STATISTICAL ANALYSIS PLAN

Version 4.0

17-Feb-2025

A Phase 3, Multicenter, Prospective, Randomized, Open-label Study for Intraoperative Ureter(s) Visualization when Using ASP5354 with Near-infrared Fluorescence (NIR-F) Imaging in Participants Undergoing Minimally Invasive and Open Abdominopelvic Surgeries

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Version History

SAP Version History Summary

The changes from the prior approved SAP that impact analyses are listed with the rationale in the table below.

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
1.0	31-Oct-2022		Not Applicable	Original Version
2.0	01-Jun-2023	2.1.1, 2.1.2.1, 3, 4.1, 5.1, 6.1, 6.4.1.1, 6.4.2	text updated	only WL/NIR-F participants with normal/mild eGFR will be included in the primary and key secondary analyses
		2.1.2.2, 5.3, 6.4.3	text regarding additional secondary endpoints added	support the new secondary endpoints
		2.1.2.3, 6.4.4	text regarding exploratory endpoint added	exploratory endpoint was added to the protocol
		2.2, 2.2.2, 3, 6.1	text regarding patients with moderate/severe eGFR added	support the addition of the new patients to the study
		4.2, 6.1	modified Intent to Treat population added	to define analysis population for other secondary analyses
		5.5.1, 6.6	PK endpoints updated and analysis text updated	for consistency with protocol updates
		6.1, 6.2.1	text added	summary added for participants who re-screen
		6.2.1	text added	to provide disposition table for all participants with informed consent
		6.2.4	imputation algorithm updated	to avoid using unblinded WL assessments in the algorithm
		6.4.3, 6.6, 6.8.1	visit windows added for CEF and PK data	to window CEF and PK data for consistency with the conspicuity data

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
		6.4.5	text added	to add subgroup analyses
		6.4.5	other efficacy analysis updated from WL conspicuity mean to WL at 30-min time point	to align the analysis with first key secondary endpoint
		6.5.1	ADE text added	to add summary table for ADEs
		6.5.1	text added	to add handling rules for AEs with missing CTCAE grade or relationship to study drug
		6.5.1.1, 6.5.1.2	tables added	to list SMQs used to identify AESIs and green coloration of urine
		6.5.2	table added	to list lab parameters that will be graded with CTCAE criteria
		Multiple	miscellaneous minor text updates throughout SAP	for consistency with protocol updates.
3.0	01-Nov-2023	2.1.2.2, 6.4.3, 6.7	text updated	to add details regarding the analysis of BICR conspicuity data
		2.2, 2.3, 3	text updated	to increase the enrollment cap of moderate/severe eGFR participants to 10
		6.2.1	text updated	clarified disposition table summaries
		6.2.3	table added	to list MedDRA coding used to identify prior inflammatory disorders of interest

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
		6.4, 6.8.1	text added	added clarification regarding how to handle efficacy data from participants that change from laparoscopic to open surgery during the procedure
		6.4.3	text updated	clarified difference between color contrast and CEF, added imputation for images that are reviewed but labelled as 'not visualized'
		6.4.4	text added	to add summary for WL conspicuity scores for index and non-index ureters by time point
		6.4.5	text added	to add efficacy summaries for non-index ureter
		6.4.5	text added	to add a summary for conspicuity scores by time point and summaries by surgeon
		6.5.1	text updated	to clarify that TEAEs with missing relationship to study drug will be counted as related
		6.5.1	new TEAE table added	to summarize TEAEs occurring in at least 5% of all participants
		6.5.1.1, 6.5.1.2	updated MedDRA AESI terms	to support the identification of AESIs and green coloration of urine
		6.5.2	text updated	to confirm lab summaries will be presented by time point

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
		6.5.6	text added	added summary for length of surgery in minutes
		6.6	text added	added additional PK urine parameters, added imputation rules for urine PK
		Multiple	text update	miscellaneous minor grammar and spelling corrections
4.0	17-Feb-2025	2.1.2.2, 5.3, 6.4.3	text added	analysis related to new objectives and endpoints added per protocol amendment 5
		2.1.2.3	objective added	added exploratory objective due to protocol update
		5.1	text added	added ureteral stent as an intercurrent event
		5.3	text updated	updated endpoint text due to protocol update
		5.4	endpoints added	added exploratory endpoints due to protocol update
		6.1	text added	to clarify which BICR assessment will be analyzed when there are multiple readings of an image
		6.2.3	age categories updated	to summarize the 12-14 and 15 to 17 age groups
		6.2.3, 6.4.5	text added	added summaries for type of device
		6.2.3, 6.5.1.1, 6.5.1.2, 9.3	text updated	specific MedDRA version removed
		6.4.1.2	text added	added sensitivity analysis excluding participants who do not receive study drug

SAP Version	Approval Date	SAP Section(s)	Change	Rationale
		6.4.3	text updated	clarified inter and intra-rater reliability will be analyzed for BICR readers
		6.4.3	text updated	clarified that the Pearson correlation coefficient will be displayed only for the overall data in the CEF analysis and not for each time point
		6.4.3	text added	Kendall's W statistic added to analysis
		6.4.3	text added	clarification added regarding instances where a BICR assessment cannot be collected
		6.4.3, 6.4.5	text added	added text for figures that will be presented
		6.4.5	text added	added new analysis for categorical summary of conspicuity scores and accidental unblinding listing, clarified that certain summaries of conspicuity data will not implement ICE1 or ICE2 imputation
		6.4.5	text added	added table and listing for ICEs
		6.4.5	text added	added table and listing for ICEs
		6.4.5	text added	clarified which efficacy analyses will have data imputation applied
		6.6	text added	added clarification for urine volume calculation

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 initial version of the SAP was finalized before the first participant was screened. Any updates to the SAP will be approved prior to database lock.

Changes from the planned analysis 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 the investigator's blinded conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment. This will be supported with the endpoint of the intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale.

2.1.2 Secondary Objectives

2.1.2.1 Key Secondary Objectives

The key secondary objective is the investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adults with normal renal function or mild renal impairment. This objective will be supported with the following two endpoints.

- Intra-participant comparison of ureter conspicuity scores for the WL 30-min time point versus the average of all NIR-F time points
- Intra-participant comparison of ureter conspicuity scores for the WL 30-min time point versus the end of surgery time point score under NIR-F

2.1.2.2 Other Secondary Objectives

Other secondary objectives are the following:

- Support the investigator's qualitative assessment of ureter conspicuity with a quantitative measure for all participants in all cohorts
- Investigator's conspicuity assessment of the ureter at the first time point for adolescents
- Investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents
- Investigator's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment

- Investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adults with moderate or severe renal impairment
- Investigator's conspicuity assessment of the ureter at the first time point for participants in all cohorts
- Investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for participants in all cohorts
- Frequency and percentage of participants with average ureter conspicuity assessed by the investigator over all time points during surgery improved under NIR-F vs WL in all cohorts
- Blinded independent central reviewer's (BICR's) conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment
- BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adults with normal renal function or mild renal impairment
- BICR's conspicuity assessment of the ureter at the first time point for adolescents
- BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents
- BICR's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment
- BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adults with moderate or severe renal impairment
- BICR's conspicuity assessment of the ureter at the first time point for participants in all cohorts
- BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for participants in all cohorts
- Concordance between investigator's intra-operative conspicuity assessment of the ureter and BICR assessment for adults with normal renal function or mild renal impairment
- Safety of ASP5354
- Investigate the pharmacokinetics of ASP5354

2.1.2.3 Exploratory Objectives

The exploratory objectives are the following:

- To summarize the investigator's conspicuity assessment with WL of the ureter at each time point.
- Concordance of conspicuity assessment of the ureter among BICR readers for participants with normal renal function or mild renal impairment.

