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

Protocol for Phase 2 Study of ASP5354

ISN/Protocol 5354-CL-0201

Version 3.0

Incorporating Substantial Amendment 2 [See Section 13]

21 Jul 2021

IND 140758

Sponsor:

Astellas Pharma Inc.
2-5-1, Nihonbashi-Honcho, Chuo-ku,
Tokyo 103-8411, Japan

Protocol History:

Version 1.0 [08 May 2019]

Version 1.1 Incorporating Nonsubstantial Amendment 1 [27 Aug 2020]

Version 2.0 Incorporating Substantial Amendment 2 [08 Feb 2021]

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SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., protocol authors and contributors, etc.) are located in [Section 14 Sponsor's Signatures].

2. INVESTIGATOR'S SIGNATURE

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

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Version 3.0, Incorporating Substantial Amendment 2

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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 subinvestigator(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: .....

.....

CONTACT DETAILS OF SPONSOR'S KEY PERSONNEL

24-hour Contact for Serious Adverse Events See [Section 12.4.5 Reporting Procedures for Serious Adverse Events]	**Please fax or email the serious adverse events/special situations worksheet to:** Astellas Pharma Global Development Inc. **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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1 PROTOCOL SUMMARY

1.1 Synopsis

Date and Version of Protocol Synopsis:	21 Jul 2021, Version 3.0
Sponsor: Astellas Pharma Inc. (API)	**Protocol Number:** 5354-CL-0201
Compound Name: ASP5354	**Phase of Development:** Phase 2
Title of Study: A Phase 2 Randomized Open-label, Dose-ranging Study for Ureter Visualization, Using ASP5354 in Subjects Undergoing Laparoscopic/Minimally Invasive Colorectal Surgery	
Planned Study Period: From approximately 1Q2020 to 4Q2021.	

Study Objectives and Endpoints:

Objectives	Endpoints
Primary	
● To determine the optimal dose of ASP5354 for ureter visualization in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	● Anatomical visualization of the index ureter(s) 30 minutes after dosing of ASP5354 and the end of surgery (Yes/No).
Secondary	
● To investigate the safety and tolerability of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	<u>Safety and Tolerability</u> ● Vital signs (blood pressure, pulse and respiratory rate) ● Routine 12-lead electrocardiograms (ECGs) ● Clinical laboratory tests (hematology [complete blood count], serum chemistry and urinalysis) ● Nature, frequency and severity of treatment-emergent adverse events (TEAEs) and serious adverse events
● To investigate the pharmacokinetics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	<u>Pharmacokinetics</u> ● Plasma and urine concentrations of ASP5354 ● Amount of ASP5354 excreted in urine (Ae) during surgery ● Percentage of drug dose excreted into urine (Ae%) during surgery

Table continued on next page

Objectives	Endpoints
Exploratory	
• To investigate the relationship between pharmacokinetics and pharmacodynamics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery. • To explore fluorescence intensity and visualization duration of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery. • To explore the benefit of visualization during surgery of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	• The Likert Scale for qualitative response about the intensity of fluorescence at the predefined time points during surgery • Duration of anatomical visualization of the index ureter(s) from the first time of positive visualization to the last time point of positive visualization • Signal background ratio (SBR) • Binary questions (Yes/No) for the benefit of visualization by ASP5354 during surgery • Ability to visualize the contralateral ureter when amenable to visualization

Ae: amount of ASP5354 excreted in urine; Ae%: percentage of drug dose excreted into urine; BMI: body mass index; ECGs: electrocardiograms; SAEs: serious adverse events; SBR: signal background ratio; TEAEs: treatment-emergent adverse events

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

Approximately 2 study sites in the US

Study Population:

Male or female subjects ≥ 18 years of age scheduled to undergo laparoscopic/minimally invasive colorectal surgery.

Number of Subjects to be Enrolled/Randomized:

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

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

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

Study Design Overview:

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

Subjects will be randomly assigned to receive single doses of ASP5354 (CCI, [redacted] or CCI) which will be administered as an iv bolus to evaluate the anatomical visualization of the index ureter(s) (and contralateral ureter when feasible). Safety, tolerability and pharmacokinetics in the study population will also be assessed.

Up to 15 subjects will be randomly assigned at each dose level. During a standard minimally invasive surgery, visualization of the surgical field will be assessed following the placement of the near-infrared fluorescence (NIR-F) imaging system (with FDA 510[k] cleared optical device system) proximal to the ureter of interest and then ASP5354 will be administered.

- Intraoperative ureter fluorescence visualization will be assessed at approximately 10, 20, 30, 45 and 60 minutes post administration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments (end of surgery).

- The contralateral ureter will also be visualized when feasible, per the investigator's judgment, at similar time points. Fluorescence images will be captured on a bright field, NIR-F and overlay view (if available) and recorded during the entire surgery and archived.
- The anatomical visualization of the index ureter(s) will be assessed by the investigator intraoperatively using a binary "Yes or No" question on the ability to visualize the ureter at each time point.
- The fluorescence intensity of the ureter will be qualitatively assessed using a Likert Scale (0 = None, 1 = Mild, 2 = Moderate and 3 = Strong).
- The duration of anatomical visualization of the index ureter(s) will be from the first time of positive visualization to the last time point of positive visualization.
- Recorded images will be used to determine the SBR.

