

The primary efficacy endpoint and the secondary efficacy endpoint visualization in mangoral-enhanced MRI vs. unenhanced MRI will be also evaluated in the following subgroups of patients:

– Subgroups of patients by the MRI scanner's magnetic field strength 1.5 Tesla and 3 Tesla.
– Subgroups of patients by the lesion type (benign, malignant, unknown, malignant: HCC, malignant: metastasis)
– Subgroups of patients with age < 65 years and ≥ 65 years.

– Subgroups of patients by eGFR: < 15, ≥ 15 to ≤ 30 and > 30 mL/min/1.73 m^2.
– Subgroups of patients by sex

These subgroup analyses will be done using the FAS population. Additional subgroup analysis may be done by race and/or ethnicity depending on the availability of data. A forest plot will be produced to show the comparison of paired differences (mean difference and 95% confidence interval) for the primary analysis and subgroup analysis in both BD and LC for combined vs. unenhanced and enhanced vs. unenhanced.

For lesions diameter (small lesion (diameter < 1 cm); medium lesion (diameter ≥ 1 to ≤ 3 cm); large lesion (diameter > 3 cm)), subgroup analyses will not be done for the primary endpoint but for the below outputs on FAS:

- Number of lesions detected by MRI method: This will summarize number of lesions by lesion diameter category and methods.
- Number of patients with at least one new lesion identified compared to unenhanced MRI: This will summarize for patients with new lesions if they are categorized as small, medium or large. The denominator for percentages will be number of patients in the FAS.

13.7.4 Pharmacokinetic Analysis

The analysis of PK parameters will be performed using the PK Set (PKS). Descriptive and graphical methods will be used to summarize the study results.

The CV and the geometric mean will be additionally included in the descriptive analyses of PK parameters, except for t_{max}. The 2-sided 95% confidence intervals for the geometric means of C_{max}, AUC_{0-t} and AUC will be constructed.

Missing data will be treated as such and will not be imputed or replaced in any way if not specified otherwise.

Individual, mean and overlay blood concentration-time profiles will be plotted on both linear and semi-logarithmic scales. Individual values and descriptive statistics for blood concentration data will be presented by the nominal time point using the Safety Population.

13.8 Safety Analysis

The analysis of safety data will be performed using the SAF. Safety data will be summarized using descriptive statistics.

13.8.1 Adverse Events

Safety analysis will include the investigation of adverse events (AEs), serious adverse events (SAEs), AEs related to IMP and SAEs related to IMP.

The following events shall be reported always as SAEs:

- seizure
- stroke
- cerebral venous thrombosis
- QTcF or QTcB greater than 480 msec
- OTcF or QTcB increase of 60 msec over baseline

For all AEs, a summary containing the following counts of patients, percentages of patients and number of events will be presented by pre and post dose (per CRF):

- Number of AEs;
- Number of related AEs;
- Number of AEs leading to study discontinuation (AE caused the patient to be discontinued from the study);
- Number of related AEs leading to study discontinuation;
- Number of SAEs;
- Number of related SAEs;
- Number of grade 3 or greater AEs;
- Number of grade 3 or greater related AEs;
- Number of AEs recovered;
- Number of AEs recovering;
- Number of AEs with outcome of death.

Further, all AEs, SAEs, AEs related to IMP and non-serious AEs will be summarized in a frequency table by MedDRA system organ class (SOC) and preferred term (PT), presented by pre and post dose (per CRF). The version of the utilized dictionary will be presented as part of the provided tables and listings. Tables will be sorted by decreasing frequency for SOC then PT within SOC using the overall column.

A summary of AEs by SOC, PT and maximum CTCAE grade will be presented.

Additionally, a summary of frequent post dose AEs (experienced by ≥ 5% of dosed patients) by PT will be displayed summarizing time to event (hours) and duration of event (hours) with summary statistics. For handling partial date/times refer to Section **15.1.4.1**. If an AE has no end date (ongoing at end of study) this will not be included in the analysis of duration of event.