2.2 Study Design

This is a phase 3, multicenter, prospective, randomized, open-label study to evaluate the clinical utility of ASP5354 with the use of NIR-F imaging devices in participants undergoing

minimally invasive and open abdominopelvic surgeries. In order to achieve reasonably balanced enrollment across surgery types, the sponsor will monitor enrollment and may cap enrollment for a specific surgery type (i.e., gynecological or other abdominopelvic) during the surgery.

This study is designed to evaluate the clinical utility of ASP5354 which enhances the conspicuity of the ureter with the use of NIR-F imaging devices. The investigator, at their discretion, will select ureter(s) of interest (i.e., right, left, or both) before surgery. If the selection is both, the investigator will further select 1 index ureter (i.e., right or left) at their discretion. The conspicuity of the ureter will be assessed by the investigator (5-Point Likert Scale) for the selected ureter(s) of interest during surgery for all participants. The investigator's qualitative assessment of ureter conspicuity will be supported with a quantitative measure and blinded independent central review assessment, which will be performed centrally using recorded images taken during the surgery.

The study will enroll approximately 107 participants (84 adults with normal renal function or mild renal impairment [eGFR ≥ 60 mL/min], up to 10 adults with moderate or severe renal impairment [eGFR 15 to < 60 mL/min] and 13 adolescents with normal renal function or mild renal impairment [eGFR ≥ 60 mL/min]) who will receive a single intravenous 3 mg dose of ASP5354. Patients with normal/mild eGFR cohort will be randomized to 2 arms: WL/NIR-F and WL-only. Randomization will be stratified by the type of surgery (gynecological; other abdominopelvic). There is no randomization for adult participants in the moderate/severe eGFR cohort or adolescent participants, and all of these participants will be evaluated with WL/NIR-F. Safety, tolerability and pharmacokinetics in the study population will also be assessed.

The conspicuity of the ureter with WL or WL/NIR-F will be assessed by the investigator (5-Point Likert Scale) during surgery for all participants.

- The investigator will attempt to locate the ureter and score the ureter conspicuity using the study-specific 5-Point Likert Scale by answering the question, "How conspicuous (easy to recognize/identify) is the ureter?"
- When assessing the 5-Point Likert Score for ureter conspicuity investigators should consider information, such as contrast, brightness (luminance), and/or fluorescence intensity.
- Conspicuity (or "How conspicuous is the ureter?") for this study is defined as "self-evident ureter location identification (jump out to capture your attention)".

Conspicuity assessment will be scored using the study specific 5-Point Likert Scale:

Score	Description of Ureter Conspicuity	Ureter Location Identification
1	None	Not self-evident
2	Poor	Somewhat self-evident
3	Sufficient	Sufficiently self-evident
4	Good	Clearly self-evident
5	Excellent	Extremely self-evident

2.2.1 Adults with Normal/Mild eGFR

Each site with adult participants will designate an unblinded study member who will randomize participants via the study's interactive response technology (IRT) system. Investigators will be blinded to the randomization assignment until after completion of the first ureter conspicuity assessment (5-Point Likert Scale) performed for ureter(s) of interest with WL at 30-min after ASP5354 administration. The first WL assessment will be done when the investigator reaches the point in surgery that visualization/identification of the ureter(s) is required. If the first assessment timeframe is missed (30 [± 15] min post-ASP5354 administration), the first conspicuity assessment for the study must be done while the investigators remain blinded to the participant's randomization assignment. The time of the conspicuity assessment will be recorded at all time points. Following this assessment, the unblinded study member will provide the investigator with the randomization assignment of the participant. If the participant was randomized to the:

- WL/NIR-F arm: the 30-min time point ureter conspicuity assessment will also be performed with NIR-F for the investigator-selected ureter(s) of interest (i.e., right, left or both). Thereafter, the investigator will assess the conspicuity of selected ureter(s) with WL and with NIR-F every 30 (± 15) min for the duration of the surgery, with the last assessment performed at the end of surgery time point.
  Note: The end of surgery time point is defined as the last time point where the ureter can be captured in the surgical field before removal of laparoscopic instruments or before the beginning of closure of the abdomen for open surgeries. If a surgical approach is switched from laparoscopic to open during surgery, ureter conspicuity assessment must be performed until the end of the laparoscopic surgery part and can be continued until the end of the open surgery part at the investigator's discretion.
- WL-only arm: only the 30-min time point after ASP5354 administration ureter conspicuity assessment will be performed with WL for the ureter(s) of interest (i.e., right, left or both).
  Note: no additional conspicuity assessments with WL will be needed at the subsequent time points.

2.2.2 Adults with Moderate/Severe eGFR

All moderate and severe eGFR participants will have conspicuity assessments performed with WL and NIR-F for all time points. The same conspicuity assessment procedures will be followed as described above for adults with normal/mild eGFR, except investigators will not be blinded at the first WL assessment time point. The WL-only arm does not apply to this cohort.

2.2.3 Adolescent Cohort

All adolescent participants will have conspicuity assessments performed with WL and NIR-F for all time points. The same conspicuity assessment procedures will be followed as described above for adults with normal/mild eGFR, except investigators will not be blinded at the first WL assessment time point. The WL-only arm does not apply to this cohort.

2.2.4 All Cohorts

All participants will have a safety follow-up assessment approximately 15 days after surgery. The anticipated duration of the study for each participant, including screening and follow-up visits, is between 5 to 53 days.

2.3 Randomization

This study will enroll approximately 107 participants (84 adults with normal renal function or mild renal impairment [eGFR ≥ 60 mL/min], up to 10 adults with moderate or severe renal impairment [eGFR ≥ 15 to < 60 mL/min] and 13 adolescents with normal renal function or mild renal impairment [eGFR ≥ 60 mL/min]) who will receive a single intravenous 3 mg dose of ASP5354. The normal/mild eGFR cohort will be randomized to 2 arms: WL/NIR-F and WL-only. Randomization will be stratified by type of surgery, (i.e., gynecological; other abdominopelvic). Details will be specified in the study randomization specification document, including allocation ratio, etc. There is no randomization for adult participants in the moderate/severe eGFR cohort or adolescent participants, and all of these participants will be evaluated with WL/NIR-F.

3 SAMPLE SIZE

Only adult participants with normal renal function/mild renal impairment randomized to WL/NIR-F will be included in the analysis of the primary and key secondary endpoints. Adolescent participants and participants with moderate/severe renal impairment will be included in other secondary analyses. Screen failures and participants who are not randomized or enrolled will not be evaluated.

Normal/Mild eGFR Cohort (Participants ≥ 18 years of age)

The sample size calculation is based on the primary efficacy endpoint. When assuming the difference in the score of the ureter conspicuity under NIR-F compared with WL is 1.0 with a common standard deviation of 2.0 and the correlation is 0.2, 84 adult participants randomized to either WL/NIR-F or WL-only at the selected allocation ratio will provide enough participants in the WL/NIR-F arm for 90% power to demonstrate a statistically significant difference from 0 at a 2-sided significance level of 0.05 using the paired t-test.

Moderate/Severe eGFR Cohort (Participants ≥ 18 years of age)

Up to 10 participants with moderate or severe renal impairment (eGFR: 15 to < 60 mL/min) will be enrolled into the WL/NIR-F arm and receive ASP5354. All participants with moderate or severe renal impairment will be assessed with both WL and NIR-F during surgery (non-randomized cohort). In the event that enrollment of participants with normal renal function/mild renal impairment and adolescents is completed prior to enrolling 10 participants with moderate/severe renal impairment, enrollment of participants in the moderate/severe eGFR cohort will end. Therefore, the final number of participants with moderate/severe renal impairment will be between 0 and 10.

