STATISTICAL ANALYSIS PLAN

Version 4.0 dated 02 September 2021

A Phase 2 Randomized Open-label, Dose-ranging Study for Ureter Visualization, Using ASP5354 in Subjects Undergoing Laparoscopic/Minimally Invasive Colorectal Surgery

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Table of Contents

I. LIST OF ABBREVIATIONS AND KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
AE	adverse event
Ae	amount of ASP5354 excreted in urine
Ae%	percentage of ASP5354 excreted in urine
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATC	anatomical therapeutic chemical
BMI	body mass index
CSR	clinical study report
%CV	coefficient of variation
DBP	diastolic blood pressure
ECG	electrocardiograms
FAS	full analysis set
Geo %CV	geometric coefficient of variation
GM	geometric mean
ICH	international conference on harmonization
IP	investigational product
IRT	interactive response technology
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute - common terminology criteria for adverse events
NIR-F	near-infrared fluorescence
PKAS	pharmacokinetic analysis set
PT	preferred term
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SBP	systolic blood pressure
SBR	signal background ratio
SD	standard deviation
SOC	system organ class
TEAE	treatment-emergent adverse event
TLF	Tables Listings Figures
ULN	upper limit of normal
VRC	visualization review committee
WHO	world health organization

List of Key Terms

Terms	Definition of terms
Baseline	Assessments of subjects as they enter a trial before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a trial.
Enroll	To register or enter a subject into a study.
Intervention	The drug, device, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety, pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the test drug or comparative drug.
Post investigational period	Period of time after the last assessment of the protocol. Follow-up observations for sustained adverse events and/or survival are done in this period.
Randomization	The process of assigning trial subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a trial.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a trial.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Study treatment	ASP5354
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

1 INTRODUCTION

This Statistical Analysis Plan (SAP) contains technical and detailed elaboration of the principal features of the analysis described in the protocol, and includes procedures for executing the statistical analysis to fulfil the objectives of the study.

The final SAP will be approved prior to first subject screened.

Changes from the planned analyses in the final SAP that impact the statistical analyses will be documented in the Clinical Study Report (CSR).

2 STUDY OBJECTIVE(S) AND DESIGN

2.1 Study Objective(s)

2.1.1 Primary Objective

The primary objective is to determine the optimal dose of ASP5354 for ureter visualization in subjects undergoing laparoscopic/minimally invasive colorectal surgery.

2.1.2 Secondary Objectives

Secondary objectives are to investigate the safety and tolerability as well as pharmacokinetics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.

2.1.3 Exploratory Objectives

Exploratory objectives are the following:

- To investigate the relationship between pharmacokinetics and pharmacodynamics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.
- To explore the fluorescence intensity and visualization duration of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.
- To explore the benefit of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.

2.2 Study Design

The study is a randomized open-label, dose-ranging study in adult subjects undergoing laparoscopic/minimally invasive colorectal surgery in which the need for anatomical visualization of the ureter is anticipated.

Subjects will be randomly assigned to receive a single dose of ASP5354 (CCI, [redacted] or CCI) which will be administered as an iv bolus to evaluate the anatomical visualization of the index ureter(s) (and contralateral ureter when feasible). The index ureter(s) is selected by the investigator before surgery. The opposite ureter is the contralateral ureter, if applicable. Safety, tolerability and pharmacokinetics in the study population will also be assessed.

Up to 15 subjects will be randomly assigned at each dose level.

Table 1 Dose Arms, ASP5354 dose levels and number of randomized subjects

Dose Arm	ASP5354 Dose Level	Number of Subjects
1	CCI	Up to 15
2	CCI	Up to 15
3	CCI	Up to 15
Total	**-**	**Up to 45**

Additional Dose Arm	ASP5354 Dose Level	Number of Subjects
4	CCI	Up to 15
5	CCI	Up to 15
6	CCI	Up to 15

* Based on Visualization Review Committee (VRC) review of the initial 3 dose levels, if none of the doses selected have visualization, then CCI and CCI dose levels will be added; if 1 dose selected has visualization, then CCI dose level will be added. The CCI dose level will only be added if only CCI dose level has visualization.

