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

ISN/Protocol 5354-CL-0301

Amendment 5 [Nonsubstantial] 24 OCT 2024

IND # 140758

IND Grantor Center for Drug Evaluation and Research (CDER)

Sponsor:

Astellas Pharma Global Development Inc.

Northbrook, IL 60062, US

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CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL

24-hour Contact for Serious Adverse Events See [Section 10.3.7]	**Please fax or email the serious adverse events/special situations/adverse device effect worksheet to:** Astellas Pharma Global Development Inc. Global Pharmacovigilance **North America fax number:** +1-888-396-3750 **North America alternate fax number:** +1-847-317-1241 **International fax number:** +44-800-471-5263 **Email:** safety-us@astellas.com
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PROTOCOL AMENDMENT SUMMARY OF CHANGES

DOCUMENT HISTORY	
Document	**Date**
Amendment 4	11 JUL 2024
Amendment 3	04 APR 2024
Amendment 2	02 OCT 2023
Amendment 1	27 APR 2023
Original Protocol	08 NOV 2022

Amendment 5 [Nonsubstantial] 24 OCT 2024

This amendment is considered to be nonsubstantial based on the criteria set forth in Regulation (EU) No 536/2014 of the European Parliament because it neither significantly impacts the safety or physical/mental integrity of participants nor the scientific value of the study.

Overall Rationale for the Amendment:

The overall rationale for this amendment is to elevate the endpoint of frequency and percentage of participants with an improvement in ureter conspicuity under NIR-F vs WL from supplementary analysis to additional secondary endpoints.

Summary of Changes

Nonsubstantial Changes

Section Number	Description of Change	Brief Rationale
1.1, 3 (Table 4), 9.3.3.2	Objectives and endpoints Elevation of supplementary analysis to additional secondary endpoints Addition of additional endpoint allowing for more substantial improvements under NIR-F vs WL	The frequency and percentage of participants with average ureter conspicuity over all time points during surgery improved under NIR-F vs WL was elevated to additional secondary endpoints. This change was requested by FDA. A difference in ureter conspicuity under NIR-F vs WL of at least 3 and 4 points was added to assess improvements beyond 2 points.
3.1, 4.2, 6.1 (Table 6), 7.1.1	Clarification added for the order of conspicuity assessments	The order of conspicuity assessment (WL followed by NIR-F) was added for clarification, based on comments received from FDA.

Section Number	Description of Change	Brief Rationale
9.3.3.3	Addition of supplementary analysis	Since comparison to WL conspicuity assessment over all time points might be biased, a supplementary analysis comparing average ureter conspicuity over all NIR-F time points to ureter WL conspicuity score at the 30-min time point was added.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title of Study:

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

Regulatory Agency Identifier Number(s):

IND number: 140758
WHO universal trial number: 1251
Chemical Abstracts Service (CAS) Registry number: 2243793-22-6
Unique Ingredient Identifier (UNII): D23R36L5QY

Planned Study Period/Duration:

From approximately 2Q2023 to 4Q2024

Planned Total Number of Study Sites and Location(s):

Approximately 6 sites in the US

Rationale:

ASP5354 (also known as pudexacianinium chloride) is a novel NIR-F imaging agent, under investigation for intraoperative anatomical identification/visualization of ureter(s) in participants undergoing minimally invasive and open abdominopelvic surgeries.

Pudexacianinium chloride is a new chemical entity which has unique hydrophilic properties through conjugation with cyclodextrin. Unlike ICG, ASP5354 is iodine free. ASP5354 is administered intravenously and has a clinically distinct pharmacokinetic profile that allows for ASP5354 to be excreted into the urine through the kidneys. Photo-optically, ASP5354 is indistinguishable from ICG with an absorption of 780 nm and a fluorescence emission at 820 nm, allowing for the use of approved (ICG) NIR-F imaging technology. In this study, ASP5354 will be used with FDA-cleared imaging devices that have been approved for use with ICG for laparoscopic (Stryker 1688 Advanced Imaging Modalities device) and open surgical procedures (Stryker SPY-PHI). These devices provide both white light (WL) and NIR-F illumination modes. The NIR-F illumination/imaging technology when used with ASP5354 facilitates anatomic ureter visualization during minimally invasive and open abdominopelvic surgeries.

This study is designed to evaluate the clinical utility of ASP5354 which enhances the conspicuity of the ureter with the use of an NIR-F imaging device. The benefit of enhanced conspicuity is to identify the ureter as soon as possible during the surgical procedures which is expected to help minimize the risk of iatrogenic ureteral injury (IUI) and associated complications. To date, there are no uniform prevention guidelines to avoid IUIs and no commercially available ureteral imaging agents. The European Association of Urology and American Society of Colon and Rectal Surgeons recommend either the use of ureteral stents and/or a cautious dissection with direct identification/visualization of the ureters during abdominopelvic procedures. Despite these recommendations, IUIs still occur and may not be evident until post-surgery. The European Association of Urology states that the best way to avoid IUIs is real-time ureter visualization. The use of ASP5354 with NIR-F imaging technologies is a promising technique that offers real-time ureter visualization for surgeons.

ASP5354 has been studied in the following completed studies: 2 phase 1 studies in healthy participants and in 1 phase 2 study in participants undergoing laparoscopic or minimally invasive colorectal surgery. ASP5354 has also been studied in a completed phase 1 study in participants with mild, moderate and severe renal impairment compared with healthy controls. In addition to this phase 3 study, another phase 3 study in adult participants undergoing minimally invasive and open abdominopelvic surgeries is being conducted. Clinical data to date shows ASP5354 has rapid renal

clearance without metabolism, and it is safe and well tolerated given as a single intravenous dose up to 24 mg. In the phase 2 study, enhanced conspicuity was observed in all 3 doses evaluated (0.3, 1 and 3 mg).

Doses of 1 mg and 3 mg provided visualization throughout the duration of the surgical procedure; however, at the 3 mg dose, fluorescent intensity was stronger and more consistent. At the 1 mg dose, the intensity of fluorescence was variable amongst the participants evaluated in the phase 2 study. The lower dose (0.3 mg) was determined to be inadequate for ureter visualization due to lower fluorescence intensity and shorter duration of visualization than doses of 1 mg and 3 mg. Given the ASP5354 safety profile, the highest dose of 3 mg was selected for the phase 3 program to provide optimal conspicuity of the ureter for the duration of surgery in all intended participants for the phase 3 clinical program, including renally impaired participants.

Using the PopPK model, plasma concentration data from the phase 2 study was used to predict urine ASP5354 concentration data. The relationship between simulated urine concentrations and the intensity of fluorescence from the Likert scale was evaluated for the 0.3, 1, and 3 mg doses. The 3 mg dose showed consistent efficacy with regards to intensity of fluorescence and was above the target urinary concentration for ureter visualization of 1 µg/mL, which was determined based on nonclinical ex vivo and in vivo minipig studies. In contrast, the 1 mg dose did not show consistency across the Likert scale regarding intensity of fluorescence and also depicted a high amount of variability even though it was above the target urinary concentration for ureter visualization of 1 µg/mL. Therefore, the 3 mg dose was selected for the phase 3 program.

Based on the data from 2 phase 1 studies in healthy participants; 1 phase 1 study in participants with mild, moderate and severe renal impairment compared with healthy controls; 1 phase 2 study in participants undergoing laparoscopic or minimally invasive colorectal surgery; and to compensate for a potential decrease in urine concentration of ASP5354 in participants with renal impairment, a single 3 mg dose is considered clinically relevant and safe. In addition, it should be adequate to evaluate the clinical utility of ASP5354 in this population.

Study Objectives, Endpoints and Estimands:

Objectives	Endpoints
Primary	
● Investigator's blinded conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment	● Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale
Key Secondary	
● 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	● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
Additional Secondary	
● Support the investigator's qualitative assessment of ureter conspicuity with a quantitative measure for all participants in all cohorts	● Using recorded images, ureter conspicuity for WL and NIR-F illumination modes will be quantified by image analysis for all time points
● Investigator's conspicuity assessment of the	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus

ureter at the first time point for adolescents	NIR-F at 30 (± 15) min after ASP5354 administration
• Investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents	• Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points • Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
• Investigator's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment	• Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
• 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	• Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points • Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
• Investigator's conspicuity assessment of the ureter at the first time point for participants in all cohorts	• Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
• 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	• Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points • Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
• 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	• 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
• BICR's conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment	• Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
• 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	• Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points • Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
• BICR's conspicuity assessment of the ureter at	• Descriptive summary of intra-participant

the first time point for adolescents	difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● BICR's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● BICR's conspicuity assessment of the ureter at the first time point for participants in all cohorts	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● Concordance between investigator's intra-operative conspicuity assessment of the ureter and BICR assessment for adults with normal renal function or mild renal impairment	● Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration by each BICR reader ● CCC, the inter-rater reliability between the investigators and BICR for the WL versus NIR-F at 30 (± 15) min after ASP5354 administration ● Intra-participant difference in ureter conspicuity for WL at 30 (± 15) min versus the average of all NIR-F time points by each BICR reader ● 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 ● Intra-participant difference in ureter conspicuity for WL at 30 (± 15) min versus the end of surgery time point score with NIR-F by each BICR reader ● 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

● Safety of ASP5354	● Nature, frequency, severity and causality of TEAEs and serious TEAEs ● Clinical laboratory tests (hematology, biochemistry and urinalysis) ● Vital signs ● Electrocardiograms/cardiac monitoring
● Investigate the pharmacokinetics of ASP5354	● Plasma and urine concentrations of ASP5354 ● Ae and Ae%
Exploratory	
● To summarize the investigator's conspicuity assessment with WL of the ureter at each time point	● 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
● Concordance of conspicuity assessment of the ureter among BICR readers for participants with normal renal function or mild renal impairment	● ICC, the inter-rater reliability among BICR readers on the intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min 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

Ae: amount of ASP5354 excreted into urine; Ae%: percentage of ASP5354 dose excreted into urine; BICR: blinded independent central review; CCC: concordance correlation coefficient; ICC: intraclass correlation coefficient; NIR-F: near-infrared fluorescence; TEAE: treatment-emergent adverse events; WL: white light

Primary Estimands

Population:

Male and female participants ≥ 18 years of age with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min undergoing minimally invasive and open abdominopelvic surgeries. The population is further defined by the eligibility criteria.

Treatment Regimen:

The treatment regimen is ASP5354, 1 single intravenous dose of 3 mg (3 [three] 1 mg vials).

Primary Endpoint:

Intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration. The first assessment will always be under WL followed by NIR-F

assessment. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale (see [Section 10.8]).

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:

- 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 with 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.

Study Population:

Male or female participants ≥ 12 years of age scheduled to undergo minimally invasive and open abdominopelvic surgery.

Number of Participants:

Approximately 107 participants (94 adults and 13 adolescents) will be enrolled.

Adult Cohorts (Participants ≥ 18 years of age)

- Normal/Mild eGFR Cohort:

  Approximately 84 adult participants with normal renal function or mild renal impairment (eGFR ≥ 60 mL/min) will be enrolled to receive ASP5354 and randomized to the WL/NIR-F arm or the WL-only arm. The allocation ratio of the randomization to WL/NIR-F versus WL-only will be specified in the study randomization specification document.

- Moderate/Severe eGFR Cohort:

  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 the enrollment of participants with normal/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 adolescent participants 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 (at least 6 participants 12 to 14 years of age and at least 4 participants 15 to 17 years of age) complete the study to allow collection of pharmacokinetic samples from at least 6 participants in age group ≥ 12 and < 15 years of age and at least 4 participants in age group ≥ 15 and < 18 years of age. 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.

Study Design Overview:

This is a phase 3, multicenter, prospective, randomized, open-label study to evaluate the clinical utility of ASP5354 with the use of an NIR-F imaging device 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 study.

This study is designed to evaluate the clinical utility of ASP5354 which enhances the conspicuity of the ureter with the use of an NIR-F imaging device. 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 (BICR) 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. The normal/mild eGFR cohort will be randomized to 2 arms: WL/NIR-F arm and WL-only arm. Randomization will be stratified by type of surgery (gynecological; other abdominopelvic). There is no randomization for adult participants with moderate/severe eGFR 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.

Efficacy Assessments

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 (by the study-specific 5-Point Likert Scale; hereafter, referred to as the 5-Point Likert Scale) performed with WL at 30-min after ASP5354 administration. This first WL assessment will be done when the investigator reaches the point in the 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 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. The first assessment will always be under WL followed by NIR-F assessment.
  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. The actual end of surgery is defined as the moment when all the procedures related to the surgery/surgery part that the investigator planned for the study are concluded.
- 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

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.

Adolescents:

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.

All Participants:

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.

Efficacy Evaluation

For all participants, the investigator will perform the ureter conspicuity assessment by 5-Point Likert Scale:

- The investigator will attempt to locate the ureter and score the ureter conspicuity using the 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)".

WL/NIR-F Arm Participants Only:

- Using recorded images, ureter conspicuity for WL and NIR-F illumination modes will be quantified by image analysis for all time points.
- The BICR will perform the ureter conspicuity assessment by 5-Point Likert Scale using recorded images taken during surgery at approximately the same timepoint as the investigator.

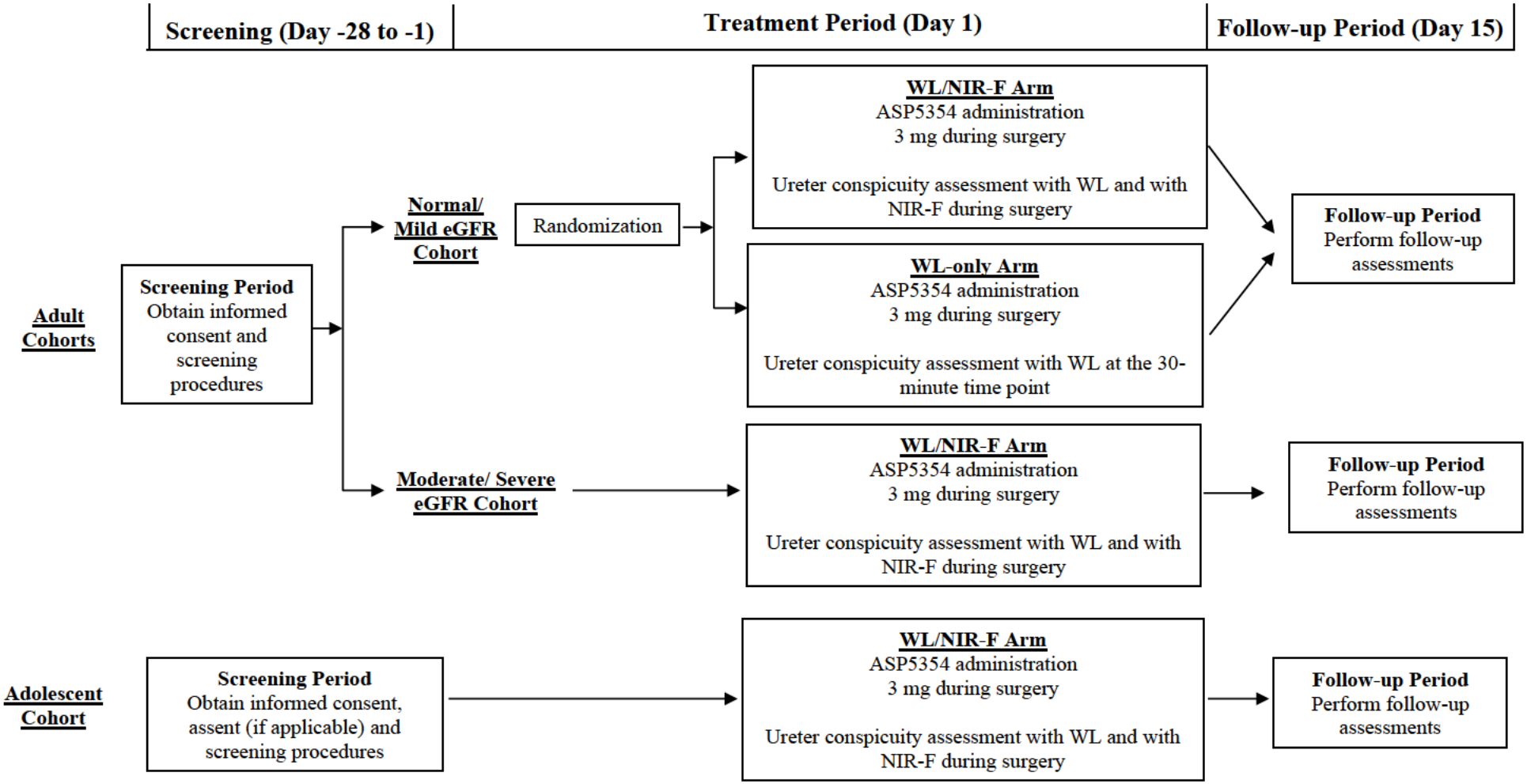
Safety Evaluation

Safety and tolerability will be assessed by adverse events (AEs), clinical laboratory evaluations (hematology, biochemistry and urinalysis), vital signs measurements, physical examinations and electrocardiograms (ECGs)/cardiac monitoring.