Adolescent Cohort (Participants ≥ 12 and < 18 years of age)

Approximately 13 adolescents with normal renal function or mild renal impairment (eGFR ≥ 60 mL/min) will be enrolled and receive ASP5354 such that a minimum of 10 evaluable adolescent participants complete the study to allow collection of pharmacokinetic samples from at least 6 participants in age group ≥ 12 and < 15 years and at least 4 participants in age group ≥ 15 and < 18 years. All adolescent participants will be assessed with both WL and NIR-F during surgery (non-randomized cohort). Assuming approximately 20% of participants might not facilitate derivation of plasma pharmacokinetic parameters, 13 adolescent participants would need to be enrolled.

To achieve the precise estimate of important plasma pharmacokinetic parameters (CL: clearance and V_d: volume of distribution) of ASP5354, prospectively the study has at least 80% power to target a 95% CI within 60% and 140% of the geometric mean estimates of CL and V_d estimated by non-compartment analysis assuming an underlying interparticipant %CV up to 42% for both parameters [Wang et al, 2012].

The data from this study is intended to confirm the similarity of pharmacokinetic and pharmacodynamic profiles between adolescents and adults and to characterize the efficacy and supportive safety in the target pediatric population under surgery. The plasma concentration of ASP5354 and the urine concentration of ASP5354 as a surrogate marker of urinary visualization intensity will be used to support dose selection for pediatrics with the age of 0 to less than 12 using the physiologically based pharmacokinetics model and population pharmacokinetic model. In addition, the mean ratio of color components of ureter (contrast enhancement factor) during surgery and the time course of contrast enhancement factor will be compared between adolescents and adults. Considering the simple pharmacokinetic and pharmacodynamic profiles of ASP5354 (intravenous bolus administration, almost completely renally cleared, no metabolism, no need for pharmacological response with the aim being to achieve sufficient concentration in the ureters to be detectable by NIR-F during the surgery), the number of participants planned is considered appropriate to confirm the similarity of pharmacokinetic and pharmacodynamic profiles by graphical exploration and model development.

4 ANALYSIS SETS

In accordance with the 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 participants are included or excluded from the analysis sets other than the pharmacokinetic analysis set will be made prior to database lock.

4.1 Intent-to-Treat Population

The intent-to-treat population (ITT) will consist of all participants in the adult normal/mild eGFR cohort randomized into WL/NIR-F. This will be the analysis set for primary and key secondary analyses.

4.2 Modified Intent-to-Treat Population

The modified intent-to-treat population (mITT) will consist of all participants in any cohort in the WL/NIR-F arm. This will be the analysis set used for other secondary analyses not considered key secondary and the exploratory endpoint analysis.

4.3 Safety Analysis Set

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

4.4 Pharmacokinetic Analysis Set

The pharmacokinetic analysis set (PKAS) consists of all participants who receive ASP5354 for which at least 1 plasma or urine concentration data are available with the time of dosing and sampling. Inclusion of participants 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 efficacy endpoint is the intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale.

Primary Estimand

Population:

Male and female participants ≥ 18 years of age and eGFR ≥ 60 mL/min undergoing minimally invasive or open abdominopelvic surgeries. The population is further defined by the eligibility criteria.

Treatment Regimen:

The treatment regimen is ASP5354, 1 single dose of 3 mg.

Primary Endpoint:

Intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale.

Intercurrent Events (ICEs) and Strategies:

ICE1: Participants who have received the medications or therapy listed below within 48 hours prior to ASP5354 administration and/or before the completion of the surgical procedure:

- Indocyanine green (ICG), unless used for anastomotic evaluation after the ureters have been visualized or for lymphatic mapping where there is a clear anatomic separation of the ureters and the lymphatics.
- Other NIR-F imaging agents
- Ureteral stent

Composite strategy for ICE1: The assessment of ureter conspicuity after ICE1 will not be considered as relevant and will be handled as missing data. For participants that experience ICE1 prior to the 30-min time point conspicuity assessment by the investigator, both conspicuity values at the 30-min time point under WL and NIR-F will be set as '1 = Not self-evident' for the primary endpoint analysis.

ICE2: Participants who are unable to have ureter conspicuity assessed by the investigator at the 30-min time point for any reason.

Treatment policy strategy for ICE2: The assessment of ureter conspicuity in the event of ICE2 will be imputed as follows. In the event that WL or NIR-F conspicuity is not assessed by the investigator at the 30-min time point, the first collected conspicuity assessment under the respective illumination mode will be used as the 30-min time point value. If a participant does not have any assessments under a respective illumination mode during the entire surgery, the 30-min time point value will be set as the 30-min value from the other illumination mode. If a participant does not have any assessments under either illumination mode during the entire surgery, both 30-min time point values will be set as '1 = Not self-evident'.

Population Level Summary:

For participants randomized to WL/NIR-F, the score of the ureter conspicuity at the 30-min time point based on the 5-Point Likert Scale with NIR-F will be compared with the score of the ureter conspicuity at the 30-min time point based on the 5-Point Likert Scale with WL using absolute difference.

5.2 Key Secondary Efficacy Endpoints

Key secondary efficacy endpoints are the following:

- Intra-participant comparison of ureter conspicuity scores for the WL 30-min time point versus the average of all NIR-F time points
- Intra-participant comparison of ureter conspicuity scores for the WL 30-min time point versus the end of surgery score under NIR-F

5.3 Secondary Efficacy Endpoints

The other secondary efficacy endpoints are as follows:

- Using recorded images, ureter conspicuity for WL and NIR-F illumination modes will be quantified by image analysis of all time points for all participants in mITT population

- Descriptive summary of intra-participant differences in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration for adolescents
- Descriptive summary of ureter conspicuity scores for WL at the 30-min time point versus the average of all NIR-F time points for adolescents
- Descriptive summary of ureter conspicuity scores for the WL 30-min time point versus the end of surgery time point score with NIR-F for adolescents
- Descriptive summary of intra-participant differences in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration for adults with moderate or severe renal impairment
- Descriptive summary of ureter conspicuity scores for WL at the 30-min time point versus the average of all NIR-F time points for adults with moderate or severe renal impairment
- Descriptive summary of ureter conspicuity scores for the WL 30-min time point versus the end of surgery time point score with NIR-F for adults with moderate or severe renal impairment
- Descriptive summary of intra-participant differences in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration for participants in the mITT population
- Descriptive summary of ureter conspicuity scores for WL at the 30-min time point versus the average of all NIR-F time points for participants in the mITT population
- Descriptive summary of ureter conspicuity scores for the WL 30-min time point versus the end of surgery time point score with NIR-F for participants in the mITT population
- Frequency and percentage of participants with an average index ureter conspicuity over all NIR-F time points at least 1 point higher than the average index ureter conspicuity over all WL time points
- Frequency and percentage of participants with an average index ureter conspicuity over all NIR-F time points at least 2, 3 or 4 points higher than the average index ureter conspicuity over all WL time points
- Intra-participant difference in ureter conspicuity assessed by BICR for WL versus NIR-F at 30 (± 15) min after ASP5354 administration for all participants in the ITT population
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL at 30 (± 15) min timepoint versus the average of all NIR-F timepoints after ASP5354 administration for all participants in the ITT population
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F for all participants in the ITT population
- Intra-participant difference in ureter conspicuity assessed by BICR for WL versus NIR-F at 30 (± 15) min after ASP5354 administration for adolescents
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL at 30 (± 15) min timepoint versus the average of all NIR-F timepoints after ASP5354 administration for adolescents

- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F for adolescents
- Intra-participant difference in ureter conspicuity assessed by BICR for WL versus NIR-F at 30 (± 15) min after ASP5354 administration for adults with moderate or severe renal impairment
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL at 30 (± 15) min timepoint versus the average of all NIR-F timepoints after ASP5354 administration for adults with moderate or severe renal impairment
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F for adults with moderate or severe renal impairment
- Intra-participant difference in ureter conspicuity assessed by BICR for WL versus NIR-F at 30 (± 15) min after ASP5354 administration for all participants in the mITT population
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL at 30 (± 15) min timepoint versus the average of all NIR-F timepoints after ASP5354 administration for all participants in the mITT population
- Intra-participant comparison of ureter conspicuity scores assessed by BICR for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F for all participants in the mITT population
- Concordance correlation coefficient (CCC), the inter-rater reliability between the investigators and BICR for the WL versus NIR-F at 30 (± 15) min after ASP5354 administration for all participants in the ITT population
- CCC, the inter-rater reliability between the investigators and BICR for the WL at 30 (± 15) min time point versus the average of all NIR-F time points for all participants in the ITT population
- CCC, the inter-rater reliability between the investigators and BICR for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F for all participants in the ITT population