During a standard minimally invasive surgery, visualization of the surgical field will be assessed following the placement of the NIR-F imaging system (with FDA 510[k] cleared optical device system) proximal to the ureter of interest and then ASP5354 will be administered.

The visualization data will be assessed by a VRC, consisting of the investigator(s) and the sponsor's medical representative. The VRC will be held at the following time points, but ad-hoc meetings may occur until the optimal dose is determined:

1. After the initial 3 subjects have completed the surgical procedures at each of the dose levels; and
2. then in increments of 3 subjects (up to a total 15 subjects per dose level) for those who have completed surgical procedures at the expanded dose levels.

The visualization data for the subjects, at each interim time point, who completed the surgical procedure will be assessed to decide whether to:

1. expand a dose under evaluation; or
2. stop a dose under evaluation in which case additional subjects would not be added to a dose level; or
3. define a dose as optimal.

Once the optimal dose is determined, the optimal dose cohort may continue enrollment up to the maximum 15 subjects.

The VRC assessment to stop, to continue or to define an optimal dose level will be based on the totality of the collected data, including but not limited to the following:

1. anatomical visualization of the ureter at both 30 minutes after ASP5354 administration and at the end of surgery; or
2. fluorescence intensity based on the Likert Scale (0 to 3).

In the case where 2 doses perform equally, the lower dose may be selected.

Based on VRC review of the initial 3 dose levels, if none of the doses selected have visualization, then CCI and CCI dose levels will be added; if 1 dose selected has visualization, then the CCI dose level will be added. The CCI dose level will only be added if only the CCI dose level has visualization. For further details of the VRC, refer to the VRC charter.

2.3 Randomization

This is an open-label study; however, enrollment, randomization and dispensation of investigational product (IP) will be performed via the interactive response technology (IRT) system.

The treatment is assigned in a 1:1:1 ratio to:

- CCI ASP5354
- CCI ASP5354
- CCI ASP5354

Doses of CCI CCI and/or CCI will be added if needed as outlined in SAP Section 2.2. Prior to initiation of treatment, study site personnel will obtain the randomization number and treatment assignment from the IRT system. Specific IRT procedures will be described in the respective study manual.

No stratification factors will be used.

3 SAMPLE SIZE

This sample size is not based on statistical power calculation. The sample size is expected to provide adequate information to determine the optimal dose of ASP5354 for ureter visualization.

Up to 15 subjects will be randomly assigned at each dose level. This results in a total expected sample size of a maximum of 45 subjects. In case, additional dose groups are added, sample size can increase to a maximum of 90 subjects (15 subjects in each of the six possible doses).

4 ANALYSIS SETS

In accordance with International Conference on Harmonization (ICH) recommendations in guidelines E3 and E9, the following analysis sets will be used for the analyses.

The determination of whether subjects are included or excluded from the analysis sets except pharmacokinetic analysis set (PKAS) will be made prior to database hard-lock.

4.1 Full Analysis Set

The full analysis set (FAS) will consist of all subjects who receive ASP5354 and have at least 1 assessment of ureter visualization during surgery. This will be the primary analysis set for efficacy analyses.

4.2 Safety Analysis Set

The safety analysis set (SAF) consists of all subjects who receive ASP5354. The SAF will be used for all summaries of the safety data, unless otherwise specified.

4.3 Pharmacokinetic Analysis Set

The PKAS consists of all subjects who receive ASP5354 for which at least 1 plasma or urine concentration data are available with the time of dosing and sampling. Inclusion of subjects in the PKAS with important protocol deviations will be considered by the pharmacokineticist on a case-by-case basis.

The PKAS will be used for all summaries of the pharmacokinetic data.

5 ANALYSIS ENDPOINTS

5.1 Primary Efficacy Endpoint

The primary endpoint is the anatomical visualization of the index ureter(s) 30 minutes after dosing of ASP5354 and the end of surgery.