Study Intervention Groups and Duration:

Intervention Name	ASP5354 (pudexacianinium chloride)
Type	drug
Dose Formulation	sterile powder for solution
Unit Dose Strength	each vial contains 1.3 mg of ASP5354 and must be reconstituted with 1.2 mL sterile water for injection, to a concentration of 1 mg/mL
Dosage Level	single dose of 3 mg (3 vials of 1 mg)
Route of Administration	intravenous
Use	experimental

1.2 Study Schema



eGFR: estimated glomerular filtration rate; NIR-F: near-infrared fluorescence; WL: white light

1.3 Schedules of Assessments

Table 1 Schedule of Assessments

Assessments	**Screening Period[1]**	**Treatment Period**				**Follow-up Period**
Visit number	**1**	**2**				**3**
Visit Label	**Screening**	**Preoperative[1]**	**Intraoperative**	**Postoperative**		**Follow-up**
Day	**-28 to -1**		**1**		**2[2]**	**15[3]**
Window	**-**	**-**	**-**	**-**	**+ 2**	**± 10**
Informed consent/assent[4]	X					
Inclusion/exclusion criteria	X					
Medical history and current conditions[5]	X					
Demographics[6]	X					
Physical examination[7]	X	X		X[8]		X
Vital signs[9]	X	X		X[8]		X
Clinical laboratory tests[10]	X[11]	X		X[12]	X	X
Height[9]	X					
Weight[9]	X					X
Pregnancy test[13]	X[11]	X				
ECG/cardiac monitoring[14]		X	X	X[8]		
Randomization (normal/mild eGFR cohort only)		X[15]				
ASP5354 dosing			X			
Blood sampling for ASP5354 pharmacokinetics[16]			See [Table 3]			
Urine sampling for ASP5354 pharmacokinetics[17]			See [Table 3]			
Visual assessment of urine output[18]			→	→	→	→
Efficacy assessments[19]			See [Table 2]			
Record surgery			X[20]			
Previous/concomitant medications	X	X	X	X	X	X[21]
AE/SAE assessment	X	X	X	X	X	X[21]
ADE assessment			X			

ADE: adverse device effect; AE/SAE: adverse event/serious adverse event; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate

1. The assessments scheduled for the screening period can be done on day 1 if all eligibility criteria can be confirmed before randomization for adults (normal/mild eGFR cohort) or can be confirmed before registering adults (moderate/severe eGFR cohort) and adolescent participants for ASP5354. If screening procedures are performed on day 1, physical examination, vital signs, clinical laboratory tests, pregnancy test (blood or urine), previous/concomitant medications and AE/SAE assessment will be done once.
2. Only when participant stays in the hospital.
3. If a participant discontinues early from the study after the study intervention administration, the follow-up visit procedures will be performed upon discontinuation. If a participant discontinues before the study intervention administration, the follow-up visit procedures will not be required.
4. For adult participants ≥ 18 years of age, consent must be obtained prior to any study-related procedures. For adolescent participants ≥ 12 and < 18 years of age, in addition to consent from a participant's parent or legal guardian, assent from an adolescent participant is obtained if required.
5. Medical history includes all significant medical conditions that have occurred or are currently ongoing (including previous abdominopelvic surgical history and prior inflammatory disorders of interest [e.g., endometriosis]).
6. Demographic information will be collected for all participants and will include age, sex, race and ethnicity.
7. A full physical examination will be performed at screening (visit 1). Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular. At visit 2 and the follow-up visit (visit 3), a symptom-directed physical examination will be performed.
8. Physical examination, vital signs and ECG/cardiac monitoring will be completed on day 1 or day 2.
9. All vital signs (blood pressure and pulse) will be measured with the participant in the sitting or supine position. Height will be collected at visit 1 and weight will be measured at visits 1 and 3 using standard institution practice and equipment.
10. Clinical laboratory tests for the central laboratory include blood collection for hematology (complete blood count), biochemistry and urine samples for urinalysis.
11. The results of these assessments have to be obtained prior to randomization for adults (normal/mild eGFR cohort) or registration of adults (moderate/severe eGFR cohort) and adolescent participants for ASP5354. If the results from the central laboratory are not yet available at time of randomization/registration, results from local laboratory within 28 days before the surgery can be used. The participant will not be excluded from the study if central laboratory results from screening are exclusionary and are available only after randomization/registration.
12. Clinical laboratory tests will be collected within 2-hours after the actual end time of surgery if participant is discharged on day of surgery (day 1). Otherwise, the test will be collected at postoperative day 2 (+ 2 days).
13. Required only for women of childbearing potential (see [Section 10.2] for contraception requirements). The pregnancy test scheduled for the screening visit can be performed by either serum or urine. For the preoperative visit, serum or urine pregnancy test will be performed locally. If no result from screening visit is available at time of randomization/registration, the result of the blood or urine pregnancy test at preoperative visit must be confirmed before randomization/registration.
14. A standard 12-lead ECG or the site's standard care of routine cardiac monitoring will be taken preoperatively prior to ASP5354 administration. After administration of ASP5354 (within 10-min after ASP5354 administration) and the postoperative ECGs can be done as per the site's standard of care routine cardiac monitoring.
15. Randomization can also be done 1 business day prior to the surgery day if eligibility is confirmed prior to randomization depending on local pharmacy availability for early morning surgery cases.
16. Blood samples for pharmacokinetics of ASP5354 will be collected from all participants. Blood samples will be collected according to [Table 3].
17. Urine samples for pharmacokinetics will be collected according to [Table 3]. Urine volume will be calculated from the weight of urine in the catheter bag. Urine volume will be recorded in milliliters. Details will be provided in a Laboratory Manual.
18. Urine output will be visually assessed throughout the study. Visual observation abnormalities such as abnormal urine color (e.g., green color in the urine), if observed, will be recorded as an AE.

19. Investigator evaluation and blinded independent central review assessments for ureter conspicuity will be assessed intraoperatively and at the end of surgery time point (see [Table 2]).
20. A video will be recorded during surgery (see [Table 2]). Additionally, the reason for surgery, surgery type, surgical duration, surgical device and time of the video for each time point will be collected. Recorded images will be used for the quantitative analysis and blinded independent central review assessments.
21. If a participant experiences an AE/SAE/ADE or change in concomitant medications between Visit 2 and Visit 3, the participant should call the study site to inform of these changes. For participants who experience hypersensitivity reaction(s), an additional blood sample should be collected to determine histamine and tryptase concentration levels. The sample should be taken as soon as possible after the onset of the hypersensitivity reaction and sent to the central laboratory.

1.3.1 Sample Collection Schedule for Pharmacokinetics

Table 2 Efficacy Assessment Schedule

Procedure	Time Point (After ASP5354 Administration)	Ureter Conspicuity by 5-Point Likert Scale		Quantitative Assessment		BICR Assessment		Assessment Window
WL/NIR-F Arm								
		WL	**NIR-F**	**WL[1]**	**NIR-F[1]**	**WL[1]**	**NIR-F[1]**	
Intraoperative	30 min	X	X	X	X	X	X	30 (± 15) min
	Every 30 min thereafter	X	X	X	X	X	X	± 15 min
End of surgery time point	-	X[2]	X[2]	X	X	X	X	-
WL-only Arm								
		WL		**WL**		**WL**		
Intraoperative	30 min	X		-		-		30 (± 15) min

BICR: blinded independent central review; NIR-F: near-infrared fluorescence; WL: white light

1. A video will be recorded during the entire surgery for all participants for retrospective review. Using recorded images, ureter conspicuity will be quantified by image analysis and used for the BICR assessment for all time points in the WL/NIR-F arm. A detailed procedure for recording and submitting images, and preparation of the recorded images will be defined in a separate manual.
2. 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.

Table 3 Sample Collection Schedule for Pharmacokinetics

Blood Collection Schedule

Procedure	Time Point (after ASP5354 administration)	PK Blood Collection	Assessment Window for Blood Collection
Intraoperative	10 min[1]	X	± 5 min
	30 min[1]	X	± 15 min
	1 h[2]	X	± 15 min
	Every 1 hour until end of surgery time point[3]	X	± 15 min
Postoperative	-[4]	X	Within 2 hours after the actual end of surgery

Urine Collection Schedule

Procedure	Time Interval (after ASP5354 administration)	PK Urine Collection	Assessment Window for Urine Catheter Bags Change[5]
Intraoperative	0- to 10 min	X	10 (± 5) min
	10- to 30 min	X	30 (± 15) min
	Every 30 min thereafter until end of surgery time point[3]	X	Every 30 (± 15) min
Postoperative	-	-	-

PK: pharmacokinetics

1. Even if surgery ends earlier than this time point, this time point would need to be collected.
2. If surgery ends earlier than this time point, this time point would NOT need to be collected.
3. 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. Regardless of switching the surgical approach during surgery, blood and urine samples for PK should be collected until the end of surgery timepoint for the open surgery part according to [Table 3]. In any case, for the time points after “3 h time point” for blood, and after “2.5 h - 3 h time point” for urine, those time points would be optional.
4. If the blood collection at 3 hours timepoint is obtained, this time point would be optional.
5. The investigator or site study staff will change the urine catheter bags during the assessment window.

2 INTRODUCTION

2.1 Study Rationale

ASP5354 (also known as pudexacianinium chloride) is a novel near-infrared fluorescence (NIR-F) imaging agent under investigation for intraoperative anatomical identification/visualization of ureter(s) in participants undergoing minimally invasive and open abdominopelvic surgeries.

Pudexacianinium chloride is a new chemical entity which has unique hydrophilic properties through conjugation with cyclodextrin. Unlike ICG, ASP5354 is iodine free. ASP5354 is administered intravenously and has a clinically distinct pharmacokinetic profile that allows for ASP5354 to be excreted into the urine through the kidneys. Photo-optically, ASP5354 is indistinguishable from ICG with an absorption of 780 nm and a fluorescence emission at 820 nm, allowing for the use of approved (ICG) NIR-F imaging technology. In this study, ASP5354 will be used with FDA-cleared imaging devices that have been approved for use with ICG for laparoscopic (Stryker 1688 Advanced Imaging Modalities device) and open surgical procedures (Stryker SPY-PHI). These devices provide both white light (WL) and NIR-F illumination modes. The NIR-F illumination/imaging technology when used with ASP5354 facilitates anatomic ureter visualization during minimally invasive and open abdominopelvic surgeries.

This study is designed to evaluate the clinical utility of ASP5354 which enhances the conspicuity of the ureter with the use of an NIR-F imaging device. The benefit of enhanced conspicuity is to identify the ureter as soon as possible during the surgical procedures, which is expected to help minimize the risk of iatrogenic ureteral injury (IUI) and associated complications. To date, there are no uniform prevention guidelines to avoid IUIs and no commercially available ureteral imaging agents. The European Association of Urology and American Society of Colon and Rectal Surgeons recommend either the use of ureteral stents and/or a cautious dissection with direct identification/visualization of the ureters during abdominopelvic procedures. Despite these recommendations, IUIs still occur and may not be evident until post-surgery. The European Association of Urology states that the best way to avoid IUIs is real-time ureter visualization. The use of ASP5354 with NIR-F imaging technologies is a promising technique that offers real-time ureter visualization for surgeons.

ASP5354 has been studied in the following completed studies: 2 phase 1 studies in healthy participants and in 1 phase 2 study in participants undergoing laparoscopic or minimally invasive colorectal surgery. ASP5354 has also been studied in a completed phase 1 study in participants with mild, moderate or severe renal impairment compared with healthy controls. In addition to this phase 3 study, another phase 3 study in adult participants undergoing minimally invasive and open abdominopelvic surgeries is being conducted. Clinical data to date shows ASP5354 has rapid renal clearance without metabolism, and it is safe and well tolerated given as a single intravenous dose up to 24 mg. In the phase 2 study, enhanced conspicuity was observed in all 3 doses evaluated (0.3, 1 and 3 mg). Given the ASP5354 safety profile, the highest dose of 3 mg was selected for the phase 3 program to provide

optimal conspicuity of the ureter for the duration of surgery in all intended participants for the phase 3 clinical program, including renally impaired participants. The physiologically based pharmacokinetic (PBPK) modeling and simulation suggests that lower urine ASP5354 concentration levels are expected in moderate and severe renally impaired participants than in healthy participants with normal renal function. However, it was suggested that mean urine concentration would still exceed 1 μg/mL for 3 hours after a single intravenous 3 mg dose of ASP5354 in participants with moderate and severe renal impairment, which is the target urine concentration for ureter visualization based on nonclinical studies [Fushiki et al, 2023]. It is suggested that ureter conspicuity can be anticipated at a dose of 3 mg in participants with moderate and severe renal impairment as well.

Based on the data from 2 phase 1 studies in healthy participants; 1 phase 1 study in participants with mild, moderate and severe renal impairment compared with healthy controls; 1 phase 2 study in participants undergoing laparoscopic or minimally invasive colorectal surgery; and to compensate for a potential decrease in urine concentration of ASP5354 in participants with renal impairment, a single 3 mg dose is considered clinically relevant and safe. In addition, it should be adequate to evaluate the clinical utility of ASP5354 in this population.

2.2 Background

The available nonclinical and clinical data collected to date indicates that the use of ASP5354 with NIR-F imaging technologies is a promising technique that offers noninvasive, real-time ureter visualization in participants undergoing minimally invasive and open abdominopelvic surgery. This is expected to help minimize the risk of IUI which is widely recognized as a "potentially devastating complication of modern surgery" [Burks & Santucci, 2014] associated with higher mortality, morbidity, longer length of hospital stays and increased healthcare cost [Halabi et al, 2014]. In a retrospective study of 53 096 hysterectomies in Norway, IUI was documented in 643 patients (1.2%). Of the 643 patients, 69 claims for compensation were received. In 77% of the claims patients required surgery to correct for IUI and 10% of these patients lost a kidney [Ravlo et al, 2022]. Numerous reports detail the seriousness of the IUI condition [Poza et al, 2022; Kaymak et al, 2021; Zhang et al, 2021].

Refer to the Investigator's Brochure (IB) for detailed information about the nonclinical and clinical data for ASP5354.

2.3 Risk/Benefit Assessment

2.3.1 Risk Assessment

In the clinical studies completed to date, ASP5354 has been shown to be safe and well tolerated given as a single intravenous dose up to 24 mg. One of the most frequently reported adverse events (AEs) was green coloration of the urine. ASP5354 is excreted into the urine through the kidneys due to its hydrophilic nature. It is expected that all participants will have green coloration of the urine with ASP5354 administration.

ASP5354 is a new chemical entity administered intravenously. Unlike ICG, it does not contain iodine and no hypersensitivity reactions have been observed for ASP5354 in the clinical studies completed to date. In general, the ICG-related hypersensitivity has been attributed to iodine [Bjerregaard et al, 2013]. Although it remains a potential risk, the use of ASP5354 will be in surgical suites/operating rooms, which are fully equipped to manage a hypersensitivity reaction should it occur.

2.3.2 Benefit Assessment

The benefit of ASP5354 is real-time visualization of the ureter in patients undergoing minimally invasive and open abdominopelvic surgeries with NIR-F optical devices currently in clinical practice, with the potential to identify intraoperative IUIs early or to minimize the risk of IUIs.

2.3.3 Overall Risk-Benefit Conclusion

ASP5354 given at 3 mg as a single intravenous dose for use in minimally invasive and open abdominopelvic surgeries with NIR-F optical devices has a favorable overall risk-benefit profile.

3 OBJECTIVES, ENDPOINTS AND ESTIMANDS

Table 4 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
● Investigator's blinded conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment	● Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration. Conspicuity will be scored individually for each illumination mode using the 5-Point Likert Scale
Key Secondary	
● 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	● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
Additional Secondary	
● Support the investigator's qualitative assessment of ureter conspicuity with a quantitative measure for all participants in all cohorts	● Using recorded images, ureter conspicuity for WL and NIR-F illumination modes will be quantified by image analysis for all time points
● Investigator's conspicuity assessment of the ureter at the first time point for adolescents	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration

Objectives	**Endpoints**
● Investigator's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● Investigator's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● Investigator's conspicuity assessment of the ureter at the first time point for participants in all cohorts	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● 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	● 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
● BICR's conspicuity assessment of the ureter at the first time point for adults with normal renal function or mild renal impairment	● Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Intra-participant comparison of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F

Objectives	**Endpoints**
● BICR's conspicuity assessment of the ureter at the first time point for adolescents	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● BICR's conspicuity assessment of the ureter when using ASP5354 with NIR-F for the duration of the surgical procedure for adolescents	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● BICR's conspicuity assessment of the ureter at the first time point for adults with moderate or severe renal impairment	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● BICR's conspicuity assessment of the ureter at the first time point for participants in all cohorts	● Descriptive summary of intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration
● 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	● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the average of all NIR-F time points ● Descriptive summary of ureter conspicuity scores for the WL 30 (± 15) min time point versus the end of surgery time point score with NIR-F
● Concordance between investigator's intra-operative conspicuity assessment of the ureter and BICR assessment for adults with normal renal function or mild renal impairment	● Intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min after ASP5354 administration by each BICR reader ● CCC, the inter-rater reliability between the investigators and BICR for the WL versus NIR-F at 30 (± 15) min after ASP5354 administration ● Intra-participant difference in ureter conspicuity for WL at 30 (± 15) min versus the average of all NIR-F time points by each BICR reader ● 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 ● Intra-participant difference in ureter conspicuity for WL at 30 (± 15) min versus the

Objectives	Endpoints
	end of surgery time point score with NIR-F by each BICR reader ● 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
● Safety of ASP5354	● Nature, frequency, severity and causality of TEAEs and serious TEAEs ● Clinical laboratory tests (hematology, biochemistry and urinalysis) ● Vital signs ● Electrocardiograms/cardiac monitoring
● Investigate the pharmacokinetics of ASP5354	● Plasma and urine concentrations of ASP5354 ● Ae and Ae%
Exploratory	
● To summarize the investigator's conspicuity assessment with WL of the ureter at each time point	● 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
● Concordance of conspicuity assessment of the ureter among BICR readers for participants with normal renal function or mild renal impairment	● ICC, the inter-rater reliability among BICR readers on the intra-participant difference in ureter conspicuity for WL versus NIR-F at 30 (± 15) min 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

Ae: amount of ASP5354 excreted into urine; Ae%: percentage of ASP5354 dose excreted into urine; BICR: blinded independent central review; CCC: concordance correlation coefficient; ICC: intraclass correlation coefficient; NIR-F: near-infrared fluorescence; TEAE: treatment-emergent adverse events; WL: white light.