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

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

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

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

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

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

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

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

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

If the investigator judges that anatomical visualization of ASP5354 is not sufficient for the subject at 30 minutes after administration, the investigator can proceed with surgery using other modalities for visualization.

Safety and tolerability will be assessed by recording TEAEs and adverse reactions associated with the use of an investigational product (IP), clinical laboratory evaluations (hematology, serum chemistry and urinalysis), 12-lead ECGs, vital signs measurements and physical examination. Blood and urine samples for pharmacokinetic assessment will be collected pre- and post-administration of ASP5354 at defined time points, up to the end of surgery. In cases where surgery is completed in less than 105 minutes, blood samples will also be collected at 180 minutes after administration.

Inclusion/Exclusion Criteria:

Inclusion Criteria:

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 authorization for US study sites) must be obtained from the subject prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Subject is scheduled to undergo laparoscopic/minimally invasive colorectal surgery.
3. Subject will need visualization of the ureter(s).
4. Subject is considered an adult (≥ 18 years of age) according to local regulation at the time of signing the informed consent form.
5. Female subject is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1 of the following conditions apply:
    a. Not a woman of childbearing potential (WOCBP; see [Appendix 12.3 Contraception Requirements])
    b. WOCBP who agrees to follow the contraceptive guidance (see [Appendix 12.3 Contraception Requirements]) from the time of informed consent through at least 30 days after final study treatment administration.
6. Female subject must agree not to breastfeed starting at screening and throughout the study period.
7. Female subject must not donate ova starting at first dose of IP and throughout the study period and for 30 days after final study treatment administration.
8. Male subject with female partner(s) of childbearing potential (including breastfeeding partner) must agree to use contraception (see [Appendix 12.3 Contraception Requirements]) throughout the treatment period and for 30 days after final study treatment administration.
9. Male subject must not donate sperm during the treatment period and for 30 days after final study treatment administration.
10. Male subject with pregnant partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy throughout the study period and for 30 days after final study treatment administration.
11. Subject agrees not to participate in another interventional study while participating in the present study.

Subjects enrolled after optimal dose determination:

12. Subject has any of the following values:
    - Body mass index > 25
    - Estimated glomerular filtration rate (eGFR) ≥ 15 mL/min/1.73 m^2 and < 60. Subjects with an eGFR ≥ 60 mL/min/1.73 m^2 may be considered after discussion with the medical monitor.

Waivers to the inclusion criteria will **NOT** be allowed.

Exclusion Criteria:

1. Subject is anticipated to require ureteral stenting during surgery.
2. Subject has a history of known retroperitoneal fibrosis.
3. Subject has an active urinary tract infection.
4. Subject has received investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to screening.
5. Subject has any condition that, in the investigator's opinion, makes the subject unsuitable for study participation.
6. Subject has a known or suspected hypersensitivity to ASP5354, indocyanine green (ICG) or any components of the formulation used.
7. Subject has had previous exposure to ASP5354.
8. Subject has moderate to severe cardiac disease that limits daily functioning (New York Heart Association Class III-IV) or other medical conditions that the investigator feels would impact safety or study compliance.
9. Subject has a mean resting heart rate ≤ 45 bpm or ≥ 115 bpm, mean systolic blood pressure ≥ 160 mmHg or mean diastolic blood pressure ≥ 100 mmHg on day -1. If the mean blood pressure exceeds the limits above, repeat readings can be taken. Subject who has adequately controlled blood pressure is eligible.
10. Subject has a mean corrected QT interval (Triplicate ECG) using Fridericia's formula (QTcF) > 430 msec (for male subjects) and > 450 msec (for female subjects) on day -1. If the mean QTcF exceeds the limits above, the mean of 1 additional triplicate ECG can be taken.
11. Subject has any of the following screening laboratory values:
    - Hemoglobin ≤ 9 g/dL
    - Absolute neutrophil count ≤ 1500/μL
    - Platelet count ≤ 100000/μL
    - eGFR < 60 mL/min/1.73 m^2 (Not applicable to subjects enrolled after optimal dose determination.)
    - Serum bilirubin ≥ 2 × upper limit of normal (ULN)
    - Aspartate aminotransferase or serum glutamic oxaloacetic transaminase ≥ 2.5 × ULN
    - Alanine aminotransferase or serum glutamic pyruvic transaminase ≥ 2.5 × ULN
12. Subject has taken ICG or other NIR-F imaging agents within 48 hours prior to study treatment administration.
13. Subject has taken diuretics or inhibitors of renal transporters defined by FDA (see [Appendix 12.6 List of Excluded Concomitant Medications]) within 48 hours prior to study treatment administration.
14. Subject has used any illicit drugs, unless legally prescribed and is not being abused (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) within 30 days prior to day -1.
15. Subject has a history of alcohol abuse. Subject should not have consumed any alcohol within 48 hours of surgery.

Waivers to the exclusion criteria will **NOT** be allowed.

Investigational Product:

Name:
ASP5354

Use:
ASP5354 solution for administration will be supplied by the sponsor or designee as a CCI aqueous solution for iv administration. The NIR-F intensity of ASP5354 will be measured at wavelength 820 nm when irradiated with near-infrared excitation light at wavelength 780 nm.

Dose(s):
ASP5354, CCI, [redacted] or CCI will be administered as a single dose once the surgical area of interest is in view.
Mode of Administration:
ASP5354 will be administered as a single iv bolus administration under fasting conditions.

Dose Modifications:
Since this is a single dose study, dose modification is not applicable.