5.4 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are as follows:

- Descriptive summary of left and right ureter conspicuity scores for WL at each time point
- Descriptive summary of index and non-index ureter conspicuity scores for WL at each time point
- Intraclass correlation coefficient (ICC), the inter-rater reliability among BICR readers on the intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) after ASP5354 administration
- ICC, the inter-rater reliability among BICR readers on the scoring in ureter conspicuity for the WL and NIR-F at 30 (± 15) min after ASP5354 administration

- ICC, the intra-rater reliability within each BICR reader on the intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
- ICC, the intra-rater reliability within each BICR reader on the scoring in ureter conspicuity for the WL and NIR-F at 30 (± 15) min after ASP5354 administration

5.5 Safety Endpoints

Safety endpoints include:

- Nature, frequency, severity and causality of treatment emergent adverse events (TEAEs) and serious TEAEs
- Clinical laboratory tests (hematology, biochemistry and urinalysis)
- Vital signs
- Electrocardiograms/cardiac monitoring

5.5.1 Adverse Events

AEs 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.
    - If the AE occurs on Day 1 and the onset check box is marked “Onset after study drug taken” or both onset check boxes “Onset before study drug taken” and “Onset after 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 study drug taken”, then the AE will not be considered treatment emergent.
    - If a participant experiences an event both during the screening period and during the treatment period, the event will be considered as TEAE only if it is reported with the 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 the eCRF, or upgraded by the Sponsor based on review of the European Medicines Agency’s list of Important Medical Events.
- The frequency and percentage of participants with iatrogenic ureteral injury (IUI) or hypersensitivity which will be considered adverse events of special interest (AESIs).

- The frequency and percentage of participants with green coloration of urine.
- Adverse device effects (ADEs) include adverse events that are flagged as related to the device by the investigator on the eCRF.

5.5.2 Clinical Laboratory Values

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

5.5.3 Vital Signs

Vital signs will include blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP]) and pulse. Vital signs will be measured as indicated by the protocol schedule of assessments.

5.5.4 Routine 12-lead Electrocardiograms and Cardiac Monitoring

A routine 12-lead ECG will be performed at the time points outlined in the protocol schedule of assessments. The overall interpretation (normal; abnormal not clinically significant; abnormal clinically significant) of the ECG will be recorded on the eCRF.

5.5.5 Physical Examination

Physical examinations will be performed as indicated on the protocol schedule of assessments and whenever there is 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.

5.6 Other Endpoints

5.6.1 Pharmacokinetic Endpoints

The pharmacokinetic endpoints are:

- Plasma and urine concentrations of ASP5354
- Ae and Ae%

6 STATISTICAL METHODOLOGY

6.1 General Considerations

Continuous data will be summarized descriptively including the number of participants (n), 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 participants 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.

All statistical comparisons will be conducted using 2-sided tests at the 5% significance level unless stated otherwise.

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.

Baseline for the safety and tolerability analysis is the last measurement taken prior to ASP5354 administration.

Efficacy summaries for the primary and key secondary endpoints will be presented for all participants in the ITT population which includes all participants in the adult normal/mild eGFR cohort randomized to WL/NIR-F. Efficacy data collected for adult participants randomized to the WL-only arm will be listed only. Efficacy summaries for the adolescent and adult moderate/severe eGFR cohort will be presented as other secondary analyses. Efficacy analyses will be presented for all participants in the mITT population as other secondary analyses.

Unless otherwise specified, efficacy analyses will use data from one ureter for each participant based on selection by the investigator. If the investigator selects either the left or right ureter as the ureter of interest before surgery, then data from the selected ureter will be used in the efficacy analyses. If the investigator selects both ureters as ureters of interest, the index ureter selected by the investigator (either left or right) will be used in the efficacy analyses.

In analyses of BICR data where BICR readers assess the same 30-min image twice, the second reading will only be used in the analysis of intra-rater reliability within BICR readers. In other analyses of BICR data, only the first assessment will be analyzed.

All safety summaries will be presented for the following groups unless otherwise specified:

- All participants who received ASP5354
- Adult participants (≥ 18 years)
- Adult participants randomized to the WL/NIR-F arm
- Adult participants randomized to the WL-only arm
- Adult and adolescent participants enrolled/randomized to the WL/NIR-F arm
- Adolescent participants (12 to < 18 years)
- Participants with moderate renal impairment (eGFR ≥ 30 to < 60 mL/min)
- Participants with severe renal impairment (eGFR ≥ 15 to < 30 mL/min)

For participants who re-screen and have a new participant ID assigned, only data collected under the new participant ID will be used in the analysis. Data collected under the old participant ID will be listed only.

6.2 Study Population

In general, data such as participant disposition, demographics and baseline characteristics will be summarized for the ITT population. The summaries will be repeated for the mITT population within the following groups (adolescents; adults with normal renal function or mild renal impairment randomized to the WL/NIR-F arm; participants with moderate renal impairment, participants with severe renal impairment; entire mITT population) and for the SAF within the participant groups listed in Section 6.1.

6.2.1 Disposition of Participants

Disposition of participants will be summarized for all participants with informed consent, the ITT population and mITT population (adolescents; adults with normal renal function or mild renal impairment randomized to the WL/NIR-F arm; participants with moderate renal impairment, participants with severe renal impairment; entire mITT population) by the participant groups listed in Section 6.1. The following analyses will be performed.

- Frequency of participants with informed consent, re-screening discontinued prior to enrollment/randomization (screen failures), enrolled/randomized for all participants with informed consent.
- Frequency and percentage of participants who were enrolled/randomized, took investigational product (IP), did not take IP (including the reason why IP was not taken), in each analysis set for all enrolled/randomized participants.
- Frequency and percentage of participants who completed/discontinued in the screening period, and primary reason for discontinuation for all participants with informed consent.
- Frequency and percentage of participants who completed and discontinued the study, and primary reason for discontinuation.

Frequency and percentage of participants who discontinued during the follow-up period as well as reasons for discontinuation will be summarized for all enrolled/randomized participants.

6.2.2 Protocol Deviations

The number and percentage of participants with the following important protocol deviation criteria will be summarized for each criterion and overall, by the ITT population and mITT population (adolescents; adults with normal renal function or mild renal impairment randomized to the WL/NIR-F arm; participants with moderate renal impairment, participants with severe renal impairment; entire mITT population). Participants deviating from a criterion more than once will be counted once for the corresponding criterion. Any participants 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 Criteria,

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 participant.

A listing of accidental unblinding events will be provided.

6.2.3 Demographic and Other Baseline Characteristics

Demographic variables (sex, age, ethnicity, race), age categories (>=12 and <15, >=15 to <18, >=18 to <65, >=65), EudraCT age categories (>=12 and <18, >=18 and <65, >=65 to <85, >=85), height, weight, body mass index (BMI), BMI categories (underweight or normal, <25 kg/m^2; overweight, ≥25 to <30 kg/m^2; obese, ≥30 kg/m^2), prior pelvic or abdominal surgery (yes; no), prior inflammatory disorders of interest (yes; no), type of surgery (gynecological; other abdominopelvic), type of surgery (minimally invasive only; open only; minimally invasive and open) and type of device (1688, SPY-PHI, Other) will be summarized by the participant groups listed in Section 6.1 for ITT population, mITT population and SAF.