3.1 Primary Estimands

Population:

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

Treatment Regimen:

The treatment regimen is ASP5354, 1 single intravenous dose of 3 mg (3 [three] 1 mg vials).

Primary Endpoint:

Intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration. The first assessment will always be under WL followed by NIR-F assessment. 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:

- 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 with 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.

4 STUDY DESIGN AND DOSE RATIONALE

4.1 Overall Study Design

This is a phase 3, multicenter, prospective, randomized, open-label study to evaluate the clinical utility of ASP5354 with the use of an NIR-F imaging device 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 study. This study will be conducted in approximately 6 clinical sites in the US.

This study is designed to evaluate the clinical utility of ASP5354 which enhances the conspicuity of the ureter with the use of an NIR-F imaging device. 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 a BICR 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. 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 (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.

For additional details on each cohort, please refer to [Sections 7.1.2, 7.1.3 and 7.1.4] for adults with normal/mild eGFR, adults with moderate/severe eGFR and adolescents, respectively.

4.2 Scientific Rationale for Study Design

There are currently no approved imaging agents for ureter identification during open or minimally invasive abdominopelvic/pelvic surgeries. A multicenter, prospective, randomized and open-label study design was chosen to evaluate the intraoperative ureter conspicuity using NIR-F by the operating investigator after administration of ASP5354 compared with ureter conspicuity by WL within each study participant in the WL/NIR-F arm. To reduce the investigator's bias, the WL-only arm is added to cast doubt in the investigator's mind as to whether NIR-F illumination mode will be used for a given participant, which may enhance more consistent behavior (similar due diligence) for evaluation with WL versus NIR-F. Conspicuity assessments in the WL/NIR-F arm will be completed throughout surgery to

evaluate the clinical utility of ASP5354 in ureter visualization. In the WL/NIR-F arm, the first assessment will always be under WL followed by NIR-F assessment. Additionally, a confirmatory blinded independent central review (BICR) will be employed to independently read all WL and NIR-F images for all timepoints for all participants in the WL/NIR-F arm.

ASP5354 has been studied in 2 phase 1 studies in healthy participants and in 1 phase 2 study in participants undergoing laparoscopic or minimally invasive colorectal surgery. ASP5354 was shown to be safe and well tolerated at a single intravenous dose up to 24 mg in the US phase 1 safety and pharmacokinetic study in healthy participants (5354-CL-0001). No treatment-related serious or non-serious AEs were reported in this study. The Japanese phase 1 study (5354-CL-0002) findings are consistent with those observed in the US phase 1 study. No safety issues were reported with no clinically significant changes in vital signs, 12-lead electrocardiogram (ECG), clinical laboratory evaluation or physical examination.

ASP5354 has also been studied in a phase 1 study in participants with mild, moderate or severe renal impairment compared with healthy controls at a 3 mg dose:

- No significant safety findings have been identified from clinical trial 5354-CL-0004 (A phase 1, open-label study to evaluate the pharmacokinetics, safety and tolerability of ASP5354 in participants with renal impairment compared to healthy participants with normal renal function). The safety data included a total of 7 non-serious treatment-emergent adverse events (TEAEs) that were reported of the 28 participants enrolled. The TEAEs were single occurrences and included: anemia aggravated, sore throat, headache, flatulence, bloating, diarrhea and hyperbilirubinemia.
- Only 2 of the non-serious TEAEs (headache grade 1 and hyperbilirubinemia grade 1) were considered related to ASP5354, which occurred in healthy participants with normal renal function:
    - The headache occurred on day 1 (day of ASP5354 administration) and resolved on day 3, as judged by the investigator.
    - The hyperbilirubinemia occurred on day 8 and resolved on day 14, as judged by the investigator.

The inclusion of adolescents in a phase 3 study was proposed because ASP5354 has shown an acceptable safety and tolerability profile similar to ICG. Pharmacokinetic modeling and simulation has suggested that systemic exposure (C_{max} and AUC_{inf}) in adolescents (12 to < 18 years of age) is comparable to those in adults (≥ 18 years of age). Also, it is expected that adolescent participants will have similar efficacy/visualization when they have similar exposures of ASP5354 as adults.

4.2.1 Participant Input into Study Design

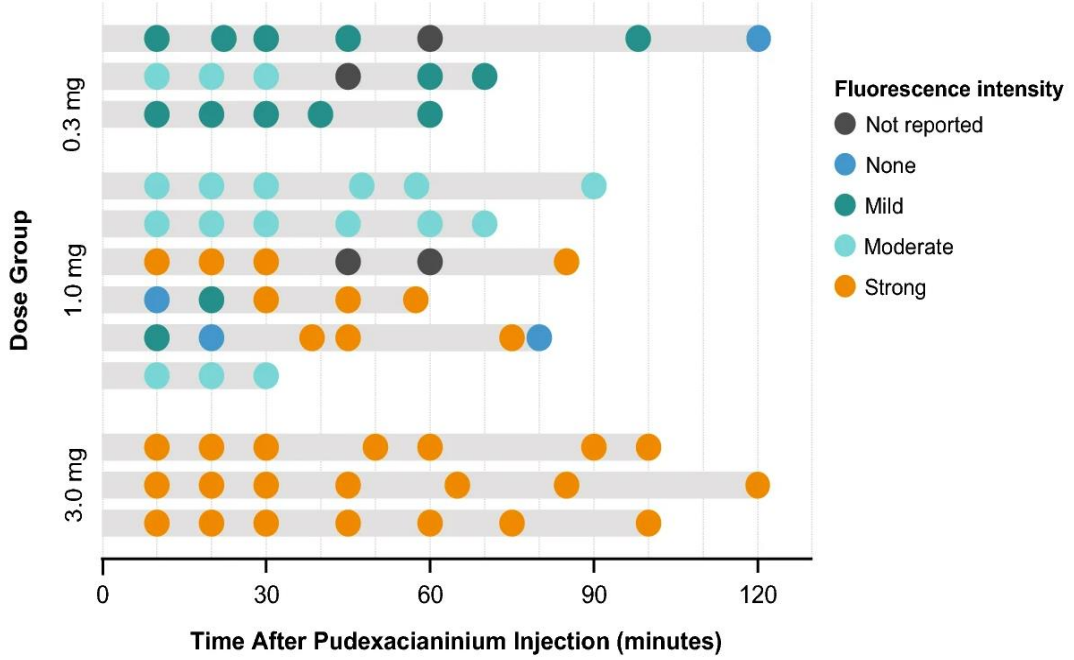
There was no participant involvement in the design of the study.

4.3 Dose Rationale

The dose selection for the phase 3 studies was based on data from the phase 2 dose-ranging study (5354-CL-0201) conducted in adult participants undergoing laparoscopic/minimally

invasive colorectal surgery. Single intravenous doses of ASP5354 (pudexacianinium chloride) 0.3, 1 and 3 mg were administered. ASP5354 was safe and well tolerated when administered as single intravenous ascending doses up to 3 mg. Doses of 1 mg and 3 mg provided visualization throughout the duration of the surgical procedure; however, at the 3 mg dose, fluorescent intensity was stronger and more consistent. At the 1 mg dose, the intensity of fluorescence was variable [Figure 1] amongst the participants evaluated in the phase 2 study. The lower dose (0.3 mg) was determined to be inadequate for ureter visualization due to lower fluorescence intensity and shorter duration of visualization than doses of 1 mg and 3 mg. Given the ASP5354 safety profile described in [Section 4.2], the highest dose of 3 mg was selected for the phase 3 program to provide optimal conspicuity of the ureter for the duration of surgery in all intended participants for the phase 3 clinical program, including renally impaired participants.

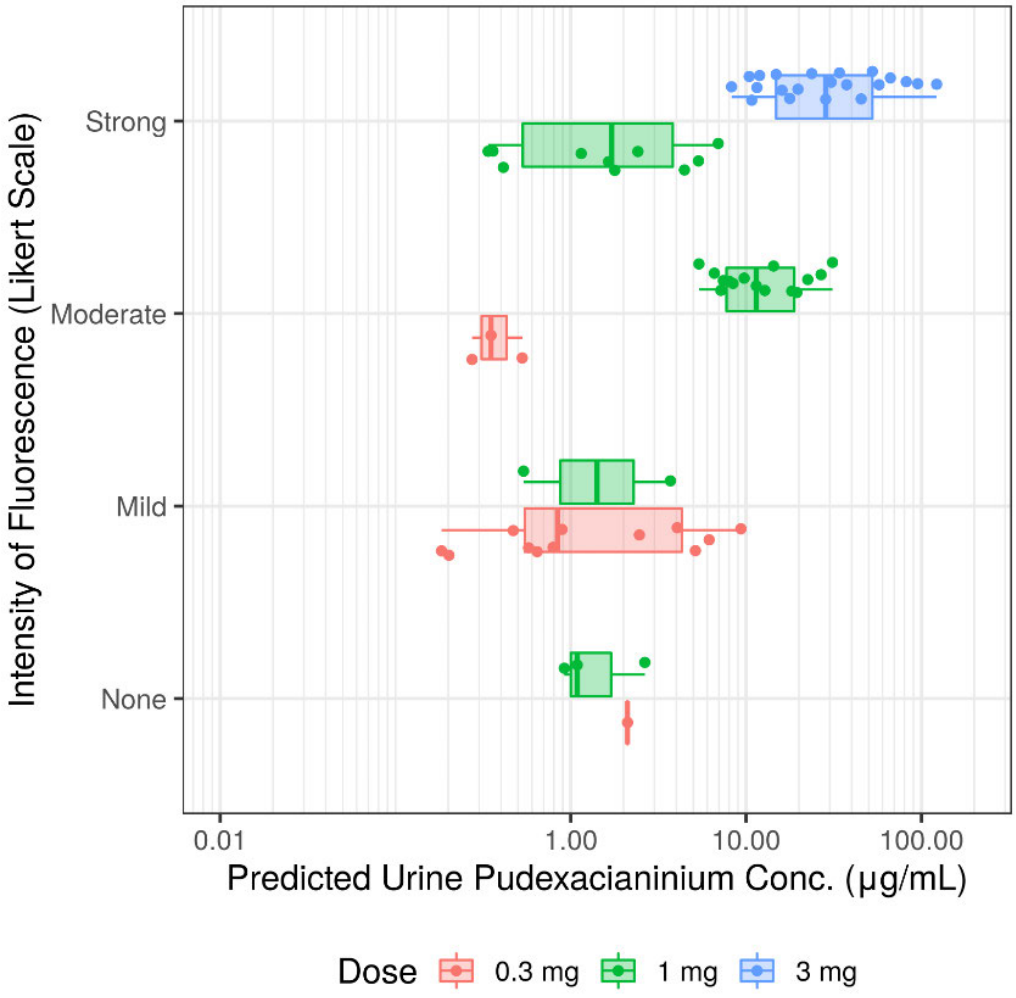
Figure 1 Fluorescence Intensity of the Index Ureter at Each Time Point During Surgery



Source: [Albert et al, 2023]

Using the PopPK model, plasma concentration data from the phase 2 study was used to predict urine ASP5354 concentration data. The relationship between simulated urine concentrations and the intensity of fluorescence from the Likert scale was evaluated for the 0.3, 1, and 3 mg doses. The 3 mg dose showed consistent efficacy with regards to intensity of fluorescence and was above the target urinary concentration for ureter visualization of 1 µg/mL, which was determined based on nonclinical ex vivo and in vivo minipig studies [Fushiki et al, 2023] [Figure 2]. In contrast, the 1 mg dose did not show consistency across the Likert scale regarding intensity of fluorescence and also depicted a high amount of variability even though it was above the target urinary concentration for ureter visualization of 1 µg/mL. Therefore, the 3 mg dose was selected for the phase 3 program.

Figure 2 Predicted Urine Pudexacianinium Concentration Data Versus the Intensity of Fluorescence (e.g., Likert Scale) From the Phase 2 Study



Source: [5354-PK-0005 PKPD report]

4.4 Start and End of Study Definitions

First act of recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of recruitment is the date the first participant signs the informed consent form (ICF) and will be the study start date.

End of study

The end of the study is defined as the last visit or assessment shown in Schedule of Assessments [Table 1] for the last participant in the study.

A participant is considered to have completed the study if the participant has completed all periods of the study including the last assessment shown in the schedule of assessments.

5 STUDY POPULATION

All screening assessments must be completed and reviewed to confirm the potential participant meets all eligibility criteria. Prospective approval of protocol deviations to eligibility criteria (also known as protocol waivers or exemptions) is not permitted.

5.1 Inclusion Criteria

Participant is eligible for participation in the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved written informed consent and privacy language as per national regulations (e.g., Health Insurance Portability and Accountability Act [HIPAA] authorization for US study sites) must be obtained from the participant or participant's parent or legal guardian, and if required, assent from adolescent participant (≥ 12 to < 18 years of age) prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Participant is ≥ 12 years of age at the time of signing an ICF.
3. Participant is scheduled to undergo minimally invasive or open abdominopelvic surgery that may require ureter identification.
4. Participant has normal renal function or has varying degrees of chronic kidney disease as defined by the National Kidney Foundation and calculated by individual eGFR using the modification of diet in renal disease (MDRD) formula for adults or the Schwartz formula for adolescents at the screening visit (see [Section 10.7]).
   - Adult normal/mild eGFR cohort: eGFR ≥ 60 mL/min
   - Adult moderate/severe eGFR cohort: eGFR ≥ 15 to < 60 mL/min
   - Adolescent cohort: eGFR ≥ 60 mL/min
5. Female participant is not pregnant (see [Section 10.2]) and at least 1 of the following conditions apply:
   a. Not a woman of childbearing potential (WOCBP; see [Section 10.2])
   b. WOCBP who agrees to follow the contraceptive guidance (see [Section 10.2]) from the time of informed consent through at least 30 days after study intervention administration.

6. Female participant must agree not to breastfeed starting at the administration of ASP5354 through 30 days after ASP5354 administration.
7. Female participant must not donate ova starting at the administration of ASP5354 through 30 days after ASP5354 administration.
8. Male participant with female partner(s) of childbearing potential (including breastfeeding partner) must agree to use contraception (see [Section 10.2]) through at least 30 days after ASP5354 administration.
9. Male participant must not donate sperm starting at the administration of ASP5354 through 30 days after ASP5354 administration.
10. Male participant with pregnant partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy from the start of ASP5354 administration through 30 days after ASP5354 administration.
11. Participant (and/or participant's parent or legal guardian) agrees not to participate in another interventional study involving unapproved study medications while participating in the present study.

5.2 Exclusion Criteria

Participant will be excluded from participation in the study if any of the following apply:

1. Participant has any physical or psychiatric condition, which, in the investigator's opinion, makes the participant unsuitable for study participation.
2. Participant is anticipated to require ureteral stenting during surgery.
3. Participant has an active urinary tract infection requiring antibiotic therapy.
4. Participant has moderate to severe cardiac disease that limits daily functioning (New York Heart Association Class III to IV) or other medical conditions that the investigator feels would impact safety or study compliance.
5. Participant has any clinically relevant laboratory abnormality that could contraindicate surgery in the opinion of the investigator.
6. Participant with body weight < 30 kg.
7. Participant has a known or suspected hypersensitivity to ASP5354, ICG or any components of the formulation used.
8. Participant has had previous exposure to ASP5354.
9. Participant has been administered ICG or other NIR-F imaging agents within 48-hours prior to ASP5354 administration, with the exception of participants who receive ICG for lymphatic mapping before the surgery.
10. Participant has received any investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to randomization.
11. Participant is on hemodialysis, hemodiafiltration or peritoneal dialysis

5.3 Lifestyle Considerations

Not applicable.

5.4 Screen Failures

A screen failure is defined as a potential participant who signed the ICF but did not meet 1 or more criteria required for participation in the study and was not enrolled/randomized.

For screen failures, the demographic data, date of signing the ICF, AEs up to the time of screen failure and reason for screen failure will be collected in the electronic case report form (eCRF).