Concomitant Treatment (Medication and Nonmedication Therapy) Restrictions or Requirements:
The following concomitant medications will not be allowed from 48 hours prior to the IP administration until the completion of the surgical procedure:

- ICG unless used for anastomotic evaluation
- Other NIR-F imaging agent
- Diuretics
- Inhibitors for renal transporters defined by FDA (see [Appendix 12.6 List of Excluded Concomitant Medications])

Duration of Treatment:
Each subject will receive a single dose of ASP5354.

Treatment Discontinuation Criteria:
Since this is a single dose study, treatment discontinuation is not applicable.

Statistical Methods:

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

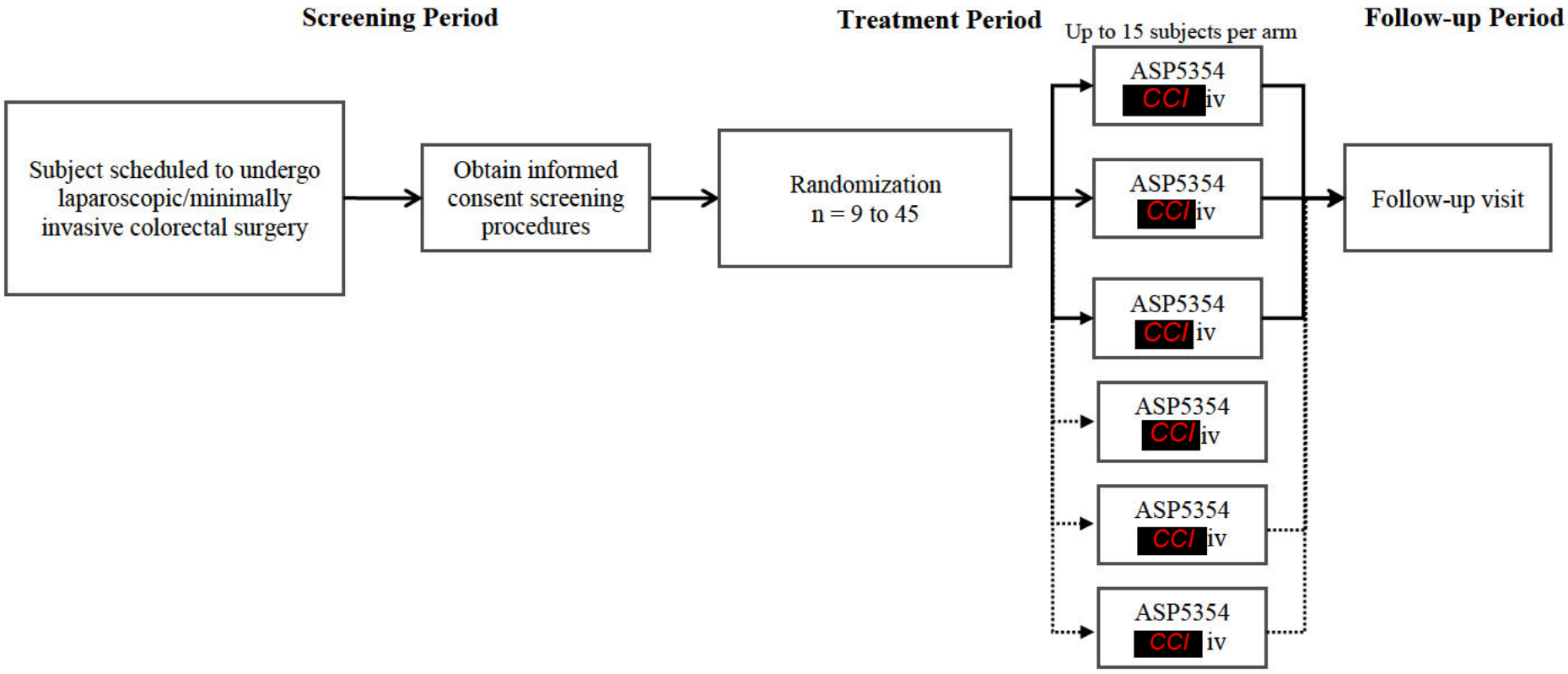
Efficacy:
The success rate of anatomical visualization of the index ureter(s) 30 minutes after dosing of ASP5354 and at the end of surgery will be estimated with exact 95% confidence interval for the full analysis set (FAS). The FAS consists of all randomized subjects who receive ASP5354 and have at least 1 assessment of ureter visualization during surgery. The FAS will be used for the summary of efficacy.
Safety and Tolerability:
The safety and tolerability endpoints will be summarized by descriptive statistics for the safety analysis set (SAF). The SAF consists of all randomized subjects who receive ASP5354.
Pharmacokinetics:
A listing and summary statistics of the individual plasma and urine concentrations and sampling times by treatment and assessment time interval will be prepared. A total of Ae, Ae% and urine volume during surgery will be listed and summarized by treatment.
Pharmacokinetic parameters will be evaluated by a population pharmacokinetic approach. Details of population analyses will be described in a separate analysis plan and a separate report.

Pharmacodynamics:
The relationship between ureter visualization (Yes/No, Likert Scale, visualization duration, etc) and pharmacokinetics will be evaluated by a population pharmacokinetic/pharmacodynamic approach. Details of the population analyses will be described in a separate analysis plan and a separate report.