The incidence of anatomical visualization of ureter(s) will be assessed at approximately 10, 20, 30, 45 and 60 minutes postadministration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments (end of surgery) by the investigator intraoperatively using a binary “Yes” or “No” question on the ability to visualize the ureter: “Can the ureter be adequately visualized with near-infrared fluorescence (NIR-F)?”

- If the answer to the question 30 minutes after ASP5354 administration and at the end of surgery is equal to “Yes”, then anatomical visualization of the index ureter(s) was a success.
- All other cases will be handled as “Not a success”.

If both ureters are index per investigator, both need to have successful visualization for a subject to have successful visualization.

In the case that if the anatomical visualization of the index ureter(s) was not assessed at 30 minutes after ASP5354 administration, the answer will be imputed as follows:

- If the answer at the nearest time points before and after 30 minutes both = Yes, then the answer to the question 30 minutes after ASP5354 administration will be imputed as = Yes.
- If the answer at the nearest time points before and after 30 minutes both = No, then the answer to the question 30 minutes after ASP5354 administration will be imputed as = No.
- All other cases will be handled as “Not Done” at 30 minutes after ASP5354 administration, and imputing will not occur.

5.2 Secondary Efficacy Endpoints

Since no efficacy endpoint is specified as secondary endpoint, it is not applicable.

5.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are the following:

- The Likert Scale for qualitative response about the intensity of fluorescence at the predefined time points during surgery
- Duration of anatomical visualization of the index ureter(s) from the first time of positive visualization to the last time point of positive visualization
- Signal background ratio (SBR)
- Binary questions (Yes/No) for the benefit of ASP5354
- Ability to visualize the contralateral ureter when amenable to visualization

5.4 Safety Endpoints

Safety and tolerability endpoints include adverse events (AEs), laboratory test results, vital signs, electrocardiograms (ECGs) and findings in physical examination.

5.4.1 Adverse Event

AE will be assessed by evaluation of the following variables:

- Treatment-emergent adverse events (TEAEs; frequency, severity, seriousness, and relationship to study drug)
    - TEAE is defined as an AE observed after ASP5354 administration and up to the follow-up period (up to Day 10).
    - If the AE occurs on Day 1 and the onset check box is marked “Onset after first study drug taken” or the both onset check boxes “Onset before first study drug taken” and “Onset after first study drug taken” are marked or left blank, then the AE will be considered treatment emergent.
    - If the AE occurs on Day 1 and the onset check box is marked “Onset before first study drug taken”, then the AE will not be considered treatment emergent.
    - If a subject experiences an event both during the pre-investigational period and during the investigational period, the event will be considered as TEAE only if it is reported with a new start date (i.e., as a new AE).
    - Any AEs with onset dates completely missing will be considered TEAEs in summaries unless the investigator has noted on the CRF the event began prior to treatment. AEs with partially missing onset dates will be assumed TEAEs unless the available portion of the date indicates that the onset was strictly before start of study medication.
    - A drug-related TEAE is defined as any TEAE with possible relationship to study treatment as assessed by the investigator or with missing assessment of the causal relationship.

- Serious adverse events (SAEs) include adverse events that are flagged as serious by the investigator on eCRF, or upgraded by the Sponsor based on review of the Sponsor's list of Always Serious terms.

5.4.2 Clinical Laboratory Variables

Refer to protocol sections 7.2.2 and protocol appendix 12.7 for a table of the laboratory tests that will be performed during the conduct of the study. Refer to the protocol schedule of assessments for the evaluations' schedule.

5.4.3 Vital Signs, Height and Weight

Vital signs will include blood pressure (systolic blood pressure (SBP) and diastolic blood pressure (DBP)), pulse and respiratory rate. Vital signs, height and weight will be measured as indicated by the protocol schedule of assessments.

5.4.4 Routine 12-lead electrocardiograms

A routine 12-lead ECG will be performed at the time points outlined in the protocol schedule of assessments. ECGs will be taken in triplicate.