5.4.1 Rescreening

Results of screening assessments that do not meet the parameters required by the eligibility criteria (e.g., clinical laboratory tests, vital signs, physical examinations, etc.) may be repeated once within the 28-day screening period without the need to register the participant as a screen failure. If more than 28 days elapse from the date of signing the ICF, the participant must be documented as a screen failure. In order for a previously screen-failed participant to re-screen, a new ICF must be signed and the participant will be registered in the IRT with a new participant identification code assigned. Rescreening is only allowed once for an individual participant.

6 STUDY INTERVENTION AND CONCOMITANT THERAPY

Study interventions are all pre-specified, investigational and non-investigational medicinal products, medical devices, vaccines and other interventions (e.g., surgical and behavioral) intended to be administered to the study participants during the study conduct.

6.1 Study Intervention(s) Administered

Table 5 Study Intervention(s) Administered

Intervention Label	ASP5354
Intervention Name	ASP5354 (pudexacianinium chloride)
Type	drug
Dose Formulation	sterile powder for solution
Unit Dose Strength(s)	each vial contains 1.3 mg of ASP5354 and must be reconstituted with 1.2 mL sterile water for injection, to a concentration of 1 mg/mL
Dosage Level(s)	single dose of 3 mg (3 vials of 1 mg)
Route of Administration	intravenous
Use	experimental
IMP and NIMP/AxMP	IMP
Sourcing	Provided centrally by the sponsor
Packaging and Labeling	ASP5354 sterile lyophilized powder will be provided in 2R amber glass vials and stored according to the instructions on the label

AxMP: auxiliary medicinal product; IMP: investigational medicinal product; NIMP: non-investigational medicinal product

Refer to the pharmacy manual for detailed information regarding preparation, handling and storage of the study intervention.

Table 6 Study Arm(s)

Arm Title	WL/NIR-F arm	WL-only arm
Arm Type	experimental	experimental
Arm Description	WL and NIR-F will be used to recognize/identify the ureter and conspicuity will be scored using the 5-Point Likert Scale. The time of the ureter conspicuity assessments will be documented. During surgery, ureter conspicuity assessment will be performed with WL and with NIR-F approximately every 30 (± 15) min from the ASP5354 administration with the last assessment performed at the end of surgery time point. The first assessment will always be under WL followed by NIR-F assessment.	Only 1 conspicuity assessment for each ureter will be performed with WL at 30 (± 15) min from ASP5354 administration.
Associated Intervention Labels	ASP5354	ASP5354

NIR-F: near-infrared fluorescence; WL: white light

6.1.1 Study Intervention Administration

All participants, adults and adolescents, will receive a single intravenous 3 mg dose (3 vials of 1 mg) of ASP5354 as an intravenous administration approximately 30 (± 15) min before the investigator expects to visualize the ureters. The time of ASP5354 administration will be documented.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and Labeling

All study intervention used in this study will be prepared, packaged and labeled under the responsibility of qualified personnel at Astellas Pharma Global Development Inc. (APGD) or sponsor's designee in accordance with APGD or sponsor's designee standard operating procedures (SOPs), current Good Manufacturing Practice (GMP) guidelines, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Each carton and vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as investigational drug. The study sites will be provided cartons each containing vials of ASP5354.

A qualified person of Astellas Pharma Europe BV or sponsor's designee will perform the final release of the study intervention according to the requirements of the European Union (EU) Clinical Trials Regulation 536/2014, current GMP guidelines, ICH GCP guidelines and applicable local laws/regulations.

Refer to the pharmacy manual for detailed information regarding packaging and labeling of the study intervention.

6.2.2 Handling, Storage and Accountability

- The investigator or designee must confirm appropriate conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.
- Only participants enrolled in the study may receive study intervention and only authorized study site personnel may supply, prepare or administer study intervention. Only study intervention with appropriate expiry/retest dating may be dispensed.
- All study intervention must be stored in a secure, environmentally-controlled and monitored (manual or automated) area in accordance with the labeled storage conditions and access must be limited to the investigator and authorized study site personnel.
- The investigator, institution or the head of the medical institution (where applicable) or authorized site staff is responsible for accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records including Certificate of Destruction or equivalent).
- The sponsor is responsible for providing further guidance and instruction on final disposition of used and unused study intervention in the pharmacy manual.

Refer to the pharmacy manual for detailed information regarding handling, storage and accountability of the study intervention.

6.3 Assignment to Study Intervention

Registration (adolescent and moderate/severe eGFR cohort[s])/randomization (normal/mild eGFR cohort only) and dispensation of study intervention will be performed via the interactive response technology (IRT) system. Each site with adult participants will designate an unblinded study member, who prior to initiation of surgery and treatment, will obtain the randomization assignment from the IRT system. Investigators will be blinded to the randomization assignment until after completion of the first ureter conspicuity assessment performed for the ureter(s) of interest with WL at the 30-min time point after ASP5354 administration. Specific IRT procedures will be described in the respective study manual.

6.4 Study Intervention Compliance

Not applicable. The exact date and time of study intervention administration will be documented and recorded in the source record and captured in the eCRF.

6.5 Dose Modification

Not applicable.

6.6 Continued Access to Study Intervention After the End of the Study

Not applicable.

6.7 Treatment of Overdose

In the event of suspected ASP5354 overdose, the participant should receive symptomatic and supportive care and monitoring. The medical monitor /expert should be contacted as applicable.

Refer to [Section 10.3.8.2] for reporting requirements for suspected overdose or other medication error.

6.8 Prior and Concomitant Therapy

The following concomitant medications listed below will not be allowed within 48 hours prior to ASP5354 administration through the completion of the surgical procedure:

- 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

Participants anticipated to require ureteral stents during surgery are excluded from the study [Exclusion Criterion No.2]. Ureteral stents however may be used based on clinical judgment of the investigator during surgery. Diuretics should be avoided 48-hours prior to ASP5354 administration until the completion of the surgical procedure but can be used based on clinical judgment of the anesthesiologist or investigator.

A list of excluded concomitant medications is provided in [Section 10.5].

Concomitant and corrective treatments (medication and non-medication therapy) will be recorded in the eCRF. However, there is no need to record supplements, nutrients or intravenous solutions for hydration.

7 STUDY PROCEDURES AND ASSESSMENTS

- Study procedures and their timing are summarized in the Schedules of Assessments [Table 1, Table 2 and Table 3]. Adherence to the study design requirements, including those specified in the Schedules of Assessments, is essential and required for study conduct. Prospective protocol waivers or exemptions are not allowed.
- Any change, divergence or departure from the study design or procedures identified in the protocol is considered a protocol deviation. All deviations from the protocol are to be recorded.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management (e.g., imaging, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the Schedule of Assessments [Table 1].
- In the event of a significant study-continuity issue (e.g., caused by a pandemic), alternate strategies for participant visits, assessments, medication distribution and monitoring may be implemented by the sponsor or the investigator, as per local health authority/ethics requirements. These alternate strategies are described in [Section 10.9].

7.1 Efficacy Assessments

Planned time points for all efficacy assessments are provided in the Efficacy Assessment Schedule [Table 2].

7.1.1 Primary and Secondary Efficacy Assessments

7.1.1.1 Ureter Conspicuity by 5-Point Likert Scale

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 first assessment will always be under WL followed by NIR-F assessment.

- The investigator will attempt to locate the ureter and score the ureter conspicuity using the 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 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

Refer to [Section 10.8] for more details regarding the conspicuity assessment by the 5-Point Likert Scale.

Training on the use of fluorescence imaging systems used during surgery and training on the use of the 5-Point Likert Scale will be provided in a separate manual.

7.1.2 Adults with Normal/Mild eGFR

Each site with adult participants will designate an unblinded study member who will randomize participants via the study's IRT system. Investigators will be blinded to the randomization assignment until after completion of the first ureter conspicuity assessment (by the study-specific 5-Point Likert Scale; hereafter, referred to as the 5-Point Likert Scale) performed for ureter(s) of interest with WL at 30-min after ASP5354 administration. This first WL assessment will be done when the investigator reaches the point in the 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 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. The actual end of surgery is defined as the moment when all the procedures related to the surgery/surgery part that the investigator planned for the study are concluded.
- 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.

7.1.3 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 for the moderate and severe eGFR participants as described in [Section 7.1.2], except investigators will not be blinded at the first WL assessment time point. The WL-only arm does not apply to this cohort.

7.1.4 Adolescents

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 for adults with normal/mild eGFR (see [Section 7.1.2]), except investigators will not be blinded at the first WL assessment time point. The WL-only arm does not apply to this cohort.

7.1.5 BICR Ureter Conspicuity by 5-Point Likert Scale

The conspicuity of the ureter with WL and WL/NIR-F will be assessed by BICR (5-Point Likert Scale) for all participants in the WL/NIR-F arm.

The BICR assessment of the ureter with WL and WL/NIR-F will be performed retrospectively using recorded images of the surgeries in this study. The ureter conspicuity will be based on the recorded images, which will be blinded for all participant identifiers

(including, but not limited to, participant identification and demographic) and time points, and provided in a random sequence. The recorded images under WL and NIR-F will be separately prepared by using the videos during the surgery and timestamp information that the investigator will provide.

The same conspicuity assessment procedures will be followed for all participants as described in [Section 7.1.1.1]. Additional details about the assessment will be provided in a separate manual.

7.1.6 Quantitative Assessment

Quantitative assessment of ureter conspicuity will be performed by image analysis using recorded images. Contrast enhancement factor (CEF) is a measure of the degree to which color contrast is enhanced in areas 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 [Table 2]. The performer of this image analysis will not have access to or be provided with the qualitative assessment score data. A separate study image analysis charter/plan will describe details of the image analysis methodology and image analysis processes.

Procedures and instructions for how to record, prepare, and submit the video files for the quantitative assessment will be described in the study's Site Imaging Manual.

7.2 Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Assessments [Table 1]. Protocol waivers or exemptions are not allowed.

Procedures conducted as part of a participant's clinical management (i.e., standard of care) obtained before signing the ICF may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame, as defined in the Schedule of Assessments [Table 1].

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.

7.2.1 Laboratory Assessments

- See [Section 10.6] for the list of clinical laboratory tests to be performed.
- The investigator must review the laboratory report, document this review and record any clinically significant changes occurring during the study as an AE (see [Section 10.3.1.1]). The laboratory reports must be filed with the source documents.
- Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or sub-investigator who is a qualified physician. Abnormal laboratory findings associated with the underlying disease are not considered

clinically significant unless judged by the investigator to be more severe than expected for the participant's condition.

- A central laboratory is used, but local results are acceptable at screening for confirming eligibility. Even if local results are used at screening for confirming eligibility, central laboratory tests should be performed at screening for safety analysis. The sites will collect blood and urine samples both for local laboratory tests and Central laboratory tests when local results are used at screening for confirming eligibility.

7.2.2 Vital Signs, Height and Weight

- All vital signs (blood pressure and pulse) will be measured with the participant in the sitting or supine position
- Height and weight will be measured using standard institution practice and equipment.

7.2.3 Physical Examination

A full physical examination will be performed at screening (visit 1). Body systems to be evaluated include general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular. At visit 2 and follow-up (visit 3), a symptom-directed physical examination will be performed.

7.2.4 Electrocardiogram and Cardiac Monitoring

A standard 12-lead ECGs and/or cardiac monitoring will be taken as indicated in the Schedule of Assessments [Table 1].

The original printout or an electronic copy of all scheduled and unscheduled ECG tracings should be maintained in the participant's medical chart. The investigator will review, sign and date the 12-lead ECG after recording to ensure participant safety. The time of the 12-lead ECG, the interval measurements, as well as an overall conclusion will be documented. Results will be recorded as normal, abnormal not clinically significant or abnormal clinically significant. Any abnormal or clinically significant 12-lead ECG or cardiac monitoring abnormalities should be recorded as an AE.

7.2.5 Order of Assessments

As a recommendation, the following order should be followed when more than 1 assessment is required at a scheduled time point.

1. Vital signs
2. ECG/cardiac monitoring
3. Blood collection

7.2.6 Other Assessments

7.2.6.1 Visual Assessment of Urine Output

Urine output will be visually assessed throughout the study. Visual observation abnormalities such as abnormal urine color (e.g., green color in the urine), if observed, will be assessed and recorded as an AE in the eCRF as per [Sections 7.3 and 10.3].

7.3 Adverse Events and Other Safety Aspects

The definitions of an AE or serious adverse event (SAE) and Adverse Device Effect (ADE) can be found in [Sections 10.3.1, 10.3.2 and 10.3.3], respectively.

Adverse events will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative [LAR]).

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE/SAE/ADE and remain responsible for following up AEs that are serious, considered related to the study intervention, or that caused the participant to discontinue the study intervention and/or study [see Section 10.3].

The method of recording, evaluating, and assessing causality of AE/SAE/ADE and the procedures for completing and transmitting SAE/ADE reports are provided in [Section 10.3].

7.3.1 Time Period and Frequency for Collecting Adverse Event and Serious Adverse Event Information

All AEs and SAEs will be collected from the signing of the ICF until the follow-up visit at the time points specified in the Schedule of Assessments [Table 1] and reported in the eCRF.

If the severity of an AE/SAE increases, an end date should be provided and the event should be relisted in the eCRF with the new National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) grade and new onset date.

If the severity decreases, an end date should be provided and the AE/SAE should be relisted in the eCRF with the new NCI CTCAE grade and new onset date. The exception is ongoing pre-dose events that continue post-dose and improve post-dose. Such events should not be re-listed.

If the severity of an SAE reduces, the details of the AE should be provided on the SAE worksheet for the medical assessor to be able to assess the course of the event.

All SAEs will be recorded and reported to the sponsor or designee without undue delay but not later than within 24 hours of obtaining knowledge of the event, as indicated in [Section 10.3]. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek any AE or SAE after conclusion of the study participation. However, if the investigator becomes aware of any SAE with a suspected causal relationship to the study intervention, including a death, at any time after a participant has been discharged from the study, the investigator must promptly report the SAE to the sponsor.

The Reference Safety Information that will be used to determine expectedness of an SAE for this study is in the IB.

7.3.1.1 Adverse Device Effects

- ADEs are to be collected via the ADE worksheet and reported to the sponsor within 24 hours of awareness (see [Section 10.3.3.1]).
- Any complaint about a device must be reported regardless of whether the defect or deficiency had any effect on a participant or on study personnel (see [Section 10.3.3.3]).

7.3.2 Method of Detecting Adverse Events and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

7.3.3 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and adverse events of special interest (AESIs) (as defined in [Section 7.3.6]) will be followed until resolution, stabilization, the event is otherwise explained or the participant is lost to follow-up (as defined in [Section 8.3]). Further information on follow-up procedures is provided in [Section 10.3].

If after the protocol-defined AE collection period (see [Section 7.3.1]), an AE progresses to an SAE, or the investigator learns of any AE/SAE including death, where the investigator considers there is reasonable possibility it is related to the study intervention or study participation, the investigator must promptly notify the sponsor.

7.3.4 Regulatory Reporting Requirements for Serious Adverse Events/Adverse Device Effects/Medical Device Deficiencies

- Prompt notification by the investigator to the sponsor of an SAE/ADE/medical device deficiency is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies and/or device manufacturer about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC and investigators.
- Investigator safety reports must be prepared for Suspected Unexpected Serious Adverse Reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

7.3.5 Disease-related Events and/or Disease-related Outcomes Not Qualifying as Adverse Events or Serious Adverse Events

Under this protocol, the following event(s) will not be considered as an AE/SAE and should not be reported to Astellas Safety:

- Preplanned and elective hospital/clinical procedures/interventions or procedures for diagnostic, therapeutic or surgical procedures for a pre-existing condition that did not worsen during the course of the study. These procedures are collected in the eCRF.

7.3.6 Adverse Events of Special Interest

Selected non-serious and serious AEs, also known as AESI, are to be collected via the SAE worksheet and reported to the sponsor within 24 hours of awareness.

In this study, the following are considered AESI:

- Hypersensitivity events (see [Section 2.3.1])
- IUI-associated events (see [Section 2.2])

7.3.7 Special Situations

Certain special situations observed in association with the study intervention, such as incorrect administration (e.g., wrong dose of study intervention) are reported as protocol deviations and/or may require special reporting, as described below. These special situations are not considered AEs but do require to be communicated to Astellas as per the timelines defined below.

If a special situation is associated with, or results in, an AE, the AE is to be assessed separately from the special situation and captured as an AE in the eCRF. If the AE meets the definition of an SAE, the SAE is to be reported as described in [Section 10.3.7] and the details of the associated special situation are to be included in the clinical description on the special situation worksheet or pregnancy reporting form.

The special situations are:

- Pregnancy
- Drug exposure via breast milk
- Medication error, overdose and use outside protocol
- Misuse/abuse
- Occupational exposure
- (Suspicion of) Transmission of infectious agent
- Suspected drug-drug interaction

Instructions and procedures for reporting special situations are provided in [Section 10.3.8].

7.4 Pharmacokinetics

7.4.1 Analysis of ASP5354 in Plasma

Blood samples for the analysis of ASP5354 in plasma will be collected as indicated in the Schedule of Assessments [Table 1] and Sample Collection Schedule for Pharmacokinetics [Table 3].

Blood samples will be collected via a peripherally or centrally placed intravenous cannula or by direct venipuncture in a suitable vein. Pharmacokinetic sample collection should not be taken from the same cannula used for the administration of ASP5354. Blood sampling, processing, storage and shipment instructions are provided in a Laboratory Manual.