Medical history is coded in the Medical Dictionary for Regulatory Activities (MedDRA), and will be summarized by System Organ Class (SOC) and Preferred Term (PT) within the participant groups listed in Section 6.1 for the SAF.

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

Prior inflammatory disorders of interest will be identified using all Preferred Terms (PTs) from Standardized MedDRA queries (SMQ), see Appendix 3 for a list of common terms. A review of medical history data will be conducted prior to database lock and additional terms may be identified.

6.2.4 Previous and Concomitant Medications

Previous medications are defined as medications that participants started and ended prior to IP administration. Concomitant medications are defined as any medications that participants 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 within the participant groups listed in Section 6.1 for the SAF. Participants 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 participants with previous and/or concomitant non-medication therapies will be summarized along with the reason for use for SAF within the participant groups listed in Section 6.1.

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

6.3 Study Drug Exposure and Compliance

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

Study drug dosing date and time will be listed.

6.4 Analysis of Efficacy

Efficacy analyses of the primary and key secondary endpoints will be conducted on the ITT population. Other secondary efficacy analyses will be conducted on the mITT population.

For participants that switch from laparoscopic to open surgery during the surgical procedure, only Likert scale conspicuity assessments collected during the laparoscopic part will be used in the efficacy analysis.

6.4.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is the investigator's blinded assessment of the intra-participant difference in ureter conspicuity for WL versus NIR-F at 30-min time point after ASP5354 administration based on a 5-point Likert scale.

6.4.1.1 Primary Analysis for Primary Efficacy Endpoint

The population used for the primary efficacy analysis will be the ITT population, which will include all adult normal/mild eGFR participants enrolled/randomized to the WL/NIR-F arm. Adolescents and moderate/severe eGFR participants that are enrolled directly into the WL/NIR-F arm will not be included in the primary analysis. All participants will have conspicuity scores from one ureter used in the analysis. The analysis ureter will be selected by the investigator using the procedure described in section 6.1. If the conspicuity assessment at the 30-min time point is missed for a participant in the adult normal/mild eGFR cohort, the first conspicuity assessment must be done while the investigator remains blinded to the participant's randomization assignment. The primary endpoint will be analyzed using a paired t-test. Prior to the analysis, data will first be imputed as follows if participants experience either ICE1 or ICE2:

- Participants who experience ICE1 will have both their NIR-F and WL conspicuity assessments at the 30-min time point after ASP5354 administration imputed as '1 = Not self-evident' for the primary endpoint analysis.
- Participants who experience ICE2 and do not have either a NIR-F or WL conspicuity assessment conducted at the 30-min time point after ASP5354 administration will have the first collected conspicuity assessment under the respective illumination mode used as

the 30-min time point value. If a participant does not have any assessments under a respective illumination mode during the entire surgery, the 30-min time point value will be set as the 30-min value from the other illumination mode. If a participant does not have any assessments under either illumination mode during the entire surgery, both 30-min time point values will be set as '1 = Not self-evident'.

Descriptive statistics will be used to summarize the ureter(s) conspicuity at the 30-min time point after ASP5354 administration with WL and the 30-min time point with NIR-F. Descriptive statistics for the differences between the ureter(s) conspicuity scores and 95% 2-sided confidence interval for the mean difference and p-value will be presented.

All conspicuity Likert scale data will be listed.

6.4.1.2 Sensitivity Analyses for Primary Efficacy Endpoint

To examine the impact of out-of-window 30-min conspicuity assessments, the same paired t-test- as the primary analysis will be run with assessments impacted by ICE1 or ICE2 left as missing.

The difference in the ureter conspicuity at the 30-min time point post-ASP5354 administration between NIR-F and WL will be evaluated using a Wilcoxon signed-rank test. The p-value will be presented. Descriptive statistics for the WL and NIR-F conspicuity scores at the 30-min time point will also be presented. The Wilcoxon signed-rank test will be conducted using the same data (with ICE1 and ICE2 imputation) as the primary analysis.

To examine the impact of participants who are randomized but do not receive study drug, the primary analysis will be repeated excluding participants who are randomized to the WL/NIR-F arm but do not receive study drug.

Sensitivity analyses will be conducted on the ITT population.

6.4.2 Analysis of Key Secondary Efficacy Endpoints

The population used for the analysis of key secondary endpoints will be the ITT population. Adolescent and moderate/severe renally impaired participants that are enrolled directly into the WL/NIR-F arm will not be included in the analysis of the key secondary endpoints.

The first key secondary endpoint of the 30-min time point WL conspicuity score versus the mean NIR-F conspicuity score across all time points will be analyzed using a sign test which will test if the median of the intra-participant time point difference means between the NIR-F score at each time point and the 30-min WL score is equal to 0. Prior to the analysis, the conspicuity scores will be windowed to time points every 30 minutes per the algorithm in Section 6.8.1.

The value used in the sign test for each participant will be derived as follows. The differences between the NIR-F scores at each time point and the 30-min WL score will be calculated for each participant. The mean of these differences will then be calculated for each participant. A sign test will be conducted using these mean differences to test if the median of the intra-

participant time point difference means between the NIR-F scores and 30-min WL scores is equal to 0.

Prior to the sign test, participants who experience ICE1 or ICE2 will have their 30-min conspicuity data imputed using the same algorithm as described in section 6.4.1.1.

Additionally, the following imputation rules will be applied to NIR-F conspicuity scores at other time points after 30-min prior to the sign test.

- Participants who experience ICE1 will have NIR-F conspicuity scores at time points after ICE1 set as their WL conspicuity assessment at the 30-min time point.
- Participants who are unable to have NIR-F conspicuity scores assessed by the investigator at time points after 30-min will have such NIR-F scores left as missing.

If a participant does not have a conspicuity assessment at a time point due to the surgery ending before the time point, the time point will be left as missing prior to the sign test.

The following results will be presented:

- For comparisons between WL and NIR-F:
  - Frequency and percentage of participants with a positive mean difference, negative mean difference and mean difference of 0.
  - Summary statistics of the mean differences between 30-min WL and NIR-F time points.

The mean differences will be used to obtain a 2-sided p-value to test if the median of the mean differences is equal to 0.

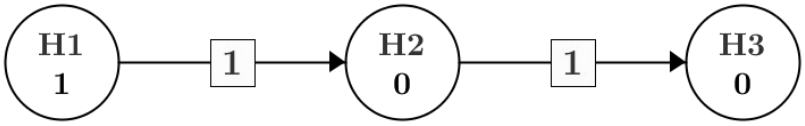
The second key secondary endpoint of the 30-min time point WL conspicuity score vs the end of surgery NIR-F conspicuity score will be analyzed using a paired t-test.

Descriptive statistics will be used to summarize the ureter conspicuity at the 30-min time point with WL and the end of surgery (EoS) time point with NIR-F. The same imputation algorithm described in the analysis of the first key secondary endpoint will be applied to the data prior to setting the EoS NIR-F value for each participant. Descriptive statistics for the differences between the ureter conspicuity scores and 95% 2-sided confidence interval for the mean difference and p-value will be presented.

A fixed sequence testing procedure will be used to adjust for multiplicity and control the type I error rate. The hypothesis tests will be conducted in the following order.

- H1: WL conspicuity at 30 minutes vs NIR-F conspicuity at 30 minutes
- H2: WL conspicuity at 30 minutes vs mean NIR-F conspicuity across all time points
- H3: WL conspicuity at 30 minutes vs NIR-F conspicuity at end of surgery

H2 will only be tested if the H1 p-value is ≤0.05. H3 will only be tested if both the H1 and H2 p-values are ≤0.05. A graphical representation of the testing procedure is given below.