To calculate QTcF (corrected QT interval using Fridericia's formula) the following formula will be applied:

$$QT_CF = \frac{QT}{\sqrt[3]{\frac{RR}{1\ s}}}$$

5.4.5 Physical Examination

Physical examinations will be performed as indicated in the protocol schedule of assessments and whenever there is a medical indication. On day 1 (pre- and postoperatively) and at the follow-up visit, a symptom-directed physical examination will be performed. If clinically significant worsening of findings from baseline is noted, the changes will be documented as AEs on the AE eCRF.

5.5 Other Endpoints

5.5.1 Pharmacokinetic Endpoints

Pharmacokinetic endpoints are:

- Plasma and urine concentrations of ASP5354
- Amount of ASP5354 excreted in urine (Ae) during surgery
- Percentage of ASP5354 excreted into urine (Ae%) during surgery

5.5.2 Pharmacodynamic Endpoints

Pharmacodynamic variables will include the primary endpoint (anatomical visualization of the index ureter) and exploratory efficacy endpoints (Likert Scale, visualization duration and SBR).

5.5.3 Green Discoloration of Urine

The incidence and the duration of green discoloration of urine will be analyzed. It will be recorded any time after dose administration. If it occurs, the color of urine will be assessed during surgery and every 120 minutes (± 30 minutes window) after the end of surgery until it resolves or the subject is discharged, whichever is earlier. The start and stop date and time will also be recorded.

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of subjects, mean, standard deviation (SD), median, minimum and maximum. In addition, for continuous PK variables, and PK parameters, coefficient of variation (%CV), geometric mean (GM), and geometric coefficient of variation (Geo %CV) will also be calculated. GM and Geo %CV will not be calculated if at least one value is below the quantification limit (BQL). Categorical data will be summarized by frequencies and percentages. Percentages by categories will be based on the number of subjects with no missing data, i.e. the percentages for the non-missing categories will add up to 100%. All non-coded free-text variables will be displayed in data listings only.

Summaries based on FAS (e.g., disposition, baseline characteristics and efficacy endpoints), safety summaries based on SAF and summaries based on PKAS will be presented by actual treatment dose received.

All data summarization and analyses will be performed using SAS® Version 9.4 or higher on Red Hat Enterprise Linux. Specifications for table, figures, and data listing formats can be found in the TLF specifications document for this study.

Unless otherwise specified, all summaries will be presented by dose group. Subjects who were randomized before determination of optimal dose are grouped by the dose received. Additional subjects who were enrolled after determination of optimal dose are in a separate group. Apart from the overall group, a separate overall group for the optimal determined dose will be presented when specified in the TLF specifications document.

6.2 Study Population

In general, data such as patient disposition, demographics and baseline characteristics will be summarized for FAS and SAF by dose group and overall, unless specifically stated otherwise. In the event when FAS is identical to SAF (i.e., all randomized subjects have at least 1 assessment of ureter visualization during surgery), then these data summaries will not need to be repeated for SAF.

6.2.1 Disposition of Subjects

Disposition of subjects will be summarized for the FAS and SAF by dose group and overall, unless otherwise specified. The following analyses will be performed:

- Number and percentage of subjects with informed consent, discontinued prior to randomization (screen failures), randomized for all subjects with informed consent.
- Number and percentage of subjects who were randomized, took IP, did not take IP (including the reason why IP was not taken), in each analysis set by dose group and overall for all randomized subjects.
- Number and percentage of subjects who completed/discontinued the screening period, and primary reason for discontinuation for all subjects with informed consent.
- Number and percentage of subjects who completed and discontinued the study, and primary reason for discontinuation by dose group and overall for all randomized subjects, FAS and SAF.

6.2.2 Protocol Deviations

The number and percentage of subjects with the following important protocol deviation criteria will be summarized for each criterion and overall, by dose group and total as well as by investigative site, for all randomized subjects. Subjects deviating from a criterion more than once will be counted once for the corresponding criterion. Any subjects who have more than one important protocol deviation will be counted once in the overall summary.