When deemed appropriate at a later date, plasma samples remaining after the pharmacokinetic analysis may be used for exploratory metabolic profiling or exploratory biomarker analysis after the study. These tests will be described in a separate report and will not be incorporated in the integrated clinical study report (CSR).

7.4.2 Analysis of ASP5354 in Urine

Urine samples for the analysis of ASP5354 in urine will be collected at the intervals indicated in the Schedule of Assessments [Table 1] and Sample Collection Schedule for Pharmacokinetics [Table 3].

A urethral catheter will be inserted before the administration of ASP5354 until the actual end of surgery. While the catheter is in place, each interval sample will be collected in a separate collection bag. Aliquots of urine samples will be collected from each collection bag. The time of ASP5354 administration, beginning of urine collection and exchange of collection bags will be recorded. Urine sampling, processing, storage and shipment instructions are provided in a Laboratory Manual.

When deemed appropriate at a later date, urine samples remaining after the pharmacokinetic analysis may be used for exploratory metabolic profiling or exploratory biomarker analysis after the study. These tests will be described in a separate report and will not be incorporated in the integrated CSR.

7.5 Pharmacodynamics

Pharmacodynamic variables will include the primary endpoint (Ureter[s] Conspicuity by the 5-Point Likert Scale by the investigator) and the secondary efficacy endpoints (Quantitative assessment and Ureter[s] Conspicuity by the 5-Point Likert Scale by the BICR).
See [Section 7.1.1] for primary and secondary efficacy assessments.

7.6 Electronic Clinical Outcome Assessment

Not applicable.

7.7 Total Amount of Blood

The total amount of blood collected from each participant will vary depending on the length of surgery. The average expected blood draw will be approximately 42.0 mL. If clinically

significant laboratory abnormalities are found, additional blood may be drawn for safety monitoring. Also, if a local laboratory is used to confirm the eligibility, additional blood will be taken for the central laboratory.

7.8 Investigator Exit Interview

Exit interviews will be conducted with investigators who have completed more than 1 procedure using ASP5354 with NIR-F. Additional details including investigator selection will be provided in a separate guidance document. The results of the exit interview will be summarized in a separate report from the CSR.

8 PARTICIPANT DISCONTINUATION

Refer to [Section 10.1.10] regarding discontinuation of study sites or of the study as a whole.

8.1 Discontinuation of Individual Participant(s) from Study Intervention

Not applicable.

8.2 Discontinuation of Individual Participant(s) from Study

The participant is free to withdraw from the study for any reason and at any time without giving reason for doing so and without penalty or prejudice. The investigator is also free to terminate a participant's involvement in the study at any time if the participant's clinical condition warrants it.

The reason for discontinuation from study must be documented in the participant's medical records.

8.3 Lost to Follow-up

Every reasonable effort is to be made to contact any participant lost to follow-up during the course of the study in order to complete study-related assessments and record outstanding data and retrieve study intervention. These contact attempts should be documented in the participant's medical record.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The primary endpoint will be analyzed using a paired t-test. The following null and alternative hypotheses will be tested:

- H_0: The mean intra-participant difference between the ureter conspicuity at the 30-min time point with WL and the ureter conspicuity at the 30-min time point with NIR-F is equal to 0.
- H_a: The mean intra-participant difference between the ureter conspicuity at the 30-min time point with WL and the ureter conspicuity at the 30-min time point with NIR-F is not equal to 0.

The difference (NIR-F - WL) in the ureter conspicuity between WL and NIR-F at the 30-min time point will be tested at a 2-sided significance level of 0.05.

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 mean differences between the NIR-F score at each time point and the 30-min WL score is equal to 0. The following null and alternative hypotheses will be tested:

- H_0: The median of the intra-participant mean differences of the NIR-F score at each time point and the 30-min WL score is equal to 0.
- H_a: The median of the intra-participant mean differences of the NIR-F score at each time point and the 30-min WL score is not equal to 0.

The difference (NIR-F - WL) in the ureter conspicuity between WL at the 30-min time point and the mean conspicuity score across all time points with NIR-F will be tested at a 2-sided significance level of 0.05.

The second key secondary endpoint of the 30-min time point after ASP5354 administration WL conspicuity score versus the end of surgery time point NIR-F conspicuity score will be analyzed using a paired t-test. The following null and alternative hypotheses will be tested:

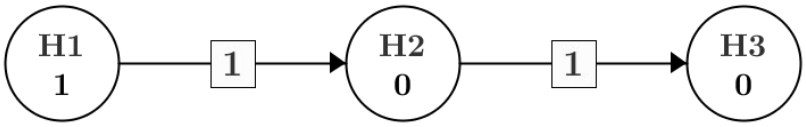
- H_0: The mean intra-participant difference between the ureter conspicuity at the 30-min time point with WL and the ureter conspicuity at the end of surgery time point with NIR-F is equal to 0.
- H_a: The mean intra-participant difference between the ureter conspicuity at the 30-min time point with WL and the ureter conspicuity at the end of surgery time point with NIR-F is not equal to 0.

The difference (NIR-F - WL) in the ureter conspicuity between WL at the 30-min time point and the end of surgery time point with NIR-F will be tested at a 2-sided significance level of 0.05.

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-min time point after ASP5354 administration versus NIR-F conspicuity at 30-min time point after ASP5354 administration
- H2: WL conspicuity at 30-min time point after ASP5354 administration versus mean NIR-F conspicuity across all time points
- H3: WL conspicuity at 30-min time point after ASP5354 administration versus NIR-F conspicuity at end of surgery time point

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.

9.2 Analysis Sets

The allocation of participants to analysis sets will be determined prior to database hard-lock.

The following populations are defined:

Population	Description
Intent to Treat (ITT)	All participants in the adult normal/mild eGFR cohort randomized to WL/NIR-F
Modified Intent to Treat (mITT)	All participants in any cohort in the WL/NIR-F arm
Safety Analysis Set (SAF)	All participants in any cohort who receive ASP5354.
Pharmacokinetic Analysis Set (PKAS)	All participants in any cohort who receive ASP5354 for which at least 1 plasma or urine concentration data are available with the time for dosing and sampling

NIR-F: near-infrared fluorescence; WL: white light

9.3 Statistical Analyses

A statistical analysis plan (SAP) will be written to provide details of the analyses, along with specifications for tables, listings and figures to be produced. Changes from the planned analyses in the final SAP that impact the statistical analyses will be justified in the CSR. The initial version of the SAP will be finalized before the first participant is screened. Any updates to the SAP will be approved prior to database lock.

9.3.1 General Considerations

In general, data will be summarized with descriptive statistics for continuous endpoints, and frequency and percentage for categorical endpoints, unless otherwise specified. Percentages by categories will be based on the number of participants with no missing data (i.e., will add up to 100%). Baseline will be defined as the last non-missing observation prior to administration of study intervention, unless otherwise specified.

Efficacy summaries for the primary and key secondary endpoints will be presented for all participants in the Intent to Treat (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.

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.

Unless otherwise specified, all safety summaries will be presented by arm and for all participants in the Safety Analysis Set (SAF). More specifically, safety summaries will be presented for the following groups of participants unless otherwise specified:

- All participants who receive ASP5354
- Adult participants (≥ 18 years of age)
- Adult participants randomized to the WL/NIR-F arm
- Adult participants randomized to the WL-only arm
- All participants enrolled/randomized to the WL/NIR-F arm
- Adolescent participants (≥ 12 to < 18 years of age)
- Moderate renal impairment participants (eGFR ≥ 30 to < 60 mL/min)
- Severe renal impairment participants (eGFR ≥ 15 to < 30 mL/min)

Additional subgroups for safety summaries may be considered based on feasibility. Details will be given in the SAP.

9.3.2 Primary Endpoint/Estimand(s) Analysis

9.3.2.1 Primary Analysis

The primary efficacy endpoint is the investigator's blinded assessment of the intra-participant difference in ureter conspicuity for WL versus NIR-F at the 30-min time point after ASP5354 administration based on a 5-Point Likert scale. The population used for the primary efficacy analysis will be the ITT population, which includes all participants in the adult normal/mild eGFR cohort randomized to the WL/NIR-F arm. Adolescents and moderate/severe renally impaired participants that are enrolled directly into the WL/NIR-F arm will not be included in the primary analysis. 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 (refer to primary estimands in [Section 3.1]):

- 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.

9.3.2.2 Sensitivity Analysis

A sensitivity analysis for the primary endpoint will be conducted. The same paired t-test as the primary analysis will be run with the assessments impacted by ICE1 or ICE2 left as missing.

An additional sensitivity analysis for the primary endpoint will be conducted. The NIR-F and WL conspicuity assessments at the 30-min time point after ASP5354 administration will be compared using a Wilcoxon signed-rank test. This analysis will be conducted using the same data (with ICE1 and ICE2 imputation) as the primary analysis.

Sensitivity analyses will be conducted on the ITT population.

9.3.3 Secondary Endpoints Analysis

9.3.3.1 Key Secondary Endpoints

The population used for the analysis of key secondary endpoints will be the ITT population which includes all participants in the adult normal/mild eGFR cohort randomized to WL/NIR-F. 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 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 mean differences between the NIR-F scores and 30-min WL scores is equal to 0. Prior to the analysis, the conspicuity scores will be windowed to time points every 30-min. Details of the windowing algorithm will be given in the SAP.

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 mean differences between the NIR-F scores and 30-min WL scores is equal to 0.

Prior to the sign test, 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 analysis of key secondary endpoint no. 1.
- Participants who experience ICE2 and do not have either an 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'.

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 the 30-min time point 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. Additional details will be given in the SAP.

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 difference 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 after ASP5354 administration WL conspicuity score versus the end of surgery time point 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 after ASP5354 administration with WL and the end of surgery 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 end of surgery 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.

9.3.3.2 Additional Secondary Endpoints

To quantify ureter conspicuity, CEF data will be summarized using descriptive statistics by time point for participants in the mITT population.

The correlation between the CEF assessments and conspicuity values will be investigated. Additional details will be given in the SAP.

The analysis of the primary and key secondary endpoints will be repeated using the following groups of participants. P-values will not be presented. Additional details will be given in the SAP.

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

To further characterize the difference of ureter conspicuity under NIR-F vs WL, the frequency and percentage of participants with an average 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. These analyses will be carried out in all cohorts.

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. The analysis of the primary and key secondary endpoints will be repeated using the BICR data. This analysis, as well as the descriptive statistics, will be presented within adolescents, adults with moderate renal impairment, adults with severe renal impairment, the ITT population and the mITT population.

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 2 assessments is considered if 2 of 3 BICR readers yield a P value of ≤ 0.05.

The concordance correlation coefficient measuring the agreement between investigator and BICR Likert conspicuity scores will be presented for the following values for each separate BICR reader.

- 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

Additional details will be provided in the SAP.

9.3.3.3 Supplementary Analysis

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

The frequency and percentage of participants with an average ureter conspicuity assessed by the investigator over all NIR-F time points during surgery at least 1 point higher than the ureter WL conspicuity score at the 30-min time point will be presented. The analysis will also be repeated using a difference of at least 2, 3 and 4 points. These analyses will be carried out in all cohorts.

9.3.3.4 Subgroup Analyses

The primary endpoint, key secondary endpoints and color contrast quantitative assessment will be summarized using descriptive statistics within the following subgroups within the ITT population:

- Body mass index (underweight or normal [< 25 kg/m^2]; overweight [≥ 25 to < 30 kg/m^2]; obese [≥ 30 kg/m^2])
- Participants with prior pelvic or abdominal surgery (yes; no)
- Participants with prior inflammatory disorders of interest (yes; no)
- Type of surgery (gynecological; other abdominopelvic)
- Type of surgery (minimally invasive and open)

Additional details will be provided in the SAP.

9.3.4 Exploratory Endpoints

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 population. Also, the same descriptive statistics summary will be applied for the ureter conspicuity under WL for both index and non-index ureters in the ITT population.

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, as defined in [Qin et al, 2019]) and details will be provided in the SAP.

9.3.5 Safety Analyses

Safety analyses will be conducted using the SAF, unless otherwise specified. No hypothesis testing will be performed.

9.3.5.1 Adverse Events

Adverse events will be coded using Medical Dictionary for Regulatory Activities and graded using the NCI CTCAE, version 5.0.

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

The number and percentage of participants with TEAEs, study intervention-related TEAEs, serious TEAEs and study intervention-related serious TEAEs will be summarized by System Organ Class and Preferred Term within the groups listed in [Section 9.3.1]. The number and percentage of TEAEs by toxicity grade will be summarized. The worst toxicity grade will be summarized if the same AE is recorded more than once for a participant.

Hypersensitivity and IUI-associated events will be considered AESIs. The frequency and percentage of participants with each type of AESI will be summarized within the groups listed in [Section 9.3.1]. 95% Clopper-Pearson confidence intervals for the proportion of participants with each type of AESI will be presented.

The frequency and percentage of participants with green coloration of urine will be summarized within the groups listed in [Section 9.3.1].

Adverse event data will be listed.

9.3.5.2 Laboratory Assessments

For quantitative clinical laboratory measurements (hematology, biochemistry, urinalysis), descriptive statistics will be used to summarize results and change from baseline by the groups listed in [Section 9.3.1] and time point.

Shift from baseline to post-baseline worst grade based on NCI CTCAE (version 5.0) until the follow-up period in laboratory tests will be tabulated.

Laboratory data will be listed.

9.3.5.3 Vital Signs

Descriptive statistics will be used to summarize vital signs results and changes from baseline by the groups listed in [Section 9.3.1] and time point.

The frequency and percentage of participants with potentially clinically significant vital signs at baseline and the worst post-baseline result for each participant will be summarized by the groups listed in [Section 9.3.1]. Additional details will be given in the SAP.

Vital signs data will be listed.

9.3.5.4 Electrocardiogram and Cardiac Monitoring

The frequency and percentage of participants with normal and abnormal ECG results for the overall interpretation will be summarized by the groups listed in [Section 9.3.1]. 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.

9.3.6 Other Analyses

9.3.6.1 Analysis of Pharmacokinetics

The analysis of 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
- Adult with severe renal impairment (eGFR ≥ 15 to < 30 mL/min): WL/NIR-F arm
- Adolescent cohort (eGFR ≥ 60 mL/min): WL/NIR-F arm

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), and cumulative amount excreted into urine by time interval.

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

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

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.

9.3.6.2 Analysis of Pharmacodynamics

The relationship between ureter visualization (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.

9.4 Interim Analysis

Not applicable.

9.5 Sample Size Determination

Only adult participants with normal/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.

9.5.1 Adult Cohorts (Participants ≥ 18 Years of Age)

9.5.1.1 Normal/Mild eGFR Cohort

The sample size calculation is based on the primary efficacy endpoint. When assuming the difference in the mean score of the averaged ureter conspicuity score at the 30-min time point after ASP5354 administration with 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. The allocation ratio of the randomization to WL/NIR-F versus WL-only will be specified in the study randomization specification document. The randomization will be stratified by type of surgery (gynecological; other abdominopelvic).

9.5.1.2 Moderate/Severe eGFR Cohort

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 the enrollment of participants with normal/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.

9.5.2 Adolescent Cohort (Participants ≥ 12 and < 18 Years of Age)

Approximately 13 adolescent participants 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 (at least 6 for 12 to 14 years of age and at least 4 for 15 to 17 years of age) complete the study to allow collection of pharmacokinetic samples from at least 6 participants in age group ≥ 12 and < 15 years of age and at least 4 participants in age group ≥ 15 and < 18 years of age. 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% 2-sided confidence interval 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 adolescent 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 adolescents 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 (CEF) during surgery and the time course of CEF 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.

9.6 Additional Conventions

Missing conspicuity scores will be imputed per the algorithm described in [Section 9.3.2.1]. Otherwise, no imputation of missing data will be done with the exception of missing start and stop dates of AEs and concomitant medications.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1 Ethical, Regulatory and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences international ethical guidelines
  - Applicable ICH GCP guidelines
  - Applicable laws and regulations
- The protocol, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent of Participants

10.1.3.1 Informed Consent Process

- The investigator or the investigator's representative will explain the nature of the study to the potential participant or their LAR (if applicable; defined as an individual, judicial or other body authorized under applicable law to consent on behalf of a prospective participant to the participant's participation in the procedure[s] involved in research (45 CFR 46.102[c])) and answer all questions regarding the study.

- The information provided shall be provided in writing and shall:
  - Enable the participant or their LAR (if applicable) to understand:
    - The nature, objectives, benefits, implications, risks and inconveniences of the clinical study
    - The conditions under which the clinical study is to be conducted, including the expected duration of the participant's participation in the clinical study
    - The possible treatment alternatives, including the follow-up measures if the participation of the participant in the clinical study is discontinued
  - Be kept comprehensive, concise, clear, relevant, and understandable to a layperson
  - Be provided in a prior interview with an appropriately qualified member of the study team. Special attention shall be paid to the information needs of specific patient populations and of individual participants, as well as to the methods used to give the information. Care should be taken to verify that the participant has understood the information.
  - Include information about the applicable damage compensation system
  - Include the study international study number (ISN) number and information about the future availability of the clinical study results in terms understandable to a layperson
- Potential participants must be informed that their participation is voluntary and shall have their protective rights and guarantees explained. In particular their right to refuse to participate and the right to withdraw from the clinical study at any time without any resulting detriment and without having to provide any justification shall be explained.
- Participants or their LAR will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, EU CTR 536/2014 (including Article 29), local regulations, ICH GCP guidelines, the Declaration of Helsinki, HIPAA requirements, where applicable, and the IRB/IEC or study center.
- If the participant is a minor, their assent, in order to participate in a clinical study, shall also be obtained.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- In case of an update to the ICF during the study, participants must be reconsented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or their LAR (if applicable).