Interim Analyses:
The determination of dose(s) to stop, continue or define a dose as optimal will occur when every 3 subjects randomly assigned to each dose level have the data for anatomical visualization during surgery reviewed via the VRC. The VRC will be held to review subject data until an optimal dose is determined.

1.2 Study Schema

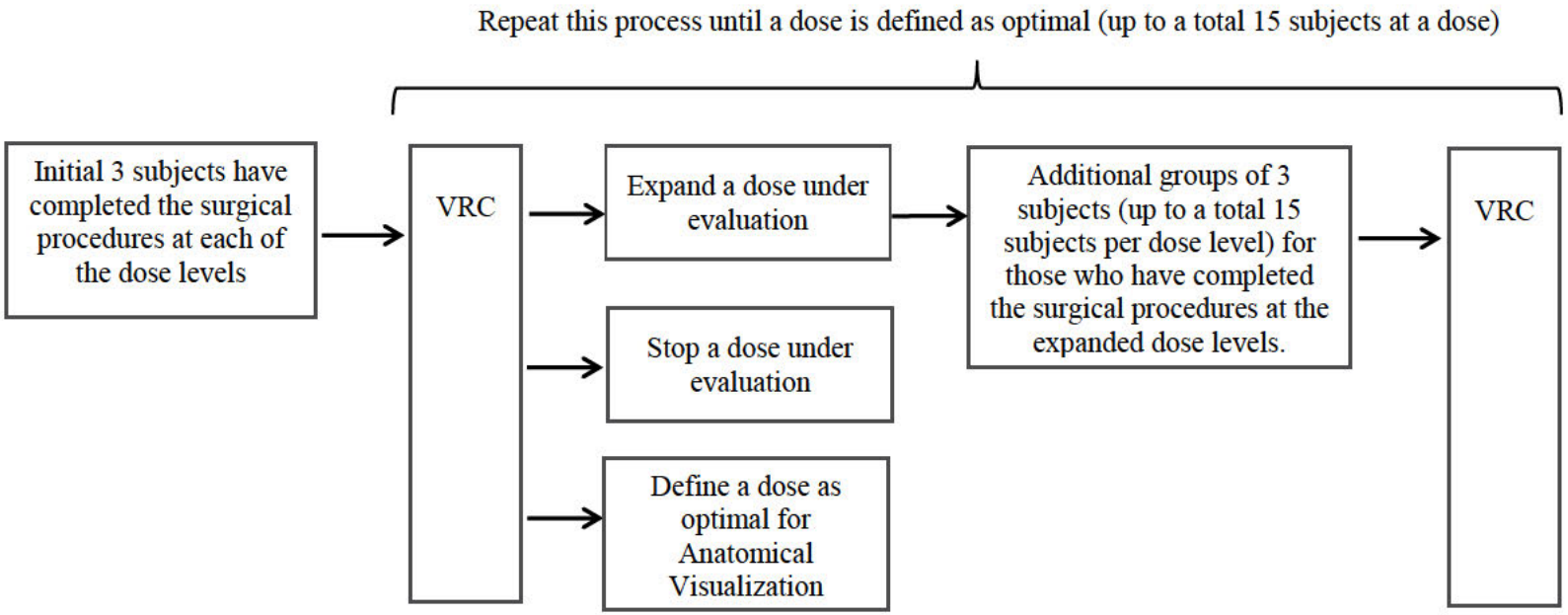
Figure 1 Study Schema



BMI: body mass index; eGFR: estimated glomerular filtration rate; iv: intravenous; n: number of subjects; VRC: Visualization Review Committee

- Up to 15 subjects will be randomly assigned to receive single doses of ASP5354 at each dose level (CCI . Once the optimal dose is determined, the optimal dose cohort may continue enrollment up to the maximum 15 subjects.
- Based on VRC review of the initial 3 dose levels, if none of the doses selected have visualization, then CCI and CCI dose levels will be added; if 1 dose selected has visualization, then the CCI dose level will be added. The CCI dose level will only be added if only the CCI dose level has visualization.

Figure 2 Dose Cohort Schema



BMI: body mass index; VRC: Visualization Review Committee

- VRC will be held at the following time points, but ad-hoc meetings may occur: 1) after the initial 3 subjects have completed the surgical procedures at each of the dose levels; and 2) then in increments of 3 subjects (up to a total 15 subjects per dose level) for those who have completed the surgical procedures at the expanded dose levels.
- The visualization data for the subjects, at each interim time point, who completed the surgical procedure will be assessed to decide whether to: 1) expand a dose under evaluation; 2) stop a dose under evaluation in which case additional subjects would not be added to a dose level; or 3) define a dose as optimal. Visualization should occur at 30 minutes post ASP5354 administration and end of surgery and Likert Scoring (0 to 3) for each time point should be recorded. Once the optimal dose is determined, the optimal dose cohort may continue enrollment up to the maximum 15 subjects.