The unique identifiers will be as follows:

PD1 – Inclusion/Exclusion,

PD2 – Withdrawal Ciriteria,

PD3 – Study Intervention,

PD4 – Excluded Concomitant Medication,

PD5 – Informed Consent,

PD6 – Safety Reporting,

PD7 – Procedures/Tests.

A data listing will be provided by investigative site and subject.

6.2.3 Demographic and Other Baseline Characteristics

Demographic variables (sex, age, ethnicity, race), age categories (>=18 to <65, >=65), EudraCT age categories (>=18 to <65, <=65 to <85, >=85), height, weight and body mass index (BMI), BMI categories (< 25, >=25 to <30, >=30 to <40, >=40) will be summarized by dose group and overall for FAS and SAF.

Medical history is coded in Medical Dictionary for Regulatory Activities (MedDRA), and will be summarized by System Organ Class (SOC) and Preferred Term (PT) by dose group and overall for the SAF.

Drug and alcohol history and medical history for each subject will be listed.

6.2.4 Previous and Concomitant Medications

Previous medications are defined as medications that patients started and ended prior to IP administration. Concomitant medications are defined as any medications that patients took at or after the day of IP administration. Medications that started prior to IP administration and continued after IP administration will be counted in both previous and concomitant medications.

Previous and concomitant medications will be summarized in separate tables by therapeutic subgroup (anatomical therapeutic chemical (ATC) 2nd level) and chemical subgroup (ATC 4th level) and preferred world health organization (WHO) name by dose group and overall for the SAF. Subjects taking the same medication multiple times will be counted once per medication. A medication that can be classified into several chemical and/or therapeutic subgroups is presented in all chemical and therapeutic subgroups.

All previous and concomitant medications will be presented in a listing.

6.2.5 Non-medication Therapies

The frequency and percentage of subjects with previous and/or concomitant non-medication therapies will be summarized along with the reason for use for SAF by dose group and overall.

All non-medication therapies will be presented in a listing.

6.3 Study Drugs Exposure and Compliance

Since this is a single dose study, the analysis of duration of exposure is not applicable.

Study drug dosing information which includes dosing date and time, total volume prepared and actual volume administered in ml, total test drug used in mg and the compliance in % will be listed only.

6.4 Analysis of Efficacy

Efficacy analyses will be conducted on the FAS. No hypothesis testing will be performed.

6.4.1 Analysis of Primary Efficacy Endpoint

The primary endpoint is the anatomical visualization of the index ureter(s) 30 minutes after dosing of ASP5354 and the end of surgery.

6.4.1.1 Primary Analysis for Primary Efficacy Endpoint

The success rate of anatomical visualization of the index ureter(s) at 30 minutes after dosing and end of surgery, will be estimated with exact 95% confidence interval (CI) by dose group.

SAS code for constructing 95% confidence interval for the success rate by Clopper-Pearson method is given as follows:

```
proc freq;
   tables success / binomial (level='1');
```

exact binomial;

run;

where the numerical variable success is either 1, in case of success, or 0 otherwise.

6.4.1.2 Secondary Analysis for Primary Efficacy Endpoint

The frequency and percentage of anatomical visualization of the index ureter(s) will be summarized by dose group and all assessed time points (including 30 minutes after dosing and end of surgery).

6.4.2 Analysis of Secondary Efficacy Endpoints

Since no efficacy endpoint is specified as secondary endpoint, it is not applicable.

6.4.3 Analysis of Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are listed in Section 5.5.

The Likert Scale of the intensity of the fluorescence will be summarized by frequency and percentage by dose group and time point for index ureter(s), and if applicable, for the contralateral ureter.

The duration of anatomical visualization of the index ureter(s) will be summarized using descriptive statistics by dose group. The duration of anatomical visualization of the index ureter(s) in minutes will be from the first time of positive visualization to the last time of positive visualization:

$$Duration = last\ time\ - first\ time + 1$$

If there are missing assessments of the anatomical visualization, then the last observed time of positive visualization will be used.