10.1.3.2 Supply of New and Important Information Influencing the Participant's Consent and Revision of the Written Information

- The investigator or the investigator's representative will immediately inform the participant verbally whenever new information becomes available that may be relevant to the participant's consent or may influence the participant's willingness to continue participating in the study (e.g., report of serious adverse drug reaction). The communication must be documented in the participant's medical records and whether the participant is willing to remain in the study or not must be confirmed and documented.

- The investigator must update the participant's ICF and submit it for approval to the IRB/IEC. The investigator or the investigator's representative must obtain written informed consent from the participant on all updated ICFs throughout their participation in the study. The investigator or the investigator's designee must reconsent participants with the updated ICF even if relevant information was provided verbally. The investigator or the investigator's representative who obtained the written informed consent and the participant should sign and date the ICF. A copy of the signed ICF will be given to the participant and the original will be placed in the participant's medical record. An entry must be made in the participant's records documenting the reconsent process.

10.1.4 Recruitment Arrangements

The activated sites will screen participants via mechanisms currently in place at each site, and review prospective participants' medical history against the study eligibility criteria.

If necessary, the sites and the sponsor will take the following study-specific recruitment strategies.

- Site database/medical records search
- Doctor to doctor letter
- Participant referrals
- Sponsor call with investigators

10.1.5 Data Protection

The sponsor will use the personal data collected from participants in order to run the study and to use and publish the results of the study. The personal data of participants will be used throughout the development program of the investigational study intervention (e.g., to develop a product, obtain permission to market the product, monitor its safety and obtain coverage by health insurance and reimbursement schemes).

Astellas relies on the permission (or "consent") in order to use the data of participants and their permission is obtained by signing the ICF.

The investigator and/or the site personnel will record information from the medical file of study participants in the eCRF and in an external (electronic) data file (e.g., central laboratory data). These study records will identify the participants with a code instead of their name or other personal data. Only the investigator and the site personnel can match the code with the name of the study participant, which will be retained in the medical file at the site only. Non-medical personnel acting on behalf of Astellas and being bound by a duty of confidentiality, as well as health authorities and/or IRB/IECs, may also be given access to this data at the site only to verify that the study is carried-out in compliance with legal and quality requirements.

The sponsor collects personal data from the participants during the study that may be used for:

- Submission to government regulatory authorities and IRB/IEC
- Use in reports or public scientific presentations, and
- Use in research, now or in the future

However, the identity of participants will not be revealed if study participants' personal data are shared for these purposes.

The personal data of participants may be shared with:

- Astellas and its present or future affiliates
- Research, collaboration and licensing partners and/or service providers (such as laboratories conducting tests on behalf of Astellas) and authorized representatives of Astellas
- Study monitors appointed by Astellas or Astellas's service providers, and
- Auditors/inspectors appointed by Astellas or Astellas's service providers or by health and regulatory authorities for GCP inspections.

The sponsor will inform the participants about their privacy rights and how to exercise them under the ICF which participants will sign in order to participate in the study. The sponsor will provide the investigator and study staff with a privacy notice explaining how their personal data will be used and how to exercise their privacy rights.

The sponsor will comply and process personal data in accordance with all applicable privacy laws and regulations.

The contract between sponsor and study sites specifies responsibilities of the parties related to data protection, including handling of data security breaches and respective communication and cooperation of the parties.

Information technology systems used to collect, process, and store study-related data are secured by technical and organizational security measures designed to protect such data against accidental or unlawful loss, alteration or unauthorized disclosure or access.

10.1.6 Committee(s) Structure

10.1.6.1 Blinded Independent Central Review Committee

A blinded committee will independently read all WL and NIR-F images for all timepoints for all participants in the WL/NIR-F arm. The committee members will be experienced surgeons not associated with the study and will have no clinical information regarding the participants.

Each BICR reader will score conspicuity for all pre-defined time points separately using a 5-point Likert scale.

A charter will be prepared to define specific procedures for BICR reader training and replacement of BICR readers.

10.1.7 Dissemination of Clinical Study Data

ICH E3 guidelines recommend and EU Clinical Trial Regulation 536/2014 requires that a final CSR that forms part of a marketing authorization application, be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator(s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of their knowledge it accurately describes the conduct and results of the study. The representative for the coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database lock.

10.1.7.1 Disclosure of Study Information and Results

Astellas complies with relevant laws, regulatory requirements and industry guidance for registration of clinical studies, posting of clinical study information, and disclosure of clinical study results.

Astellas commits to registering all interventional clinical studies sponsored by Astellas that seek to evaluate the safety and/or efficacy profile of an Astellas owned or in-licensed product. Clinical studies sponsored by Astellas that are covered under Astellas policy are registered on a publicly accessible clinical study registry (e.g., www.clinicaltrials.gov). In addition, other Astellas-sponsored studies are registered on national registries, if required by local/regional laws or regulations.

Astellas commits to disclosing summary results in Astellas clinical study websites for all phase 1 to 4 interventional clinical studies for products sponsored by Astellas, conducted in the target patient population, with Astellas products that have health authority approval. In addition, summary results for other Astellas-sponsored studies are disclosed on national registries, if required by local/regional laws or regulations.

Summary of end of study results will be available only after all data have been obtained from all participating countries/sites. Statistical analyses of end of study results are conducted when all relevant data are available.

10.1.7.2 Access to Study Data

Subject to compliance with the applicable laws and regulations relevant to protection of personal data, Astellas provides a platform (www.clinicalstudydatarequest.com) where researchers may request access to participant level data, study level data and protocols from Astellas-sponsored clinical studies conducted in patients that are completed after 01 JAN 2010.

Access to this data is granted for products and indications approved in any country after the request has been reviewed and approved by an independent panel of experts (“Scientific Review Board”) based on scientific merit and the qualifications of the researcher. Access is given by Astellas after review and approval by the Scientific Review Board and execution of a data sharing agreement.

Before participant-level data are shared, it is anonymized to respect the rights of the clinical study participants to privacy and to protection of their personal health information.

10.1.8 Data Quality Assurance

- All participant data relating to the study will be recorded in the eCRF unless transmitted to the sponsor or designee electronically in an external data file (e.g., central laboratory data). The investigator is responsible for verifying that data entries in the eCRF are accurate and correct by physically or electronically signing the eCRF.
- Guidance on completion of eCRFs will be provided in a separate eCRF Completion Guideline.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy, including definition of study critical data items and processes (e.g., risk-based initiatives in operations and quality such as risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., contract research organizations [CRO(s)]).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator according to ICH or applicable local regulatory requirements, whichever is longer, after study completion. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.9 Source Documents

- Source data must be available at the study site to document the existence of the participants and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are handwritten on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved or transmitted electronically via computerized systems (and/or other kind of electronic devices) as part of regulated study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol-related assessments, AE tracking, electronic clinical outcome assessment and/or drug accountability.

- Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments, and audit trail information, if applicable). All printed records must be kept in the participant file and be available for archiving.
- The sponsor or designee will perform ongoing source data review to confirm that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- The documents of the blinded independent review committee (minutes and charters and others) and judgement committee outside the study sites (minutes and charters and others) shall be retained by the sponsor.

10.1.10 Study and Site Closure

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further investigational study intervention development

For site termination:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures or GCP guidelines
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants enrolled earlier than expected

If the study is prematurely terminated or suspended, the sponsor or designee shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any CRO(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

10.1.11 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Publication of the study results is discussed in the study agreement.

10.1.12 Insurance of Participants and Others

The sponsor has covered this study by means of an insurance of the study according to national requirements. The name and address of the relevant insurance company, the

certificate of insurance, the policy number and the sum insured are provided in the investigator's file.

10.1.13 Quality Assurance

The sponsor is implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP and applicable regulatory requirement(s). Where applicable, the QA and QC systems and written SOPs of the CRO will be applied.

The sponsor or sponsor's designee may arrange to audit the study at any or all study sites and facilities. The audit may include on-site review of regulatory documents, eCRFs and source documents. Direct access to these documents will be required by the auditors.

10.2 Appendix 2 Contraception Requirements

WOCBP who are eligible for participation in the study, including those who choose complete abstinence, must have pregnancy tests as specified in the Schedule of Assessments [Table 1]. Pregnancy test results must confirm that the participant is not pregnant.

WOMEN OF CHILDBEARING POTENTIAL DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A female is considered fertile (i.e., WOCBP) following menarche and until becoming postmenopausal unless permanently sterile.

Females in the following categories are not considered WOCBP

- Premenarchal
- Premenopausal with 1 of the following (i.e., permanently sterile):
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy
- Postmenopausal

A postmenopausal state is defined as at least 12 months after last menstrual bleeding without an alternative medical cause.

In case the last menstrual bleeding cannot be clearly determined, confirmation with more than 1 follicle-stimulating hormone (FSH) measurement of at least > 40 IU/L (or higher per local institutional guidelines) is required.

Females on hormone replacement therapy (HRT) and whose menopausal status is in doubt will be required to use 1 of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status by repeated FSH measurements before study enrollment.

Documentation of any of these categories can come from the study site personnel's review of the female participant's medical records, medical examination or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILDBEARING POTENTIAL

Female participants of childbearing potential are eligible for participation in the study if they agree to use one of the highly effective methods of contraception listed below from the time of signing the ICF and until the end of relevant systemic exposure, defined as 30 days after the final study intervention administration.[a]

Highly effective methods of contraception (failure rate of < 1% per year when used consistently and correctly)[b]:

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation
  - Oral
  - Injectable
  - Implantable
- Other combined (estrogen- and progesterone-containing) methods
  - Vaginal ring
  - Injectable
  - Implantable
  - Intrauterine hormone-releasing system or intrauterine device
- Bilateral tubal occlusion or bilateral tubal ligation
- Vasectomized partner
  A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

[b] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILDBEARING POTENTIAL.

Male participants with female partners of childbearing potential are eligible for participation in the study if they agree to the following during treatment and until the end of relevant systemic exposure defined as 30 days after study intervention administration.[a]

- Inform any and all partner(s) of their participation in a clinical study and the need to comply with contraception instructions as directed by the investigator
- Use a condom
- Female partners of male participants who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

Contraception for Pediatric Male Participants of Reproductive Potential

Male children/adolescents in the following categories are not considered of reproductive potential:

1. Tanner stage 1 development
2. Documented surgically sterile

Documentation from the site personnel's review of the male participant's medical records, medical examination, and medical history interview is necessary.

Contraception guidance for male children/adolescents of reproductive potential:

- Male children/adolescents of reproductive potential (Tanner Stage 2 and above) receiving nongenotoxic agents should use a condom through at least 30 days after study intervention administration.
- Male participants should inform their female partners that they are participating in a clinical study and effective methods of contraception should be used.
- Female partners of male participants who have not undergone bilateral orchiectomy should consider use of effective methods of contraception through at least 30 days after study intervention administration.

Contraception for Pediatric Female Participants of Childbearing Potential

Female children/adolescents in the following categories are not considered of childbearing potential:

1. Premenarchal
2. Documented surgically sterile (hysterectomy, bilateral salpingectomy, bilateral oophorectomy)

Documentation from the site personnel's review of the female participant's medical records, medical exam, and medical history interview is necessary.

Contraception guidance for female children/adolescents of childbearing potential:

- Female children/adolescents of childbearing potential receiving nongenotoxic agents should use effective contraception during treatment through at least 30 days after ASP5354 administration.
- Pregnancy testing for female children/adolescents of childbearing potential
    - At screening and preoperative visits

One of the highly effective methods of contraception listed below is required at the time of informed consent and until the end of relevant systemic exposure as defined above.

Highly Effective Birth Control Methods-failure rate < 1%/year

1. Combined estrogen- and progesterone-containing hormonal contraception
   a. Oral
   b. Intravaginal
   c. Transdermal (e.g., Patch)
   d. Injectable (e.g., Cyclofem, Mesigyna)
2. Progestogen-only hormonal contraception
   a. Oral
   b. Injectable (e.g., depot-medroxyprogesterone acetate intramuscular or subcutaneous)
   c. Implantable (e.g., Norplant)
3. IUD
4. IUS
5. Bilateral tubal occlusion
6. Vasectomized male partner
7. True abstinence*

*True abstinence: When this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (such as calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. It is not necessary to use any other method of contraception when complete abstinence is elected.

10.3 Appendix 3 Adverse Events/Adverse Device Effects: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

10.3.1 Definition of Adverse Events

AE Definition:

An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.

NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention. This includes events related to the (study) procedures.

Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, biochemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, biochemistry or urinalysis) or other safety assessment (e.g., vital signs, physical examination, or ECGs), including those that worsen from baseline, that is considered to be clinically significant in the medical and

scientific judgment of the investigator and not related to underlying disease, is to be reported as an AE/SAE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment, which is associated with the underlying disease, does not require reporting as an AE/SAE, unless judged by the investigator to be more severe than expected for the participant's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

10.3.1.2 Potential Cases of Drug-induced Liver Injury

Refer to [Section 10.4] for detailed instructions on drug-induced liver injury (DILI). Abnormal values in aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) concurrent or with abnormal elevations in total bilirubin (TBL) that meet the criteria outlined in [Section 10.4], in the absence of other causes of liver injury, are considered potential cases of DILI (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 10.3.7].

10.3.2 Definition of Serious Adverse Events

An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death
- Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization
  - In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
  - Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity
  - The term disability means a substantial disruption of a person's ability to conduct normal life functions.
  - This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza,

and accidental trauma (e.g., sprained ankle), which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect
- Other situations:
  - Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious.
  - Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

If an event is not an AE per definition in [Section 10.3.1], then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

10.3.3 Adverse Device Effect (ADE)

10.3.3.1 Definition of ADE

- Any adverse event related to the use of device (includes all hardware components).
  - Includes any event that is a result of a user error or intentional misuse.

10.3.3.2 Definition of Unanticipated adverse device effect (UADE)

- UADE is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants. Note the reporting of UADEs should follow reporting requirements for SAEs/serious adverse device effects.

10.3.3.3 Definitions of Technical Complaints and Device Deficiencies

- Device Complaint: A quality complaint received in writing, electronically, or orally that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a device product. (In this definition, "effectiveness" refers to the actual function of the device, not to how the participant responds to the action of the device.
- Device Deficiency: Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance. Device deficiencies include malfunctions, use errors and inadequate labelling.

10.3.3.4 Risks/Precautions

Refer to the SPY Portable Handheld Imager (SPY-PHI) and 1688 Advanced Imaging Modalities (AIM) 4k Platform Manuals for a full description of the risks and precautions associated with all components of the SPY-PHI and 1688 AIM systems, respectively. The entirety of the Operator Manuals should be read before using the device(s). Failure to follow the instructions and warnings in the manual may result in unsafe operation of the system and/or injury to the subject or operator.

10.3.4 Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention, imaging device(s), study procedures and each occurrence of each AE/SAE/ADE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the IB and/or product information, for marketed products, in the assessment.
- For each AE/SAE/ADE, the investigator must document in the medical notes that they have reviewed the AE/SAE/ADE and has provided an assessment of causality.
- There may be situations in which an SAE/ADE has occurred and the investigator has minimal information to include in the initial report to the sponsor. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE/ADE data to the sponsor.
- The investigator may change their opinion of causality in light of follow-up information and send a SAE/ADE follow-up report with the updated causality assessment.
- The causality assessment between study intervention is 1 of the criteria used when determining regulatory reporting requirements.

Following a review of the relevant data, the causal relationship between the study intervention and each AE/SAE/ADE will be assessed by answering "yes" or "no" to the question "Do you consider that there is a reasonable possibility that the event may have been caused by the study intervention?"

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a "reasonable possibility" that an AE/SAE/ADE may have been caused by the study intervention (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Has the participant been administered study intervention?
- Plausibility (i.e., could the event have been caused by the suspect study intervention? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)

- Dechallenge/rechallenge:
  - Dechallenge: Did the AE/SAE/ADE resolve or improve after only stopping the dose of the suspect study intervention without any treatment?
  - Rechallenge: Did the AE/SAE/ADE reoccur if the suspected study intervention was reintroduced after having been stopped?
- Laboratory or other test results: a specific laboratory investigation supports the assessment of the relationship between the AE/SAE/ADE and the study intervention (e.g., based on values pre-, during and post-treatment)
- Available alternative explanations independent of study intervention exposure, such as other concomitant interventions, device malfunctions, medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation
- Temporal relationship between exposure to the study intervention and AE/SAE/ADE onset and/or resolution. Did the AE/SAE/ADE occur in a reasonable temporal relationship to the administration of the study intervention?
- Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausibility will be provided.