The values within the circles indicate the initial allocation of the 0.05 significance level. Therefore, at the initial test, the primary endpoint (H1) will be tested at the full significance level of 0.05 and the key secondary endpoints will not be tested. The values on the lines indicate the significance level from the prior step that is allocated to the next step if the null hypothesis is rejected. Therefore, if the null hypothesis for the primary endpoint is rejected, the first key secondary endpoint (H2) will be tested at the full significance level of 0.05. Subsequently, if the null hypothesis for the first key secondary endpoint is rejected, the second key secondary endpoint (H3) will be tested at the full significance level of 0.05.

6.4.3 Analysis of Secondary Efficacy Endpoints

The secondary efficacy endpoints are listed in section 5.3. The analysis of additional secondary endpoints including CEF data will be conducted using all participants in the mITT population. CEF data will be mapped to time points using the windowing algorithm described in Section 6.8.1.

Quantitative assessment of ureter conspicuity will be performed by image analysis using recorded images. CEF is a measure of the degree to which color contrast is enhanced in area in which ASP5354 fluorescence signal is present when compared to areas in which ASP5354 fluorescence signal is absent. The CEF value is utilized as a quantitative analog for the qualitative assessment of "conspicuity" by the surgeon. An independent vendor will perform the image analysis using WL and NIR-F images extracted from the video files for all time points specified in the protocol schedule of events. A separate study image analysis charter/plan will describe details of the image analysis methodology and image analysis processes. The contrast enhancement factor (CEF) is derived by first normalizing the green signal to remaining colors (red and blue) for the same area as of the tissue within the WL and NIR-F images. The WL and NIR-F color contrasts are then calculated as a ratio of this normalized green signal inside and outside of the ureter. The CEF value is then calculated by taking the NIR-F color contrast and dividing by the WL color contrast.

WL and NIR-F color contrast values and CEF values will be summarized using descriptive statistics by time point.

The overall Pearson correlation coefficient between the WL color contrast values and WL conspicuity scores as well as the NIR-F color contrast values and NIR-F conspicuity scores will be presented.

In the event that WL or NIR-F images are reviewed and the ureter is unable to be located by the CEF reader, the color contrast for that image will be imputed as 1 for the analysis. Images which are collected after a patient experiences ICE1 will have the color contrast imputed as 1 for the analysis.

CEF data will be listed.

Boxplots for the distribution of WL and NIR-F CEF values for the index and non-index ureters will be presented. Boxplots for the distribution of CEF values by conspicuity score for the index and non-index ureters will be presented.

The analysis of the additional secondary endpoints will be conducted using the following groups of participants. P-values and confidence intervals will not be presented. This analysis will be performed after applying the ICE1 and ICE2 imputation as well as the visit windowing algorithm described in section 6.8.1.

- Adolescents
- Adults with moderate renal impairment (eGFR ≥ 30 to < 60 mL/min)
- Adults with severe renal impairment (eGFR ≥ 15 to < 30 mL/min)

To further characterize the difference of ureter conspicuity under NIR-F vs WL, the frequency and percentage of participants with an averaged ureter conspicuity assessed by the investigator over all NIR-F time points during surgery at least 1 point higher than the average ureter conspicuity over all WL time points during surgery will be presented. The same analysis will be repeated using a difference of 2, 3 and 4 points. The frequency and percentage of participants with an averaged ureter conspicuity within NIR-F during surgery at least 1 point higher than their WL conspicuity at the 30-min time point during surgery will be presented. The analysis will also be repeated using a difference of at least 2, 3 and 4 points.

The qualitative assessment of index ureter(s) conspicuity scored on a 5-point Likert Scale under WL and NIR-F (1 = None, 2 = Poor, 3 = Sufficient, 4 = Good and 5 = Excellent) performed by a BICR will be summarized descriptively at all time points post-ASP5354 administration by WL and NIR-F for each separate BICR reader. The descriptive analysis of the primary and key secondary endpoints will be repeated for each separate BICR reader. This analysis will be conducted within adolescents, adults with moderate renal impairment, adults with severe renal impairment, the ITT population and the mITT population. The BICR data will be assigned to the same analysis time points as the corresponding investigator assessments. The same ICE1 and ICE2 imputation will also be applied within the BICR data. In the event that a BICR assessment cannot be collected for a corresponding investigator assessment, the corresponding analysis time point will be considered missing in the BICR analysis. If a backup reader is utilized in place of a primary reader, the primary reader and their backup will be considered the same BICR reader in the analysis.

The concordance analysis between BICR readers and investigators will be conducted for the ITT population. The hypothesis testing for the primary and key secondary endpoints will be repeated for each separate BICR reader. This will be the primary measure of concordance between the BICR readers and investigator data. A high concordance between the two assessments is considered if 2 of 3 BICR readers yield p-value ≤0.05.

The CCC measuring the agreement between investigator and BICR Likert conspicuity scores will be presented for the following values for each separate BICR reader for the ITT population. This is considered a secondary measure of reliability compared to the hypothesis testing as differences between the raw conspicuity scores of the BICR readers and investigator data may be expected due to the different environments under which conspicuity is assessed.

- Difference between NIR-F and WL at the 30-min time point
- Mean difference between NIR-F across all time points and WL at the 30-min time point
- Difference between NIR-F at the end of surgery time point and WL at the 30-min time point

In addition to the CCC, Kendall's coefficient of concordance along with 95% jackknife CI will be presented in order to assess the agreement across all raters.

BICR data will be listed.

6.4.4 Analysis of Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are listed in section 5.4.

Descriptive statistics will be used to summarize the ureter conspicuity by time point under WL for both the left and right ureters of participants in the ITT and mITT population. In addition, the same descriptive statistics summary will be applied for the ureter conspicuity under WL for both index and non-index ureters in the ITT and mITT populations.

Inter- and intra-rater reliability within the BICR readers will be measured using the assessments at the 30-min time point for the ITT population and will be assessed using an ICC with a 95% CI. Inter-rater reliability of the differences between the 30-min WL and 30-min NIR-F values as well as the raw values under WL and NIR-F will be assessed. Intra-rater reliability of the repeat readings of the 30-min WL and 30-min NIR-F images will also be assessed. Intra-rater reliability of the differences between the 30-min WL and 30-min NIR-F values will also be assessed. ICC will be calculated using Shrout-Fleiss formulae for estimation of inter- and intra-rater reliability (agreement) (ICC [2, 1] , as defined in Qin et al., 2019). As a primary indicator, ICC for inter-rater and intra-rater (also called test-retest) reliability will be assessed using separate ANOVA models. Inter-rater reliability will be assessed using a random-effects ANOVA, estimating random effects of BICR reader and image. Intra-rater reliability will be assessed using a mixed-effect ANOVA, estimating random effects of BICR reader, image and fixed effect of time point. Parameters from the outputs obtained in these analyses will be used in the formula below to calculate two ICCs (for inter-and intra-rater reliability):

$$\frac{MS_B - MS_E}{MS_B + (k-1)MS_E + k/n(MS_W - MS_E)}$$

where MS_B = mean squares for image (for inter-rater reliability), MS_E = mean square for error, n = number of images (for inter-rater reliability), k = number of surgeon raters (for inter-rater reliability), and MS_w = mean square for rater (for inter-rater reliability).

In the case of intra-rater reliability, the above formula has a slightly different interpretation: MS_w = mean square for time point and k = number of time points (here 2) and n = number of raters multiplied by the number of images.

6.4.5 Other Efficacy Analysis

All analyses of the 5-Point Likert Scale for conspicuity and CEF will be conducted using conspicuity assessments mapped to time points using the windowing algorithm described in Section 6.8.1.

Subgroup analysis will be conducted on the primary endpoint, key secondary endpoints and color contrast quantitative assessment (CEF) within the ITT population. Data imputation described in the analysis of the primary and key secondary endpoints will also be applied to the subgroup analysis.