Based on fluorescence images recorded during the entire surgery (predose and approximately 10, 20, 30, 45 and 60 minutes postadministration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments), the SBR will be derived as follows:

$$SBR = \frac{mean\ region\ of\ interest}{mean\ signal\ background}$$

based on Hoogstins et al, 2019. The SBR for index ureter(s) will be summarized using descriptive statistics by dose group and time point.

The investigator will be asked the following questions:

- Was the location of the index ureter(s) as expected (Yes or No)?
- Did visualization of the index ureter(s) occur with white light at 30 minutes after ASP5354 administration (Yes or No)?
- Did visualization of the index ureter(s) occur with white light at the end of surgery (Yes or No)?

- Was NIR-F superior to white light in terms of visualization of the index ureter(s) (Yes or No)?
- Did the location of the ureter visualized by NIR-F alter the operative plan (Yes or No)?
- Were any ureter anomalies or abnormalities observed (Yes or No)?

These binary questions for the benefit will be summarized by frequency and percentage by dose group.

The success rate of anatomical visualization of the contralateral ureter at 30 minutes after dosing and end of surgery will be estimated with exact 95% CI by dose group (for SAS code, see Section 6.4.1.1), if applicable. In addition, the frequency and percentage of anatomical visualization of the contralateral ureter will be summarized by dose group and time point, if applicable.

6.5 Analysis of Safety

Safety analyses will be conducted on the SAF by dose group and overall, unless otherwise specified. No hypothesis testing will be performed.

6.5.1 Adverse Events

AEs will be coded using MedDRA and graded using the National Cancer Institute-common terminology criteria for AE (NCI-CTCAE, version 5.0).

A TEAE is defined as an AE observed after administration of the IP and up to the follow-up period. An drug-related TEAE is defined as any TEAE with a causal relationship assessed as "yes" by the investigator.

An overview summary table will include number and percentages of subjects with TEAEs, drug-related TEAEs, serious TEAEs, drug-related serious TEAEs, TEAEs leading to death, drug-related TEAEs leading to death and of all deaths. Frequencies and percentages of TEAEs and drug-related TEAEs by worst NCI CTCAE will be presented in the overview, too.

The number and percentage of subjects with TEAEs, drug-related TEAEs, serious TEAEs, drug-related serious TEAEs will be summarized by SOC and PT. The number and percentage of TEAEs by NCI CTCAE grade will also be summarized. The worst toxicity grade will be summarized if the same AE is recorded more than once for a subject.

All AE data will be listed.

6.5.2 Clinical Laboratory Evaluation

Baseline is the last measurement taken prior to IP administration.

For quantitative clinical laboratory measurements (hematology, biochemistry, urinalysis), descriptive statistics will be used to summarize results and change from baseline by dose group and time point (no overall presentation).

Quantitative laboratory test results by NCI-CTCAE Grade (version 5.0) will be summarized with frequencies and percentages by time point. Shift from baseline to the postbaseline worst grade based on NCI-CTCAE until the follow-up period in laboratory tests will be tabulated.

All laboratory data will be listed.

6.5.2.1 Liver Safety Assessment

The liver safety assessments will be summarized by the following categories below based on the measurements from Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), total bilirubin, Aspartate Transaminase (AST) and their combination.

The subject's highest value post-baseline of each parameter will be used; (upper limit of normal (ULN).

- ALT: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- AST: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALT or AST: > 3xULN, > 5xULN, > 10xULN, > 20xULN
- ALP: > 1.5xULN
- Total Bilirubin: > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN
- (ALT or AST > 3xULN) and Total Bilirubin > 2xULN and ALP < 2xULN

The last 2 criteria where 2 or more parameters are evaluated will use the measurements on the same day or up to 1 day apart. The number and percentage of subjects meeting the criteria post-baseline will be summarized.

6.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline by dose group and time point. Baseline is the last measurement taken prior to IP administration.