There may be situations in which an SAE/ADE has occurred and the investigator has minimal information to include in the initial report to the sponsor. While it is very important that the investigator always assesses causality for every event before the initial transmission of the SAE data to the sponsor, the initial report should be submitted without delay (i.e., within 24 hours of awareness). With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of "no" is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise their assessment of causality in light of new information regarding the SAE/ADE and shall send an SAE/ADE follow-up report and update the eCRF with the new information and updated causality assessment.

Device Relatedness

Events will be classified as device related if they meet the following criteria:

- Not Related**:** Any reaction that does not follow a reasonable temporal sequence from administration of the device or surgical procedure, or study intervention AND that is likely to have been produced by the subject's clinical state or other modes of therapy administered to the subject.
- Related**:** A reaction that follows or may follow a reasonable temporal sequence from administration of the device, surgical procedure, or study intervention AND that follows a known response pattern to the suspected device/surgical procedure/study intervention.

10.3.5 Assessment of Severity

Adverse events, including abnormal clinical laboratory values, will be graded using the NCI CTCAE guidelines, version 5.0. The items that are not stipulated in the NCI CTCAE version 5.0 will be assessed according to the criteria below and entered into the eCRF:

Table 7 Grading Scale Defining the Severity of an Adverse Event

Grade	Assessment Standard
1 - Mild	Asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
2 - Moderate	Minimal local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[1]
3 - Severe	Medically significant but not immediately life-threatening, hospitalization or prolonged hospitalization indicated; disabling; limiting self-care ADL[2]
4 - Life-threatening	Life-threatening consequences, urgent intervention indicated
5 - Death	Death related to AE

ADL: activities of daily living; AE: adverse event

1 Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

2 Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden.

10.3.6 Recording and Follow-up of AEs/SAEs and/or ADEs

AE, SAE and ADE Recording

- When an AE/SAE/ADE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE/ADE information in the eCRF.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to the sponsor in lieu of completion of the eCRF.
- There may be instances when copies of medical records for certain cases are requested by the sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to the sponsor.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE/ADE.

Follow-up of AEs/SAEs/ADEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the sponsor to elucidate the nature and/or causality of the AE/SAE/ADE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other healthcare professionals.

- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated SAE or ADE data to the sponsor within 24 hours of receipt of the information.

10.3.7 Reporting Procedures for SAEs/ADEs/Medical Device Deficiencies

The investigator must complete and submit an SAE or ADE worksheet containing all information that is required by local and/or regional regulations to the sponsor by fax or email immediately (no later than within 24 hours of obtaining knowledge of the event).

The SAE or ADE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE/ADE data, as well as the investigator causality assessment including the explanation for the causality assessment.

For contact details, see [Contact Details of Sponsor's Key Personnel]. Fax or email the SAE/special situations or the ADE worksheet to:

Astellas Pharma Global Development Inc.
Global Pharmacovigilance
North America fax number: +1-888-396-3750
North America alternate fax number: +1-847-317-1241
International fax: +44-800-471-5263
Email: safety-us@astellas.com

If there are any questions, or if clarification is needed regarding the SAE/ADE/medical device deficiency, please contact the sponsor's medical monitor/study physician or their designee [Contact Details of Sponsor's Key Personnel].

Follow-up information for the event should be sent promptly (as soon as available, but no longer than within 7 days of the initial notification).

Full details of the SAE/ADE/medical device deficiency should be recorded on the medical records and SAE/special situation or ADE worksheet.

The following minimum information is **required**:

- International study number/study number
- Participant number, sex and age
- Date of report
- Description of the SAE/ADE/medical device deficiency (event and seriousness criteria)
- Causal relationship to the study intervention (including reason)
- Drug provided (if any)

The sponsor or sponsor's designee will medically evaluate the SAE/ADE/medical device deficiency and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., SUSAR reporting) according

to current local/regional regulatory requirements. The sponsor or sponsor's designee will submit expedited safety reports to competent authorities and concerned ethics committee per current local regulations, and will inform the investigator of such regulatory reports as required. Investigators must submit safety reports as required by their IRB/IEC within timelines set by regional regulations (e.g., European Medicines Agency, Food and Drug Administration [FDA]) where required. Documentation of the submission to and receipt by the IRB/IEC of expedited safety reports should be retained by the study site. In the US, FDA expedited IND reporting guidelines will be followed.

The sponsor or sponsor's designee will also evaluate any ADEs/medical device deficiencies and determine if the report meets the requirements for expedited reporting.

The sponsor will notify all investigators responsible for ongoing clinical studies with the study intervention of all SUSARs, which require submission per local requirements (IRB/IEC).

Investigators should provide written documentation of IRB/IEC notification for each report to the sponsor.

The investigator may contact the sponsor's medical monitor/study physician for any other problem related to the rights, safety or well-being of the participant.

10.3.8 Reporting Procedures for Pregnancy and Special Situations

10.3.8.1 Contraceptive Guidance and Collection of Pregnancy Information

- Details of all pregnancies in female participants and, if indicated, female partners of male participants will be collected after the start of study intervention and until 30 days after the study intervention administration.
- If a pregnancy is reported, the investigator will record pregnancy information on the appropriate form and submit it to the sponsor within 24 hours of learning of the female participant or female partner of male participant (after obtaining the necessary signed informed consent from the female partner) pregnancy.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs and will be reported as such.
- The participant/pregnant female partner will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant/pregnant female partner and the neonate and the information will be forwarded to the sponsor.
- Any pregnancy-related SAE reported after the participant's last study visit, considered reasonably related to the study intervention by the investigator, will be reported to the sponsor as described in [Section 10.3.7]. While the investigator is not obligated to actively seek this information in former study participants/pregnant female partners, they may learn of pregnancy-related SAE through spontaneous reporting.

- Any female participant who becomes pregnant while participating in the study will be withdrawn from the study.

10.3.8.2 Medication Error, Overdose and "Off-label Use"

If a medication error (defined as an unintended failure in the treatment process that leads to, or has the potential to lead to, harm to the participant), overdose or "off-label use" (i.e., use outside of the target disease defined in the protocol) is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated AEs/SAEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7] together with the details of the medication error, overdose and/or "off-label use."

10.3.8.3 Misuse/Abuse

Definition of misuse: Situations where the study intervention is/are intentionally and inappropriately used not in accordance with the intended use as defined in the protocol.

Definition of abuse: Persistent or sporadic, intentional excessive use of medicinal products which is accompanied by harmful physical or psychological effects.

If misuse or abuse of the study intervention is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated AEs/SAEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7] together with details of the misuse or abuse of the study intervention.

10.3.8.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the study intervention of study site personnel while preparing it for administration to the participant) to the study intervention occurs, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated AEs/SAEs occurring to the individual associated with or resulting from the special situation are to be reported on the special situations worksheet.

10.3.8.5 (Suspicion of) Transmission of Infectious Agent

If transmission of an infectious agent associated with the study intervention is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness) and any associated AEs/SAEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7] together with the details of the suspected transmission of infectious agent.

10.3.8.6 Suspected Drug-drug Interaction

If a drug-drug interaction associated with the study intervention is suspected, the investigator must forward the special situation worksheet to the sponsor by fax or email immediately

(within 24 hours of awareness). Any associated AEs/SAEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 10.3.7] together with details of the suspected drug-drug interaction.

10.3.9 Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary to allow for proper conduct of the study, the sponsor will inform all investigators involved in the study, as well as the appropriate regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

The investigator will also inform the participants, who will be required to sign an updated ICF in order to continue in the study.

10.3.10 Urgent Safety Measures

An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant competent authorities, IRB/IEC, where applicable, in order to protect participants from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate a USM. The cause of a USM can be safety-, product- or procedure-related.

10.3.11 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the study physician (within 24 hours of awareness). Full details of the potential USM are to be recorded in the participant's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be a USM, the sponsor will take appropriate action to ensure the safety and welfare of the participants. These actions may include, but are not limited to, a change in study procedures or study intervention, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify the relevant competent authorities and concerned ethics committee within the timelines required per current local regulations, and will inform the investigators, as required. When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

10.4 Appendix 4 Liver Safety Monitoring and Assessment

The purpose of this appendix is to provide guidance for the monitoring DILI during the course of the study. It should be noted that this section does not specify the end of study analyses of liver enzymes. The end of study liver enzymes analyses will be described in the SAP.

Testing for Liver Abnormalities

Conduct the following for any participant enrolled in a study receiving active study intervention with an increase of serum aminotransferase (AT) to > 3 × upper limit of normal (ULN) (to > 5 x ULN in participants with liver metastases) or total bilirubin (TBL) > 2 × ULN

- Repeat testing for liver enzymes (including at least ALP, ALT, AST, TBL, and INR) within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate or severe liver abnormality to inform the investigator and study team.
- Participants should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN is as shown below.

Table 8 Moderate and Severe Liver Abnormalities

	ALT or AST		**TBL**
Moderate	> 3 × ULN (in participants without liver metastases), > 5 × ULN (in participants with liver metastases)	or	> 2 × ULN
Severe	> 3 × ULN	and†	> 2 × ULN

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBL: total bilirubin; ULN: upper limit of normal

†Samples taken simultaneously or within maximum 24 hours.

In addition, the participant should be considered to have severe hepatic abnormalities if any of the following apply:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks (in the absence of liver metastases)
- ALT or AST > 3 × ULN and† TBL > 2 × ULN or INR > 1.5 (if INR testing is applicable/evaluated)
- ALT or AST > 5 × ULN and† (TBL > 2 × ULN in participants with liver metastases)
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%)

† Samples taken simultaneously or within a maximum of 24 hours.

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures for Moderate or Severe Abnormalities

- Follow participants with confirmed moderate or severe abnormal liver function by repeat testing 2 to 3 times weekly, and then weekly or less frequently if abnormalities stabilize or the study intervention has been discontinued and the participant is asymptomatic.
- Complete the LA-CRF
- Thoroughly characterize moderate or severe abnormalities in hepatic functions by obtaining appropriate expert consultations, detailed pertinent history, physical examination and clinical laboratory tests.
    - Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new onset diseases are to be recorded as "AEs" within the eCRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic participants and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
    - Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications are to be entered in the eCRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
    - Obtain a history of exposure to environmental chemical agents.
    - Based on the participant's history, other testing may be appropriate including:
        - Acute viral hepatitis (A, B, C, D, E or other infectious agents such as cytomegalovirus, Epstein–Barr virus)
        - Ultrasound or other imaging to assess biliary tract disease
        - Other clinical laboratory tests, including INR and direct bilirubin
    - Consider gastroenterology or hepatology consultations.
    - Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology may be considered an important medical event and may be reported as an SAE. The sponsor should be contacted and informed of all participants for whom severe hepatic liver function abnormalities possibly attributable to study intervention are observed.

Study Intervention Discontinuation

Since this is a single dose study, treatment discontinuation is not applicable.

10.5 Appendix 5 List of Excluded Concomitant Medications

Please note that this is not an exhaustive list. Investigators should verify the participant's concomitant medications for all prohibited and restricted drug classes.

Prohibited Medications

From 48 hours prior to the ASP5354 administration until the completion of the surgical procedure.

Class	Drug
ICG[1]	Indocyanine green
Other NIR-F imaging agent	Methylene blue
	Other agents which emit fluorescence when excited by NIR-F spectrum

ICG: indocyanine green; NIR-F: near-infrared fluorescence

1. 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.

10.6 Appendix 6 Clinical Laboratory Assessments

Laboratory tests will be performed according to the Schedule of Assessments [Table 1] and sent to a central laboratory for analysis.

Table 9 Clinical Laboratory Tests

Panel/Assessments	Parameters to be Analyzed
Hematology	Hemoglobin Hematocrit Erythrocytes Leukocytes Differential leukocytes Platelets
Biochemistry	Albumin Alanine aminotransferase Alkaline phosphatase Aspartate aminotransferase Blood urea nitrogen Calcium Chloride Creatinine Creatine kinase Estimated glomerular filtration rate Gamma-glutamyl transferase Glucose Inorganic phosphorus Lactate dehydrogenase Magnesium Potassium Sodium Total bilirubin Total cholesterol Total protein Triglycerides Uric acid
Allergic reactions	Histamine and tryptase (only in participants with hypersensitivity reaction)
Urinalysis Urine, dipstick as applicable (if protein, blood, leukocytes or nitrites are abnormal, microscopy will be performed)	Protein Glucose pH Blood Leukocytes Urobilinogen Bilirubin Ketones Nitrite Color
	Microscopy (if needed) Casts Crystals Epithelial cells Leucocytes

Panel/Assessments	Parameters to be Analyzed
	Erythrocytes Bacteria
Pregnancy	Human chorionic gonadotropin (female participants of childbearing potential only)

10.7 Appendix 7 Assessment of Renal Function

10.7.1 Adult Participants

Individual eGFR (mL/min):
= eGFR (mL/min/1.73 m^2) (by MDRD) × BSA/1.73

- Formula for eGFR from the MDRD [Levey et al, 2006]:
  eGFR (mL/min per 1.73 m^2) = 175 × $(S_{cr, std})^{-1.154}$ × $(age)^{-0.203}$ × (0.742 if female) × (1.212 if African American)
- Formula for BSA calculated by Du Bois Formula [Du Bois & Du Bois, 1916]:
  BSA (m^2) = 0.007184 × weight $(kg)^{0.425}$ × height $(cm)^{0.725}$

BSA = body surface area; eGFR: estimated glomerular filtration rate; MDRD: modification of diet in renal disease; $S_{cr, std}$: serum creatinine (mg/dL) measured with a standardized assay

10.7.2 Adolescent Participants

Individual eGFR (mL/min):
= eGFR (mL/min/1.73 m^2) (by Schwartz formula) × BSA/1.73

- Formula for eGFR from the Schwartz formula [Schwartz et al, 2009]:
  eGFR (mL/min per 1.73 m^2) = 0.413 × (height [cm] / $S_{cr, std}$ [mg/dL])
- Formula for BSA calculated by Du Bois Formula [Du Bois & Du Bois, 1916]:
  BSA (m^2) = 0.007184 × weight $(kg)^{0.425}$ × height $(cm)^{0.725}$

BSA = body surface area; eGFR: estimated glomerular filtration rate; $S_{cr, std}$: serum creatinine (mg/dL) measured with a standardized assay

10.8 Appendix 8 Ureter Conspicuity by 5-Point Likert Scale

Primary and Secondary Efficacy Assessments

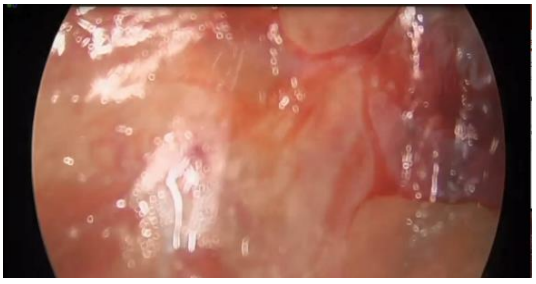
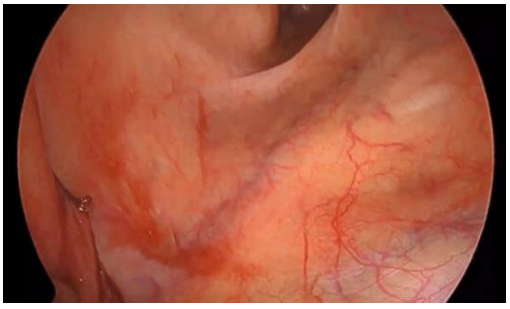
10.8.1 Ureter Conspicuity by 5-Point Likert Scale

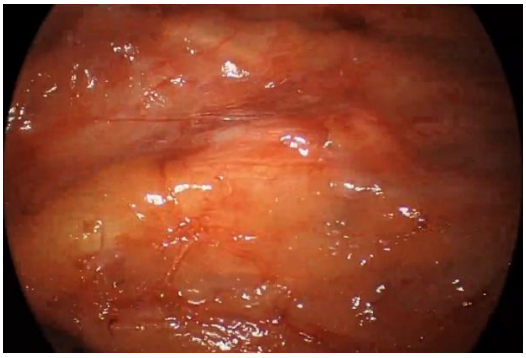
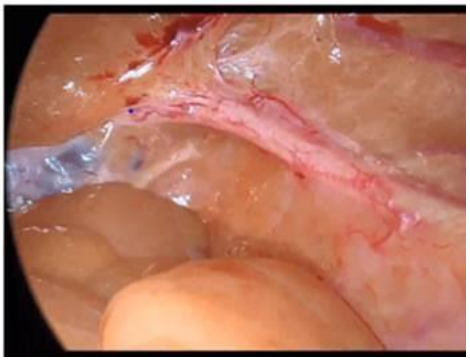
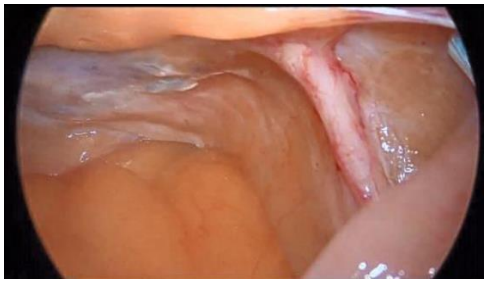
The conspicuity of the ureter with WL or WL/NIR-F will be assessed by the investigator (5-Point Likert Scale) during surgery for all participants.