1.3 Schedules of Assessments

Table 1 Schedule of Assessments

Assessments	**Screening Period**	**Treatment Period**			**Follow-up Period**
Day	**-28 to -1**	**1 (Operation)**			**7 (Outpatient) [13]**
		Preoperative	**Intraoperative**	**Postoperative**	
Window	**-**	**-**	**-**	**-**	**+3**
Visit Number	**1**	**2**			**3**
Informed Consent	X				
Inclusion/Exclusion Criteria	X				
Medical History	X				
Demographics	X				
Drug and Alcohol Screen [1]	X†				
Randomization		X			
Physical Examination [2]	X	X		X	X
Vital Signs [3]	X	X		X [12]	X
Clinical Laboratory Tests [4]	X†	X		X [12]	X
Height and Body Weight [3]	X				X
Serum Pregnancy Test [5]	X†				X
Routine 12-lead ECG [6]	X	X		X	X
Dosing ASP5354			X		
Blood Sampling for ASP5354 Pharmacokinetics and Metabolite Profiling [7]		X	X	X	
Urine Point Sampling for ASP5354 Pharmacokinetics and Metabolite Profiling [7]		X	X		
Total Urine Sampling in Catheter Bag and Amount of ASP5354 Excreted in Urine During Surgery [8]			X		
Confirmation of urine discoloration [9]			→	→	→
Investigator Evaluation for Ureteral Visualization [10]			X		
Record Surgery			X [11]		
Previous/Concomitant Medications	X	X	X	X	X [14]
AE Assessment	X	X	X	X	X [14]

AE: adverse event, ECG: electrocardiogram

†These assessments will be performed by local laboratory and the results of these assessments have to be obtained prior to subject randomization.

Footnotes continued on next page

1. Drug screen (e.g., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and alcohol screen will be performed by the study site's preferred method.
2. 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.
3. Vital signs include blood pressure, pulse and respiratory rate. All vital signs will be measured with the subject in the sitting or supine position. Height and weight will be measured using standard institution practice and equipment.
4. Clinical laboratory tests include blood collection for hematology (complete blood count) and serum chemistry and urine samples for urinalysis.
5. This assessment will be required only for subjects who are women of childbearing potential (see [Appendix 12.3 Contraception Requirements]).
6. Routine 12-lead ECGs will be taken in triplicate.
7. Blood and urine samples for pharmacokinetics of investigational product and possible metabolite(s) (if applicable) will be collected from every subject. A single pharmacokinetic blood sample will be collected at each of the following time points: prior to dose, approximately 10 minutes postdose, either approximately 30 or 60 minutes postdose and at the end of surgery. In cases where surgery is completed in less than 105 minutes, blood samples will also be collected at approximately 180 minutes after administration. Urine point sampling will be performed prior to dose and at the end of surgery (the end of surgery is an optional time point if a sample is available). For a detailed procedure of pharmacokinetic sampling, refer to the Sample Handling and Processing Manual.
8. Total urine volume of urine in the catheter bag will be calculated by dividing the weight of urine in the catheter bag by predefined density. Urine volume will be recorded in millimeters, and urine sample for pharmacokinetics of investigational product and possible metabolite(s) (if applicable) will be collected from the urine in the catheter bag to calculate Ae and Ae%. For a detailed procedure of total urine volume calculation, refer to the Sample Handling and Processing Manual.
9. The incidence of green discoloration of the urine will be recorded any time after dose administration. If it occurs, the color of urine will be assessed during surgery and every 120 minutes (± 30 minute window) after the end of surgery until it resolves or the subject is discharged, whichever is earlier. The start and stop date and time will also be recorded.
10. Surgeon evaluation for ureteral visualization includes incidence of anatomical visualization of the ureter(s), questions for the surgical benefit of ASP5354 and the Likert Scale for qualitative response about the intensity of fluorescence at the predefined time points during surgery.
11. Fluorescence images will be captured and recorded during the entire surgery and archived.
12. Postoperative vital signs and clinical laboratory tests will be collected within 2 hours after the surgery.
13. If a subject discontinues early from the study, day 7 outpatient visit procedures will be performed upon discontinuation.
14. If a subject experiences an AE or change in concomitant medications during days 2 to 6, he or she should call the study site to inform any of these changes. Should subjects develop a hypersensitivity reaction, an additional blood sample for determination of histamine and tryptase concentrations should be taken as soon as possible after the onset of the hypersensitivity reaction.

1.3.1 Sample Collection and Timed Procedure Schedule

Table 2 Sample Collection and Timed Procedure Schedule

Day	Operation	Time Point	Sample Collection		Efficacy Assessment	Collection Window
			Blood Sampling for Pharmacokinetics	Urine Point Sampling for Pharmacokinetics	Surgeon Evaluation for Ureteral Visualization	
Day 1	Preoperative	Predose	X	X	[d]	Within 1 h before dosing
	Intraoperative	10 min [a]	X		X	5 min ≤ and <15 min
		20 min [a]			X	15 min ≤ and < 25 min
		30 min [a]	(X) [e]		X	25 min ≤ and < 38 min
		45 min [a]			X	38 min ≤ and < 53 min
		60 min [a]	(X) [e]		X	53 min ≤ and < 75 min
		T min [a, b]			X	T-15 min ≤ and < T+15 min
	End of surgery	-	X	X [f]	X	-
	Postoperative	180 min [c]	X			105 min ≤ and < 240 min

a. If end of surgery is earlier than this time point, this time point(s) would not need to be collected.

b. This time point(s) would need to be evaluated every 30 minutes from 60 minutes after administration.

c. In cases where the surgery is completed in less than 105 minutes, blood samples would be collected at approximately 180 minutes after administration.

d. At the predose time point, fluorescence images will be captured and recorded on a bright field, near infrared fluorescence and overlay view (if available) for signal background ratio analysis, as well as the other time points.

e. Either 30 or 60 minutes after administration need to be collected.

f. Urine collection at end of surgery time point is optional (if a sample is available). The urine sample for the amount of ASP5354 excreted in urine during surgery will be collected from the urine in the catheter bag after the end of surgery.