Tables for potentially clinically significant vital signs will be generated using baseline value and worst value obtained post-baseline for each subject. If the criterium is checking for values greater (or equal) a specific value, the worst value is referring to the highest value obtained. If the criterium is checking for values less (or equal) a specific value, the worst value is referring to the lowest value obtained. If the criterium is a combination of two conditions, the conditions need to be fulfilled for values obtained on the same post-baseline time points to meet the criterium.

The following potentially clinically significant criteria are defined for each parameter:

Vital Sign Variable	Criteria
SBP	≥180 mmHg AND ≥20 mmHg change from baseline
SBP	≤ 80 mmHg
DBP	≥105 mmHg AND ≥15 mmHg change from baseline
Pulse Rate	≥120 bpm AND ≥15 bpm change from baseline

All vital signs data will be listed.

6.5.4 Electrocardiograms

ECG will be taken in triplicate. The mean values of available triplicate results will be used in the analyses.

Descriptive statistics will be used to summarize routine 12-lead ECG results and changes from baseline by dose group and time point. Baseline is the last measurement taken prior to IP administration.

Number and percentage of subjects with normal and abnormal results for the overall interpretation will be tabulated by time point. A shift analysis table showing shift in overall ECG interpretation from baseline to each time point will be provided. Percent of subjects on different kind of abnormality will also be reported.

The QT interval corrected by Fridericia's Correction formula (QTcF interval) will be summarized using frequency tables for values of clinical importance using the range criteria below.

QTcF Interval Criteria	QTcF Interval Value (msec)
Normal	≤ 450
Borderline	> 450 to <=480
Prolonged	> 480 to <=500
Clinically significant	> 500

QTcF interval: Fridericia-corrected QT interval

Cumulative tabulation for >450, >480 and >500 msec will also be presented.

The QTcF interval will also be summarized by the frequencies of subjects with a change from baseline of clinical importance using the criteria identified below.

Variable	Change from Baseline
QTcF Interval (msec)	<=0
	>0 to <=30
	>30 to <=60
	> 60

QTcF interval: Fridericia-corrected QT interval

Baseline value is latest pre-dose assessment. Cumulative tabulation for >0, >30 and >60 msec will also be presented.

All routine 12-lead ECG data and interpretations will be listed.

6.5.5 Pregnancies

A listing of all pregnancy tests will be provided.

6.6 Analysis of Pharmacokinetics

Descriptive statistics will be used to summarize plasma and spot urine concentrations of ASP5354 by dose group and time point. In addition, for each dose, descriptive statistics will be used to summarize Ae and Ae%.

All sampling dates, times, urine volumes and plasma and urine concentrations will be listed.

Pharmacokinetics will be evaluated by a population pharmacokinetics approach using concentrations of ASP5354. All details of population analyses will be described in a separate analysis plan and a separate report will be written.

6.7 Analysis of Pharmacodynamics

6.7.1 Concentration-response Relationship Analysis

The relationship between pharmacokinetics and anatomical visualization or other exploratory endpoints will be explored by a population pharmacokinetics/pharmacodynamics approach. All details of population analyses will be described in a separate analysis plan and a separate report will be written.

6.8 Other Analyses

6.8.1 Green Discoloration of Urine

The frequency and percentage of patients who experience green discoloration of urine will be summarized by dose group. The duration of green discoloration in hours will be summarized using descriptive statistics by dose group. The duration is calculated from first date/time point of green discoloration to last date/time point of green discoloration. If the green discoloration is still present at discharge, the duration is from first date/time point of green discoloration to the date/time of discharge.

Additional to descriptive statistics, if applicable, the duration will be analyzed using Kaplan Meier estimation. For this purpose, subjects with green discoloration of urine at discharge are censored at the time point of discharge.

6.9 Interim Analysis (and Early Discontinuation of the Clinical Study)

The determination of dose(s) to stop, continue or define a dose as optimal will occur when every 3 subjects randomly assigned to each dose level have the data for anatomical visualization during surgery reviewed via the VRC.