- The investigator will attempt to locate the ureter and score the ureter conspicuity using the 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)".

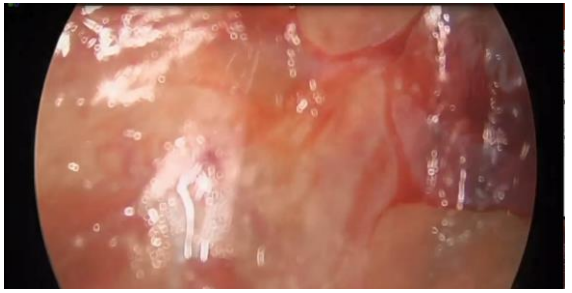
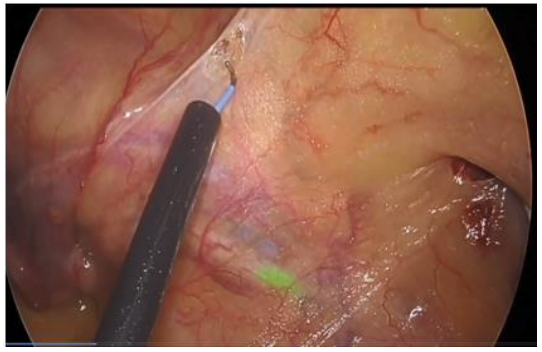
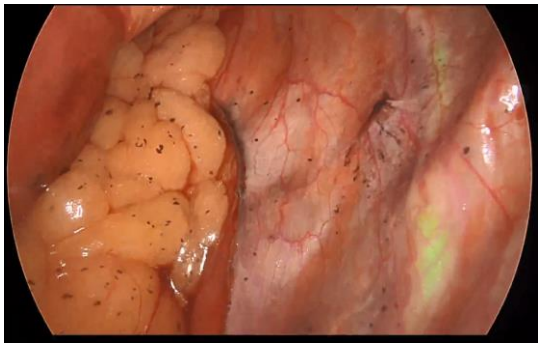
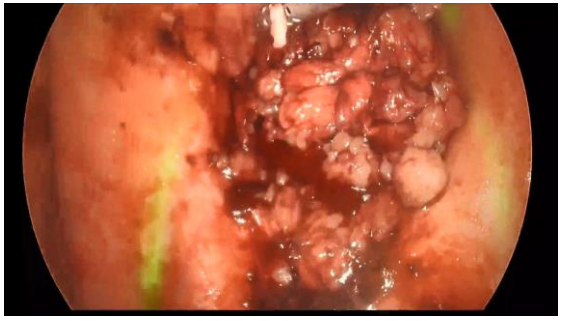
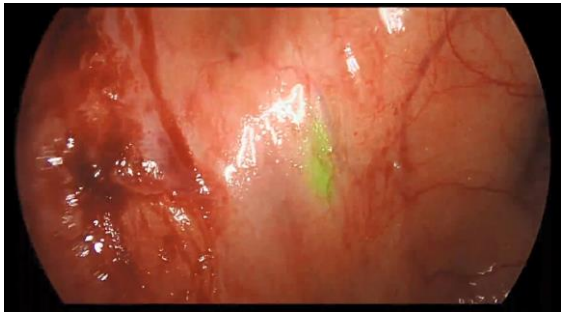
Conspicuity assessment will be scored using the 5-Point Likert Scale below for WL [Section 10.8.1.1] and NIR-F [Section 10.8.1.2]:

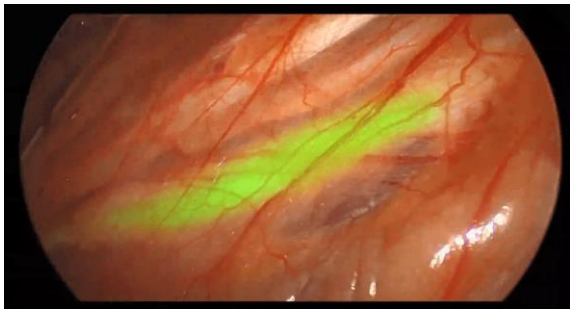
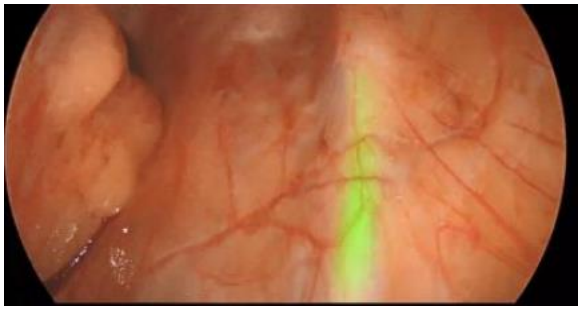
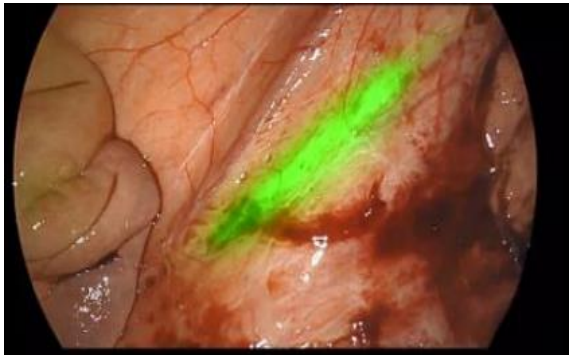
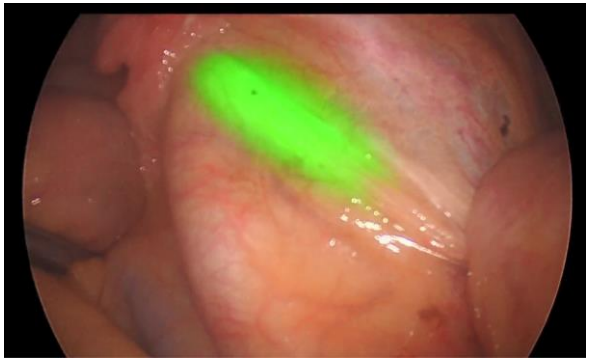
10.8.1.1 White Light Illustrative Examples

Score	Description of Ureter Conspicuity	Ureter Location Identification	Illustrative Example of Images
1	None	Not self-evident	
2	Poor	Somewhat self-evident	

Score	Description of Ureter Conspicuity	Ureter Location Identification	Illustrative Example of Images
3	Sufficient	Sufficiently self-evident	
4	Good	Clearly self-evident	
5	Excellent	Extremely self-evident	

10.8.1.2 NIR-F Illustrative Examples

Score	Description of Ureter Conspicuity	Ureter Location Identification	Illustrative Example of Images
1	None	Not self-evident	
2	Poor	Somewhat self-evident	 
3	Sufficient	Sufficiently self-evident	 

Score	Description of Ureter Conspicuity	Ureter Location Identification	Illustrative Example of Images	
4	Good	Clearly self-evident		
5	Excellent	Extremely self-evident		

10.9 Appendix 9 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during any crisis (e.g., natural disaster, pandemic).

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during any crisis. The site is expected to follow the protocol and associated Schedule of Assessments [Table 1] unless the site principal investigator discusses the need with the Astellas medical monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study-required assessments and procedures if study activities are unable to be performed as described in [Section 7] due to a crisis.

INFORMED CONSENT

Participants who need to follow any or all of the alternate measures outlined in this appendix will be required to provide informed consent, which explicitly informs them of the nature of and rationale for these changes, and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the participant followed by written documentation as soon as is feasible. A separate addendum to the study ICF will be provided to document the participant's consent to the changes.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if 1 or more of the following conditions are met due to the crisis:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible.
- Site facilities have been closed for clinical study conduct.
- Site has been restricted to treating patients with conditions outside of the scope of the study.
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel.
- Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel.

- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel.
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety.

Adherence to the original protocol as reflected in the Schedule of Assessments [Table 1] is expected, where plausible, in the case of a crisis. The alternate measures as noted in [Table 10] below are only permissible in the event of a crisis, and after discussing the need with the Astellas medical monitor to implement the alternate measures. This is to allow for continuity of receiving study intervention and maintaining critical safety and efficacy assessments for participants in the study at a time of crisis.

If 1 or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

Table 10 Alternative Schedule of Assessments in Response to a Crisis

Assessments	**Treatment Period**		**Follow-up Period**	**Alternate Approaches**
Visit Number	**2**		**3**	
Visit Label	**Postoperative**		**Follow-up**	
Day	**1**	**2[1]**	**15[2]**	
Window	**-**	**+ 2**	**± 10**	
Physical examination[3]	X[4]		X	Can be obtained at local clinic
Vital signs[5]	X[4]		X	Can be obtained at local clinic
Clinical laboratory tests[6]	X[4]	X	X	Visit collection of samples at local facility acceptable if results can be made available to investigator's site.
Weight[5]			X	Can be obtained at local clinic
Previous/concomitant medications	X	X	X[7]	Remote/virtual/telemedicine visits allowed
AE/SAE assessment	X	X	X[7]	Remote/virtual/telemedicine visits allowed

AE: adverse event; ECG: electrocardiogram; SAE: Serious adverse event

1. Only when participant stays in the hospital.
2. If a participant discontinues early from the study after the study intervention administration, the follow-up visit procedures will be performed upon discontinuation
3. At visit 2 and the follow-up visit (visit 3), a symptom-directed physical examination will be performed.
4. Physical examination, vital signs and ECG/cardiac monitoring will be completed within 2-hours after surgery. Clinical laboratory tests will be collected within 2 hours after surgery if participant is discharged on day of surgery (day 1) or on day 2, if participant remained hospitalized.
5. All vital signs will be measured with the participant in the sitting or supine position. Weight will be measured using standard institution practice and equipment.
6. Clinical laboratory tests for the central laboratory include blood collection for hematology (complete blood count), biochemistry and urine samples for urinalysis.
7. If a participant experiences an AE/SAE or change in concomitant medications between Visit 2 and Visit 3, the participant should call the study site to inform of these changes. For participants who experience hypersensitivity reaction(s), an additional blood sample should be collected to determine histamine and tryptase concentration levels. The sample should be taken at a local laboratory as soon as possible after the onset of the hypersensitivity reaction.

STUDY INTERVENTION SUPPLY

If any of the conditions outlined above in the Participants Procedures Assessment are met, 1 or all of the following mitigating strategies will be employed, as needed, to ensure continuity of study intervention supply to the participants:

- Increase stock of study intervention on-site to reduce number of shipments required, if site space will allow.

DATA COLLECTION REQUIREMENTS

Additional data may be collected in order to indicate how participation in the study may have been affected by a crisis and to accommodate data collection resulting from alternate measures implemented to manage the conduct of the study and participant safety.

- Critical assessments for safety and efficacy based on study endpoints to be identified as missing or altered (performed virtually, at alternative locations, out of window or other modifications) due to the crisis.

10.10 Appendix 10 List of Abbreviations and Definition of Key Study Terms

List of Abbreviations

Abbreviations	Description of abbreviations
ADE	adverse device effect
AE	adverse event
Ae	amount of ASP5354 excreted into urine
Ae%	percentage of ASP5354 dose excreted into urine
AESI	adverse event of special interest
ALT	alanine aminotransferase
APGD	Astellas Pharma Global Development Inc.
AST	aspartate aminotransferase
AT	aminotransferases
BICR	blinded independent central review
BSA	body surface area
CEF	contrast enhancement factor
CFR	Code of Federal Regulations
CRO	contract research organization
CSR	clinical study report
DILI	drug-induced liver injury
ECG	electrocardiogram
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EU CTR	European Union Clinical Trial Regulation
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HIPAA	Health Insurance Portability and Accountability Act
HRT	hormone replacement therapy
IB	Investigator's Brochure
ICC	intraclass correlation coefficient
ICE	intercurrent event
ICF	informed consent form
ICG	indocyanine green
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IND	investigational new drug
INR	international normalized ratio
IRB	Institutional Review Board
IRT	interactive response technology
ISN	international study number
ITT	Intent to Treat

Abbreviations	Description of abbreviations
IUI	iatrogenic ureteral injury
LA-CRF	liver abnormality case report form
LAR	legally authorized representative
MDRD	modification of diet in renal disease
mITT	Modified Intent to Treat
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIR-F	near-infrared fluorescence
PBPK	physiologically based pharmacokinetic
PK	pharmacokinetics
PopPK	population pharmacokinetics
QA	quality assurance
QC	quality control
SAE	serious adverse event
SAF	Safety Analysis Set
SAP	statistical analysis plan
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TBL	total bilirubin
TEAE	treatment-emergent adverse event
UADE	unanticipated adverse device effect
ULN	upper limit of normal
USM	urgent safety measure
WL	white light
WOCBP	woman of childbearing potential

Definition of Key Study Terms

Terms	Definition of Terms
Baseline	Assessments of participants as they enter a study before they receive any treatment.
Blinded independent central review	A committee that consists of experienced surgeons not associated with the study with no clinical information regarding the participants. The reviewers will review recorded images to evaluate ureter conspicuity.
Conspicuity	Self-evident ureter location identification (jump out to capture your attention).
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study. Note: Not all endpoints are themselves assessments since certain endpoints might apply to populations or emerge from analysis of results. That is, endpoints might be facts about assessments (e.g., prolongation of survival).
Enroll	To register or enter a participant into a study.
Randomization	The process of assigning participants to dose groups using an element of chance to determine assignments in order to reduce bias.
Screen failure	Potential participant who signed the ICF, but did not meet one or more criteria required for participation in the study and was not enrolled.
Screening	A process of active consideration of potential participants for enrollment in a study. Note: Once a participant has signed ICF, the protocol applies to the participant.
Screening period	Period of time before entering the investigational period, usually from the time when a participant signs the consent form until just before the test product or comparative drug (sometimes without randomization) is given to a participant.
Study period	Period of time from the first study site initiation date to the last study site completing the study.
Variable	Any quantity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

11 REFERENCES

Albert M, Delgado-Herrera L, Paruch J, Gerritsen-van Schieveen P, Kishimoto T, Takusagawa S, et al. Pudexacianinium (ASP5354) chloride for ureter visualization in participants undergoing laparoscopic, minimally invasive colorectal surgery. Surg Endosc. 2023;37:7336-47.

Bjerregaard J, Pandia MP, Jaffe RA. Occurrence of severe hypotension after indocyanine green injection during the intraoperative period. A A Case Rep. 2013;1:26-30.

Burks FN, Santucci RA. Management of iatrogenic ureteral injury. Ther Adv Urol. 2014;6:115-24.

Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med. 1916;17:863-71.

Fushiki H, Yoshikawa T, Matsuda T, Sato T, Suwa A. Preclinical development and validation of ASP5354: A near-infrared fluorescent agent for intraoperative ureter visualization. Mol Imaging Biol. 2023;25:74-84.

Halabi WJ, Jafari MD, Nguyen VQ, Carmichael JC, Mills S, Pigazzi A, et al. Ureteral injuries in colorectal surgery: an analysis of trends, outcomes, and risk factors over a 10-year period in the United States. Dis Colon Rectum. 2014;57:179-86.

Kaymak Ş, Özer MT, Demirbaş S, Kaya E, Kozak O. Renal autotransplantation due to iatrogenic ureter injury: A case report. Ulus Travma Acil Cerrahi Derg. 2021;27:260-4.

Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med. 2006;145:247-54.

Poza A, Marzullo L, García G, Reig C. Reparación de lesiones ureterales complejas: presentación de tres casos clínicos y análisis de las diferentes opciones terapéuticas [Surgical repair of complex ureteral injuries: three clinical cases and analysis of different technical approaches]. Arch Esp Urol. 2022;75:77-81.

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. Qual Life Res. 2019;28:1029-33.

Ravlo M, Moen MH, Bukholm IRK, Lieng M, Vanky E. Ureteric injuries during hysterectomy-A Norwegian retrospective study of occurrence and claims for compensation over an 11-year period. Acta Obstet Gynecol Scand. 2022;101:68-76.

Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD, J Amer Soc Nephrol. 2009;20:629-37.

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

Zhang Z, Chen D, Deng L, Li W, Wang X, Zhang Y et al. Iatrogenic ureteral injury during retroperitoneal laparoscopy for large renal cysts: What we learned and a review of the literature. J Xray Sci Technol. 2021;29:185-96.

12 INVESTIGATOR'S SIGNATURE

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

ISN/Protocol 5354-CL-0301, Amendment 5 [Nonsubstantial]

24 OCT 2024

I have read all pages of this protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that sub-investigator(s) and other relevant members of my personnel have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: .....

Date (DD MMM YYYY)

Printed Name: .....

<Insert name and qualification of the investigator>

Address of study site: .....

.....

13 COORDINATING INVESTIGATOR'S SIGNATURE

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

ISN/Protocol 5354-CL-0301, Amendment 5 [Nonsubstantial]

24 OCT 2024

I have read all pages of this protocol for which Astellas is the sponsor. I agree that it contains all the information required to conduct this study.

Coordinating Investigator:

Signature: _____

Date (DD MMM YYYY)

Printed Name: _____ _____

<Insert name and qualification of the coordinating investigator>

Address: _____

_____

14 SPONSOR'S SIGNATURE

Required sponsor signatures as required by ICH GCP 4.5.1 are located in the first attachment

Attachment 1	Electronic Sponsor Signatures