All AEs, SAEs (other than death) and AEs leading to study discontinuation will be listed. In addition, a listing for AEs leading to death will be presented. Listings of AEs leading to death and SAEs (other than death) will be presented on screened patients and other AE listings will be presented on enrolled patients.

13.8.2 Vital Signs

Summary statistics for vital signs specified in Section **9.2.4.1** and their changes from baseline will be presented.

All vital signs data will be listed.

13.8.3 Physical Examination and Neurological Assessments

Parameters of physical examination (specified in Section **9.2.4.2**) and neurological assessments (specified in Section **9.2.4.3**) there will be summarized using descriptive statistics.

All physical and neurological examination data will be listed.

13.8.4 12-Lead ECG

Summary statistics for 12-lead ECG parameters specified in Section **9.2.4.4** and their changes from baseline will be presented.

Additionally, the number and percentage of patients with maximum QT value in certain ranges per ICH E14 will be summarized (≤ 450 msec, 450 to 480 msec, 480 to 500 msec and > 500 msec). The number and percentage of patients with maximum change from baseline in QT value in certain ranges will be summarized (≤ 30 msec, 30 to 60 msec and >60 msec). All ECG data will be listed.

13.8.5 Laboratory Parameters

Summary statistics for laboratory parameters specified in Section **9.2.4.5** will be presented by International System of Units overall. Shift-tables for hematology and biochemistry with respect to CTCAE v5 will be presented as well.

For urinalysis parameters, categories such as “positive” and “negative” will be used. The positive category could be further classified as positive “+” or borderline positive “(+)”.

For presentation of laboratory values as out of normal range, the flags are created using the normal ranges as provided by the central laboratory.

Box plots will be provided for hematology and biochemistry parameters showing the reference ranges for the parameters.

All laboratory data will be listed. In addition, all out of range laboratory data will be listed.

14.0 References

[1] Koh DM, Brown G, Riddell AM, et al. Detection of colorectal hepatic metastases using MnDPDP MR imaging and diffusion-weighted imaging (DWI) alone and in combination. Eur Radiol. 2008;18(5):903-10.

[2] Brismar TB, Kartalis N, Kylander C, Albiin N. MRI of colorectal cancer liver metastases: comparison of orally administered manganese with intravenously administered gadobenate dimeglumine. Eur Radiol. 2012;22(3):633-41.

[3] FDA Guidance for Industry. Developing medicinal imaging drug and biological products, Part 3: Design, analysis, and interpretation of clinical studies. June 2004.

[4] Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986; 1(8476):307–10.

Further references are given in the study protocol.

15.0 APPENDIX

15.1 Data derivation and analysis rules

The purpose of this section is to give technical details for the implementation of the SAP.

15.1.1 General specifications

15.1.1.1 Visit windows

The exact times at which IMP administration and mangoral-enhanced MRI are performed are defined in Section 5.1.5 of the protocol.

15.1.2 Disposition

All presentations for patient disposition will be done overall.

15.1.2.1 Patient discontinuation

Reasons for patient discontinuation as specified in the End-of- study page of the CRF will be used.

15.1.3 Demographics and other baseline characteristics

15.1.3.1 Patient demographics

Age as collected in CRF demographics form will be used.

15.1.3.2 Prior and concomitant medication

Prior and concomitant medication is collected as of enrollment in the CRF and described like that in the study protocol. For the analysis, the definition as described in the following is used.

The following rules are used to define the categories “prior” and “concomitant” medication.

Pre-requisite is a complete date/time of IMP administration (entered or imputed).

Stop of medication Date/time	Condition	Category
Complete date/time is available	Stop date/time is earlier than date/time of first dose of IMP.	Prior
Missing month	Year of stop date is earlier than year of first dose of IMP.	Prior
Missing day	Month/year of stop date are earlier than month/year of first dose of IMP.	Prior
Missing hours	Day/month/year of stop date are earlier than day/month/year of first dose of IMP.	Prior
Missing minutes	Day/month/year/hours of stop date/time are earlier than day/month/year/hours of first dose of IMP.	Prior
Otherwise		Concomitant

Medication ticked in the CRF as “continuing” will be classified as concomitant.