The endpoints listed above will be summarized using descriptive statistics within the following subgroups:

- BMI (underweight or normal, <25 kg/m^2; overweight, ≥25 to <30 kg/m^2; obese, ≥30 kg/m^2)
- Prior pelvic or abdominal surgery (yes; no)
- Prior inflammatory disorder (yes; no)
- Type of surgery (gynecological; other abdominopelvic)
- Type of surgery (minimally invasive only; open only; minimally invasive and open)
- Type of device (1688; SPY-PHI; Other)
- Sex (female; male)
- EudraCT Age (>=12 and <18; >=18 and <65; >=65 to <85; >=85)
- Ethnicity (Hispanic or Latino; Not Hispanic or Latino)
- Race (American Indian or Alaska Native; Asian; Black or African American; Native Hawaiian or Other Pacific Islander

Summaries by surgeon will also be presented.

Further, the following analyses of conspicuity data will only use observed records mapped to time points using the windowing algorithm but will not have data imputation applied.

The descriptive summaries of the primary end point, key secondary endpoints and CEF will be repeated using data collected from non-index ureters on participants in the ITT population.

Descriptive statistics for the number of NIR-F conspicuity time points for each participant during surgery will be presented.

Descriptive statistics for WL and NIR-F conspicuity scores will be presented by time point for the ITT and mITT populations. A line chart displaying the NIR-F and WL scores by time point will be provided for the mITT population.

The frequency and percentage of participants with each WL and NIR-F conspicuity score will be presented by time point for the ITT and mITT populations. This will be repeated for the non-index ureter for the ITT population.

The frequency and percentage participants who experience ICEs throughout the study will be presented for the ITT and mITT populations. The specific categories and subcategories will be as follows:

- Any ICE
- ICE1 Total
    - ICG unless used for anastomotic evaluation after the ureters have been visualized or for lymphatic mapping where there is a clear anatomic separation of the ureters and lymphatics
    - Other NIR-F imaging agents
    - Ureteral stent
- ICE2 Total
    - ICE2, first conspicuity assessments collected after 30-min window
    - ICE2, first conspicuity assessment collected in 30-min window but either WL or NIR-F not collected
    - ICE2, no conspicuity assessments collected

A listing of ICEs will be provided. And a swimmer plot displaying NIR-F conspicuity scores for the index ureter and assessments impacted by ICEs for each patient will be provided.

6.5 Analysis of Safety

Safety analyses will be conducted on the SAF and will be presented within the participant groups listed in Section 6.1 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. A 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 participants 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 ADEs, TEAEs and drug-related TEAEs by worst NCI-CTCAE will also be presented in the overview.

The number and percentage of participants with TEAEs, drug-related TEAEs, serious TEAEs and 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 participant. The number and percentage of participants with ADEs will be summarized by SOC and PT separately from TEAEs.

If a participant has multiple TEAEs with the same SOC or PT, but with differing CTCAE grade, then the participant will be counted once with the worst nonmissing grade. If a participant has multiple TEAEs with the same SOC or PT, but with differing relationship, then the participant will be counted once with the highest degree of relationship. If grade is missing for all episodes of the event, the participant will be counted under missing severity. If relationship is missing for all episodes of the event, the TEAE will be counted as related to study drug.

The number and percentage of TEAEs occurring in at least 5% of all participants in the SAF will be summarized by SOC and PT.

All AE data will be listed.

6.5.1.1 Adverse Events of Special Interest

Hypersensitivity and IUI-associated events will be considered adverse events of special interest (AESIs). The frequency and percentage of participants with each type of AESI will be summarized within the participant groups listed in Section 6.1. 95% Clopper-Pearson confidence intervals for the proportion of participants with each type of AESI will be presented.

AEs of special interest will be identified using all Preferred Terms (PT) from Standardized MedDRA queries (SMQ), see Table 1.

Table 1 Standardized MedDRA Queries for AESIs

AESI	MedDRA SOC/PT coding or SMQs
Hypersensitivity	Hypersensitivity SMQ - Narrow search
Iatrogenic ureteral injury (IUI)	Iatrogenic injury PT - 10061213 Ureteric injury PT - 10046405 Ureteric rupture PT – 10049810 Ureteric perforation PT – 10065809 Urinary tract injury PT – 10061397

6.5.1.2 Green Coloration of Urine

The frequency and percentage of participants who experience green coloration of urine will be summarized within the participant groups listed in Section 6.1.

Green coloration of urine will be identified using all Preferred Terms (PT) from Standardized MedDRA queries (SMQ), see Table 2.

Table 2 Standardized MedDRA Queries for Green Coloration of Urine

Event	MedDRA SOC/PT coding or SMQs
Green coloration of urine	Lower Level Terms (LLTs) that fall under Chromaturia PT – 10008796 Chromaturia LLT - 10008796 Urine color abnormal LLT - 10064752 Urine colour abnormal LLT - 10064748 Discoloration urine green LLT - 10070811 Discolouration urine green LLT - 10070812 Urine coloring green LLT - 10070848 Urine colouring green LLT - 10070862 Urine discoloration LLT - 10046628 Urine discolouration urine LLT - 10046629 Discoloration urine LLT - 10013080 Discolouration urine LLT - 10056126

6.5.2 Clinical Laboratory Evaluation

For quantitative clinical laboratory measurements (hematology, biochemistry, urinalysis), descriptive statistics will be used to summarize results and change from baseline by time point within the participant groups listed in Section 6.1. Postoperative laboratory values collected after surgery on Day 1 and postoperative laboratory values collected on Day 2 will be considered separate time points in the summary tables.

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 post-baseline worst grade based on NCI-CTCAE until the follow-up period in laboratory tests will be tabulated.

Table 3 Lab Parameters with CTCAE Grading

Panel/Assessments	Parameters with CTCAE Grading
Hematology	Eosinophils Hemoglobin Leukocytes Lymphocytes Neutrophils Platelets
Biochemistry	Albumin Alanine aminotransferase Alkaline phosphatase Aspartate aminotransferase Creatinine Creatinine kinase Gamma-glutamyl transferase Glucose Lactate dehydrogenase Magnesium Potassium Sodium

Panel/Assessments	Parameters with CTCAE Grading
	Total bilirubin Total cholesterol Triglycerides

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 participant'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

6.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline within the participant groups listed in Section 6.1 and time point.

Tables for potentially clinically significant vital signs will be generated using baseline value and worst value obtained post-baseline for each participant. If the criterium is checking for values greater (or equal) to a specific value, the worst value is referring to the highest value obtained. If the criterium is checking for values less (or equal to) 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 Electrocardiogram and Cardiac Monitoring

Number and percentage of participants with normal, abnormal not clinically significant and abnormal clinically significant results for the overall interpretation will be tabulated within the participant groups listed in Section 6.1. A shift analysis table showing shift in overall interpretation from baseline to each time point will be provided.

Standard 12-lead ECG interpretations will be listed.

6.5.5 Pregnancies

A listing of all pregnancies will be provided.

6.5.6 Other Safety Analysis

Descriptive statistics for the duration of the surgical procedure in minutes will be presented.

Descriptive statistics for the time between ASP5354 dosing and the first conspicuity assessment will be presented.

6.6 Analysis of Pharmacokinetics

The analysis of the pharmacokinetics will be reported by the following 5 groups of participants.

- Adults with normal/mild eGFR (eGFR ≥ 60 mL/min): WL/NIR-F arm
- Adults with normal/mild eGFR (eGFR ≥ 60 mL/min): WL-only arm
- Adults with moderate renal impairment (eGFR ≥ 30 to < 60 mL/min): WL/NIR-F arm
- Adults with severe renal impairment (eGFR ≥ 15 to < 30 mL/min): WL/NIR-F arm
- Adolescent cohort (eGFR ≥ 60 mL/min): WL/NIR-F arm

Plasma and urine concentrations will be mapped to time points using the windowing algorithm described in Section 6.8.1.

For ASP5354 in plasma, descriptive statistics will be used to summarize plasma concentrations for ASP5354 by time point. Individual overlay (spaghetti) and mean plasma concentration-time profiles (linear and semi-logarithmic scale) will be produced.