2 INTRODUCTION

2.1 Background

Iatrogenic ureteral injury (IUI) is a rare but serious complication of abdominal or pelvic surgery. More than half of IUIs occur during gynecological procedures, followed by colorectal and vascular surgeries and urologic procedures [Burks & Santucci, 2014].

In patients undergoing hysterectomy for benign disease, minimally invasive hysterectomy was associated with increased risk in IUI relative to open procedures [Packlam et al, 2016]. In a US national series of 101021 patients, rates of ureteric repair were 0.5% for laparoscopic hysterectomy compared with 0.3% for open hysterectomy and 0.1% for vaginal hysterectomy [Wallis et al, 2016]. Laparoscopic procedure (versus vaginal), uterine size and endometriosis were associated with an increased risk for urologic intervention posthysterectomy.

In a 10-year retrospective analysis of ureteral injury in colorectal surgery, 6027 (0.28%) cases were identified in approximately 2 million US procedures [Halabi et al, 2014]. Though rare, IUIs were independently associated with higher mortality, morbidity, longer length of hospital stay and increased hospital charges. The highest risk factors for ureteral injuries were rectal cancer, adhesions and metastatic cancer. Postoperative complications associated with IUI included acute renal failure, urinary tract infection, wound complications and anastomotic leak.

Intraoperative diagnoses of IUIs are important to both patients and surgeons to minimize postoperative complications and improve surgical outcome; however, most IUIs are not detected intraoperatively [Burks and Santucci, 2014]. The identification of ureters can often be challenging in patients with advanced endometriosis, previous surgery or radiotherapy. Laparoscopic procedures may make it more difficult to detect the ureter using visual inspection and palpation, potentially increasing the risk for IUI [Slooter et al, 2019].

Preoperative ureteral stenting has long been proposed as a technique to identify the ureters and avoid IUI. However, this is an invasive procedure with associated complications, and reduction in the injury rate is not significant. Other preoperative techniques to reduce the risk of ureteral injury require radiation or complex nuclear medical equipment [Yeung et al, 2016].

Image guided surgery using near-infrared fluorescence (NIR-F) imaging is a promising technique that offers real-time visual information for surgeons and potentially makes operations safer for patients. Two dyes, methylene blue (MB) and indocyanine green (ICG), are commercially available but each has limitations with respect to intraoperative identification of the ureter [Slooter et al, 2019].

MB can be used for the identification of ureters during surgery [Verbeek et al, 2013]; however, the penetration depth of MB imaging is not sufficient for the identification of ureters under some circumstances due to its 700 nm excitation wavelength. Improved contrast agents with 800 nm fluorescence, a higher extinction coefficient and a higher quantum yield would be needed [Verbeek et al, 2013].

ICG is the only approved iv administered NIR-F imaging agent for intraoperative fluorescence imaging such as angiography. It has an NIR-F wavelength, which permits photons to penetrate relatively deeply through tissue and blood without altering the surgical field. However, ICG is minimally excreted into urine due to its lipophilicity; therefore it is not suitable for ureteral imaging.

ASP5354 is an ICG-derivative which shows hydrophilic properties by conjugating with cyclodextrins while maintaining the near-infrared fluorescent property similar to ICG. ASP5354 has an absorption of 780 nm and an emission of 820 nm, similar to that of ICG. ASP5354 is almost entirely excreted into the urine through the kidneys after iv administration due to its hydrophilic nature. Therefore, the detection of excretory flow of ASP5354 from the kidneys to the bladder using NIR-F imaging technology has the potential to facilitate accurate identification of the ureter during surgery.

2.1.1 Nonclinical and Clinical Data

Nonclinical and clinical data for ASP5354 available as of the writing of this protocol are summarized below. Please refer to the current version of the ASP5354 Investigator's Brochure (IB) for the most recent data.

2.1.1.1 Summary of Nonclinical Data

[redacted]

[redacted]
CCI
[redacted]
CCI
[redacted]
CCI
[redacted]

Please refer to the current ASP5354 IB for the most recent nonclinical data.

2.1.1.2 Summary of Clinical Data

2.1.1.2.1 Phase 1 Results (Study 5354-CL-0001)

As of the writing of this protocol, 1 clinical study of ASP5354 has completed dosing and study report preparation is ongoing. Study 5354-CL-0001 is a phase 1, double-blind, randomized, placebo controlled, single ascending dose study assessing the safety and pharmacokinetics of ASP5354 in healthy adult volunteers. IP was administered as a single iv bolus injection under fasting conditions.

2.1.1.2.1.1 Pharmacokinetics and Product Metabolism in Humans

Following a single iv administration, ASP5354 was rapidly eliminated. The observed pharmacokinetic parameters suggested a dose proportional increase in the investigated dose range from CCI to CCI. Almost all fraction of ASP5354 administered was excreted unchanged in urine. Renal clearance was nearly equivalent to the total body clearance.

For details please refer to the current version of the ASP5354 IB.

2.1.1.2.1.2 Safety

Single doses of up to CCI were considered safe and tolerable, with no patterns noted for safety concerns in healthy subjects. Twenty subjects received ASP5354 at doses of CCI, CCI, CCI CCI and CCI (each in 4 subjects). Ten subjects received placebo for ASP5354 (2 subjects per cohort).