15.1.4 Safety analysis

15.1.4.1 Adverse events

The result of all imputation strategies (e.g., incomplete start dates of adverse events) and new derived information must be stored in the corresponding analysis dataset.

Handling of missing date information

The term missing date/time refers to a completely missing date/time or to an incomplete date/time where parts are not available, e.g., missing hours.

Missing start and end date/times will be imputed conservatively, i.e., missing values will be imputed in such a way that the duration of the adverse event is considered with the longest possible duration.

Imputation

I1: Impute AE start date with:

A replacement of missing year for AE start information is not foreseen. If needed, this will be considered on a case-by-case decision which must be documented.

a) If year of AE is the same as year of treatment then set start date to the date of treatment, otherwise use first calendar day and/or first calendar month.

Imputation will be done based on the available partial information starting with month and then day:

Missing date	Date of treatment	Imputed date
2014-Mar	2014-Mar-XX	2014-Mar-XX
2014-Mar	Not 2014-Mar	2014-Mar-*01*
2014	2014-XXX-XX	2014-XXX-XX
2014	Not 2014	2014-*Jan-01*

b) First hour and/or first minute.

Imputation will be done based on the available partial information starting with hour and then minutes:

Missing time	Date time of treatment	Imputed time
11:--	11:XX	11:XX
11:--	Not 11	11:*00*
--:--	Known	Time of treatment
--:--	Unknown	00:00

I2: Impute AE stop date with:

a) Last calendar day and/or calendar last month.

Imputation will be done based on the available partial information starting with month and then day. The respective last month and day will be chosen for imputation:

Missing date	Imputed data
2014-Mar	2014-Mar-*31*
2014	2014-*Dec-31*

For February, leap years must to be taken into account when calculating the last day in February.

b) Last hour and/or last minute.
Imputation will be done based on the available partial information starting with hour and then minutes. The respective last hour of a day and last minute will be chosen for imputation. “23:59” will be considered as the last hour/last minute per day.

Missing time	Imputed time
11:--	11:59
--:--	*23:59*

List of deaths
Death will be identified by the outcome of adverse event if the outcome equals “fatal” or reason for end of study is “dead”.

Patient experiencing a non-serious adverse event
All patients who had at least 1 non-serious AE will be taken into account regardless of the experience of a serious AE.

15.1.4.2 Laboratory parameters, vital signs and further safety parameters

The tables will display the number of patients still in the study (n) at the time point/visit. For the overall presentation, all post-baseline values on treatment are taken into account.

For all ordinary levels of the categorical parameter including missing values, both the number and the corresponding percentage is displayed.

For descriptive statistics of continuous parameters, n is the number of patients with recorded values at the respective time point/visit. The analysis of changes from baseline (e.g., baseline versus End of treatment) is based on patients with non-missing values at both visits; for all other patients, the change is missing.

Unscheduled visits
Unscheduled visits are time points not planned in the protocol.

In listings, unscheduled visits will be listed as recorded. All visits will be ordered chronologically including the dates of unscheduled visits.

Unscheduled visits will be incorporated in the overall post-baseline summary in tables. If the date is incomplete, but it can be determined whether values were measured in the on-treatment period, they will be incorporated in overall post-baseline summaries. Unscheduled visits will be excluded from the per time point/visit presentation.

Not exact values
Not exact laboratory values such as < x, > x will not be included in the analysis of continuous parameters. The frequency of occurrence of not exact values will be displayed in the respective table where applicable.

Handling of not exact values in a categorical analysis is described in the section below (“Values out of range”).

Ordering of parameters

Laboratory parameters, vital signs and further safety parameters will be ordered alphabetically within their parameter group (e.g., hematology, clinical chemistry and urinalysis). Time points/visits will be sorted chronologically. If changes from baseline are displayed by time point/visit, all visits will be displayed first followed by all the changes from baseline.

15.2 Statistical output documentation

The outputs of statistical procedures for primary analysis will be included in the statistical output documentation.