For ASP5354 in urine, descriptive statistics will be used to summarize urine concentrations for ASP5354, urine volume, amount excreted into urine (Ae), cumulative amount excreted into urine by time interval, duration of urine collection interval and urine production rate (mL/min).

Urine volume at a time point will be calculated by dividing the urine weight in grams by 1.018. Urine weight will be calculated as the difference between the full urine bag weight (g) and the empty urine bag weight (g).

Individual overlay (spaghetti) and mean cumulative amount of ASP5354 excreted into urine-time profiles will be produced.

Ae and the percentage of ASP5354 dose excreted into urine (Ae%) during surgery will be summarized.

Participants with 'unable to void' reported on the urine collection eCRF at a time point will have their urine weight imputed as 0 g and their urine volume reported as 0 mL for the analysis. Participants with a reported urine weight that is negative will have their urine weight imputed as 0 g.

Pharmacokinetic data will be listed.

Plasma and urine concentration data of ASP5354 will be subjected to population pharmacokinetic analysis and the similarity of pharmacokinetic profiles will be investigated by comparing adults and adolescents.

Details of the population analyses will be described in a separate analysis plan and a separate report.

6.7 Analysis of Pharmacodynamics

The relationship between ureter conspicuity (5-Point Likert Scale score by the investigator and BICR, CEF, etc.) and pharmacokinetics will be evaluated by a population pharmacokinetic/pharmacodynamic approach and compared between adults and adolescents. Details of the population analyses will be described in a separate analysis plan and a separate report.

6.8 Additional Conventions

Missing data will be imputed in the following scenarios. Details are given in the respective sections.

- Likert scale conspicuity data impacted by ICE1 or ICE2 (see Sections 6.4.1.1 and 6.4.2)
- CEF data where images are reviewed and the ureter is marked as 'not visualized' (see Section 6.4.3)
- Start and stop dates of AEs and concomitant medications if they are partial or missing (see Section 6.8.2)
- Urine weight and volume for which a participant was unable to void (see Section 6.6)

6.8.1 Analysis Windows

For 5-Point Likert Scale analysis of ureter conspicuity, assessments will be mapped to a time point based on the following table. The latest NIR-F conspicuity assessment performed before removal of laparoscopic instruments or before the beginning of closure of the abdomen for open surgeries will be considered the end of surgery value. All assessments up to the end of surgery value will be considered when applying the mapping algorithm. Unscheduled assessments collected after the end of surgery value will be listed only.

Table 4 Mapping Actual Assessments to Time Points, 5-Point Likert Scale and CEF Data

Time point (post ASP5354 administration)	Analysis Window
30-min	0 ≤ and < 45 min
60-min	45 ≤ and < 75 min
T-min (every 30 minutes from 60 minutes)	T-15 min ≤ and < T+15 min

For the 30 minute time point, the first record within the window will be used in the analysis. For other time points, 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.

After applying the windowing algorithm, missing data and data occurring after a participant experiences ICE1 will be imputed per the algorithm described in Section 6.4.1.1. In the event that an assessment is not collected within the 30-min window for a participant and a late out-of-window assessment is assigned to 30-min per the imputation algorithm, that assessment will not be considered when applying the windowing algorithm to other time points for the analysis. For the sensitivity analysis described in Section 6.4.1.2, such assessments will not be mapped to the 30-min time point and the 30-min NIR-F score will be considered missing. CEF data will be mapped to a time point using the visit windows in Table 4. No missing data imputation will be applied to CEF data.

Plasma and urine concentration data will be mapped to a time point based on the following table. Urine concentrations will be mapped based on the time the urine bag is collected at the end of the interval. The postoperative time point for plasma will be mapped to a numeric time point based on the collection time.

Table 5 Mapping Actual Assessments to Time Points, Plasma and Urine Concentration Data

Time point (post ASP5354 administration)		Analysis Window
Plasma Concentration	**Urine Concentration**	
10-min	0- to 10 min	5 ≤ and < 15 min
30-min	10- to 30 min	15 ≤ and < 45 min
60-min	30- to 60 min	45 ≤ and < 75 min
T-min (every 30 minutes from 60 minutes)	[T - 30]- to T min (Every 30 min from 60 minutes)	T-15 min ≤ and < T+15 min

For analyses other than 5-Point Likert Scale, quantitative assessment of ureter conspicuity and plasma and urine concentrations of ASP5354, no mapping to analysis windows will be performed. The visit as collected in the CRF will be used as the analysis visit. Baseline is the last non-missing assessment prior to ASP5354 administration.

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

Both scheduled and unscheduled assessments will be listed.

6.8.2 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 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 the 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 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 dose of study drug, then the imputed onset date will be the **latest** of the following non-missing dates:
  - Date of the 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

The SAP has been updated to support protocol amendment 5, dated October 24, 2024. A detailed list of changes is given in the version history section on page 4.

8 REFERENCES

ICH Harmonized Tripartite Guideline E 3. Structure and Content of Clinical Study Reports, November 1995. (www.ich.org; Guidelines; "Efficacy" Topics)

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

Qin S, Nelson L, McLeod L, Eremenco S, Coons SJ. Assessing test-retest reliability of patient-reported outcome measures using intraclass correlation coefficients: recommendations for selecting and documenting the analytical formula. Quality of Life Research. 2019;28(4):1029-1033

Wang Y, Jadhav PR, Lala M, Gobburu J. Clarification on precision criteria to derive sample size when designing pediatric pharmacokinetic studies. J Clin Pharamcol. 2012 Oct; 52(10):1601-6

9 APPENDICIES

9.1 Appendix 1 List of Abbreviations

Abbreviations	Description of abbreviations
ADE	Adverse device effect
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
BICR	blinded independent central review
BMI	body mass index
CCC	concordance correlation coefficient
CEF	contrast enhancement factor
CS	clinically significant
CSR	clinical study report
%CV	coefficient of variation
DBP	diastolic blood pressure
ECG	electrocardiograms
EoS	end of surgery
Geo %CV	geometric coefficient of variation
GM	geometric mean
ICC	intraclass correlation coefficient
ICG	indocyanine green
ICH	international conference on harmonization
IUI	iatrogenic ureteral injury
IP	investigational product
IRT	interactive response technology
ITT	Intent to Treat
LS	least squares
MedDRA	Medical Dictionary for Regulatory Affairs
mITT	modified Intent to Treat
NCS	not clinically significant
NIC-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
SD	standard deviation
SOC	system organ class
TEAE	treatment-emergent adverse event
TLF	Tables Listings Figures
ULN	upper limit of normal
WHO	world health organization
WL	white light

9.2 Appendix 2 List of Key Terms

Terms	Definition of terms
Baseline	Assessments of participants 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 participant 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).
Treatment 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 participant, and continues until the last assessment after completing administration of the test drug of comparative drug.
Follow-up 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 participants 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 participants for enrollment in a trial.
Screen failure	Potential participant who did not meet 1 or more criteria required for participation in a trial
Screening period	Period of time before entering the treatment period, usually from the time when a participant signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a participant.
Study period	Period of time from the first site initiation date to 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.

9.3 Appendix 3: Pre-Specified Criteria for Inflammatory Disorders of Interest

Standardized MedDRA Queries for Inflammatory Disorders of Interest

	MedDRA SOC/PT coding or SMQs
Inflammatory Disorders	Crohn's disease PT - 10011401 Colitis ulcerative PT - 10009900 Colitis PT - 10009887 Proctitis PT - 10036774 Enteritis PT - 10014866 Diverticulitis PT - 10013538 Retroperitoneal fibrosis PT - 10038979 Appendicitis PT - 10003011 Cholecystitis PT - 10008612 Pancreatitis PT - 10033645 Gastritis PT - 10017853 Coeliac disease PT - 10009839 Irritable bowel syndrome PT – 10023003 Endometriosis PT - 10014778

9.4 Appendix 4: Author and Approver Signatures

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