Of the 30 subjects treated with ASP5354 or placebo, 5 subjects experienced at least 1 treatment-emergent adverse event (TEAE; oral herpes, pyelonephritis, urinary tract infection, headache, presyncope, dysuria, nausea, vomiting and incontinence [each in 1 subject]). Two (20.0%) subjects who received placebo experienced TEAEs of nausea, vomiting and incontinence (each in 1 subject); 1 (25.0%) subject who received CCI ASP5354 experienced TEAEs of oral herpes and presyncope; 1 (25.0%) subject who received CCI ASP5354 experienced a TEAE of urinary tract infection; and 1 (25.0%) subject who received CCI ASP5354 experienced TEAEs of dysuria, headache and pyelonephritis. None of the subjects experienced TEAEs considered to be at least possibly related to ASP5354. One subject who received ASP5354, CCI experienced a ≥ grade 3 TEAE; the grade 3 event of pyelonephritis was also an SAE that was considered by the investigator to be not related to ASP5354; rather, it was assessed as related to the use of a urinary catheter. TEAEs leading to withdrawal of IP were not applicable, as it was a single dose study. No deaths were reported in the study.

For details please refer to the current version of the ASP5354 IB.

2.1.2 Summary of Key Safety Information for Investigational Product(s)

The safety profile of ASP5354 is based on the results of the nonclinical studies and the clinical phase 1 study (Study 5354-CL-0001). Please refer to the current version of the ASP5354 IB for the most recent information.

In nonclinical studies, ASP5354 caused very slight macrophage accumulation in the alveolus in the lungs of rats and increased plasma histamine levels in dogs. Abnormal urine and skin color (pale green, greenish yellow or green) and green discoloration of the kidneys, epididymides and/or lymph nodes in gross pathology were also observed in rats and monkeys. These findings disappeared with discontinuation of drug administration. These findings were considered to be caused due to the green color of ASP5354 and were not considered toxicologically significant.

Though very slight macrophage accumulation in the alveolus in the lung was observed in the 4-week repeated iv dose toxicity study in rats (Study 5354-TX-0004), the possibility that ASP5354 induces this effect in the clinic is considered to be low.

Increased plasma histamine levels and clinical signs which are associated with increased plasma histamine concentration levels were induced by the single administration in dogs; however, the possibility of ASP5354 inducing this effect in the clinic is considered to be low.

ASP5354 has shown no discernible genotoxicity, phototoxicity or embryofetal toxicity potential in the nonclinical species tested.

As of the writing of this protocol, 1 phase 1 first-in-human study of ASP5354 has completed dosing, and study report preparation is ongoing (Study 5354 CL 0001). ASP5354 was well tolerated, with no IP-related TEAEs, or deaths reported in the study up to CCI. ASP5354 had no clinically significant effect on vital signs, electrocardiogram (ECG), blood chemistry, hematology or urinalysis.

In conclusion, the nonclinical and phase 1 clinical safety data support the initiation of a phase 2 dose-finding study in patients undergoing abdominal surgery.

2.2 Study Rationale

IUI is a devastating rare but serious complication of abdominal or pelvic surgery. More than half of IUIs occur during gynecological procedures, followed by colorectal surgery and vascular surgeries and urologic procedures [Burks and Santucci, 2014]. In retrospective analysis of ureteral injury in colorectal surgery, IUIs were independently associated with higher mortality, morbidity, longer length of hospital stay and increased hospital charges [Halabi et al, 2014]. The highest risk factors for ureteral injuries were rectal cancer, adhesion and metastatic cancer. Postoperative complications associated with IUI included acute renal failure, urinary tract infection, wound complications and anastomotic leak.

Intraoperative diagnoses of IUIs are important to both patients and surgeons to minimize postoperative complications and improve surgical outcomes; however, most IUIs are not detected intraoperatively [Burks and Santucci, 2014]. Preoperative ureteral stenting has long been proposed as a technique to identify the ureters and avoid IUI. However, this is an invasive procedure with associated complications and reduction in the injury rate is not significant; in addition, there are increased costs related to the procedure as well as increased operative times [Yeung et al, 2016]. Image guided surgery using NIR-F imaging is a promising technique that offers real-time visual information for surgeons and potentially

make operations safer for patients. There are 2 commercially available dyes, MB and ICG, but each has limitations with respect to intraoperative identification of the ureter [Slooter et al, 2019]. Neither are FDA approved for ureter visualization.

ASP5354 has hydrophilic properties, unlike ICG, with an absorption and emission similar to that of ICG. After IV administration, ASP5354 is mostly excreted through the kidney and into the urine. Therefore, the detection of excretory flow of ASP5354 from the kidneys to the bladder using NIR-F imaging technology can enable the surgeon, through a noninvasive means, to accurately and conveniently identify the ureters during surgery.

Based on this preclinical data confirming ureter visualization and the safety data in healthy volunteers, there is a strong rationale to define the dose in subjects undergoing minimally invasive surgery. As no noninvasive imaging agents are FDA approved for ureter imaging, ASP5354 provides a simplified and safe way to visualize ureters without adding operating time and expense or risk to subjects.