6.10 Additional Conventions

As a general principle, no imputation of missing data will be done. Exceptions are the start and stop dates of AEs and concomitant medications if they are missing (see Section 6.10.2).

For the primary efficacy endpoint of anatomical visualization at 30 minutes after dosing and end of surgery, imputation will be performed in special cases when the assessment is not done at 30 minutes after dosing (see Section 5.1).

6.10.1 Analysis Windows

For efficacy analysis of anatomical visualization, assessments are summarized per time point mapped according to the following table:

Table 2 Mapping actual assessments times to time points

Time Point (post ASP5354 administration)	Collection Window
10 min	5 min ≤ and < 15 min
20 min	15 min ≤ and < 25 min
30 min	25 min ≤ and < 38 min
45 min	38 min ≤ and < 53 min
60 min	53 min ≤ and < 75 min
T min (every 30 minutes from 60 minutes)	T-15 min ≤ and < T+15 min
End of surgery	-

If more than one record is mapped to a specific time point, the record which is closest to the scheduled time point will be used in the analysis. In case of ties, the earlier one will be used.

For safety analyses, no mapping to analysis windows will be performed. The visit as collected in CRF will be used as analysis visit. Baseline is the last non missing assessment before ASP5354 administration.

In case of unscheduled assessments on Day 1 for vital signs, they will not be taken into account to derive Baseline, because unscheduled vital signs assessments do not provide information about the assessment time.

In safety analyses of post-baseline results (i.e. potentially clinical significant vital signs), not by time point, both scheduled and unscheduled assessments will be included to define the worst case. See also Section 6.5.2, Section 6.5.3 and Section 6.5.4.

Both scheduled and unscheduled assessments will be listed.

6.10.2 Imputation Rules for Incomplete Dates

In case of missing partial start and stop dates for concomitant medications, the following rules will be used:

If the start date is missing or partial:

- if the month is missing, use January
- if the day is missing, use the first day of the month under consideration
- if the year is missing, use year of the informed consent date
- if the entire date is missing then:
  a) If the concomitant medication did not start more than 28 days prior to treatment administration (operation day), use the informed consent date.
  b) Otherwise, use the 29th day prior to treatment administration.

If the stop date is missing or partial and the concomitant medication is not ongoing then:

- if the month is missing, use December
- if the day is missing, use the last day of the month under consideration
- if the year or the entire date is missing, set the stop date to December 31st, 2099

If the imputed start date is after the stop date, then the imputed start date will be 1 day prior to the stop date.

For AEs, a missing or incomplete onset date will be imputed according to the following conventions.

If only the year is known for the AE onset date, the imputed onset date will be the latest of the following non-missing dates:

- Date of first dose of study drug
- January 1 of the year of AE onset date

If only the month and year is known for the onset date, set the surrogate onset date to the first day of that month and then apply the following rules.

- If the month and year of the onset date is prior to the month and year of the first dose of study drug, then the surrogate onset date will be the imputed onset date.
- If the month and year of the onset date is on or after the month and year of the first dose of study drug, then the imputed onset date will be the **latest** of the following non-missing dates:
  - Date of first dose of study drug
  - Surrogate onset date

If the imputed onset date is after the adverse event end date, the imputed onset date will be the same as the adverse event end date.

7 REVISION AND RATIONALE

All updates in the revision of SAP v1.0 only have clarification purpose, e.g. detailed visit windows and updated imputation rules for missing AE and concomitant medication dates. SAP v2.0 does not include major revisions compared to SAP v1.0. SAP v3.0 includes updates of the protocol substantial amendment 1, updates of unique identifiers for protocol deviations and removal of mapping to analysis window for safety analyses. SAP v4.0 includes updates of the protocol substantial amendment 2.

8 REFERENCES

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ICH Harmonized Tripartite Guideline E 9. Statistical Principles for Clinical Trials, February 1998. (www.ich.org; Guidelines; "Efficacy" Topics)

9 APPENDICES

9.1 Appendix 1: Author and Approver Signatures

(E-signatures are attached at the end of document.)