2.3 Risk Benefit Assessment

As no FDA approved imaging agents for ureter identification are available, current options include stents or in some cases, off-label use of NIR-F agents. Stents have the issue of increased time in the operating room and medical costs, as well as potential for ureteral injury. ASP5354 provides a noninvasive way to visualize the ureters intraoperatively. ASP5354 has been safely administered up to CCI. ASP5354 was well tolerated, with no IP-related TEAEs reported. As such, there are no safety concerns for its use in subjects undergoing minimally invasive surgery. The benefits of avoiding ureters intraoperatively will minimize ureteral injuries and associated complications, potentially decrease operating times, avoid repeat surgeries and not increase medical costs like stents.

One important potential risk, hypersensitivity reactions including anaphylactic reactions with fatal outcome, are reported to have occurred after treatment with iodine based ICG. The reported incidence for ICG-related anaphylactic reactions is 1:42000 administrations or lower. Hypersensitivity reactions are well known to occur, in varying frequencies, with all kinds of contrast agents (iodinated contrast agents for computed tomography, gadolinium based contrast agents for magnetic resonance imaging and contrast agents for image enhancement during ultrasound). No such reactions have been observed for ASP5354 during the phase 1 study (Study 5354-CL-0001). The risk with ASP5354 is estimated to be lower, as ASP5354 is not iodinated, whereas ICG is.

The benefit of ASP5354 outweighs the anticipated risk, as no toxicities have been seen in doses up to CCI. Safety will be monitored during the study for all enrolled subjects.

3 STUDY OBJECTIVE(S) AND ENDPOINT(S)

Table 3 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
● To determine the optimal dose of ASP5354 for ureter visualization in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	● Anatomical visualization of the index ureter(s) 30 minutes after dosing of ASP5354 and the end of surgery (Yes/No).
Secondary	
● To investigate the safety and tolerability of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery. ● To investigate the pharmacokinetics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	<u>Safety and Tolerability</u> ● Vital signs (blood pressure, pulse and respiratory rate) ● Routine 12-lead ECGs ● Clinical laboratory tests (hematology [complete blood count], serum chemistry and urinalysis) ● Nature, frequency and severity of TEAEs and SAEs <u>Pharmacokinetics</u> ● Plasma and urine concentrations of ASP5354 ● Ae during surgery ● Ae% during surgery
Exploratory	
● To investigate the relationship between pharmacokinetics and pharmacodynamics of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery. ● To explore the fluorescence intensity and visualization duration of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery. ● To explore the benefit of visualization during surgery of ASP5354 in subjects undergoing laparoscopic/minimally invasive colorectal surgery.	● The Likert Scale for qualitative response about the intensity of fluorescence at the predefined time points during surgery ● Duration of anatomical visualization of the index ureter(s) from the first time of positive visualization to the last time point of positive visualization ● SBR ● Binary questions (Yes/No) for the benefit of visualization by ASP5354 during surgery ● Ability to visualize the contralateral ureter when amenable to visualization

Ae: amount of ASP5354 excreted in urine; Ae%: percentage of drug dose excreted into urine;
ECGs: electrocardiograms; SAEs: serious adverse events; SBR: signal background ratio;
TEAEs: treatment-emergent adverse events

4 STUDY DESIGN AND DOSE RATIONALE

4.1 Study Design

The study is a randomized open-label, dose-ranging study in adult subjects undergoing laparoscopic/minimally invasive colorectal surgery in which the need for anatomical visualization of the ureter is anticipated. This study will be conducted at approximately 2 study sites in the US.

Subjects will be randomly assigned to receive single doses of ASP5354 (CCI, CCI or CCI) which will be administered as an iv bolus to evaluate the anatomical visualization of the index ureter(s) (and contralateral ureter when feasible). Safety, tolerability and pharmacokinetics in the study population will also be assessed.

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

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

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

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

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

- Intraoperative ureter fluorescence visualization will be assessed at approximately 10, 20, 30, 45 and 60 minutes postadministration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments (end of surgery).
- The contralateral ureter will also be visualized when feasible, per the investigator's judgment, at similar time points. Fluorescence images will be captured on a bright field, NIR-F and overlay view (if available) and recorded during the entire surgery and archived.
- The anatomical visualization of the index ureter(s) will be assessed by the investigator intraoperatively using a binary "Yes or No" question on the ability to visualize the ureter at each time point.
- The fluorescence intensity of the ureter will be qualitatively assessed using a Likert Scale (0 = None, 1 = Mild, 2 = Moderate and 3 = Strong).
- The duration of anatomical visualization of the index ureter(s) will be from the first time of positive visualization to the last time point of positive visualization.
- Recorded images will be used to determine the signal background ratio (SBR).

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

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

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

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

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

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

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

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

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

If the investigator judges that anatomical visualization of ASP5354 is not sufficient for the subject at 30 minutes after administration, the investigator can proceed with surgery using other modalities for visualization.

Safety and tolerability will be assessed by recording TEAEs and adverse reactions associated with the use of an IP, clinical laboratory evaluations (hematology, serum chemistry and urinalysis), 12-lead ECG, vital signs measurements and physical examination. Blood and urine samples for pharmacokinetic assessment will be collected pre- and post-administration of ASP5354 at defined time points, up to the end of surgery. In cases where surgery is completed in less than 105 minutes, blood samples will also be collected at 180 minutes after administration.

4.2 Dose Rationale

A dose of CCI was considered to provide sufficient ureter visualization for 3 hours after iv administration, based on ureter visualization data in nonclinical studies and pharmacokinetic data of the phase 1 healthy volunteer study (Study 5354-CL-0001). ASP5354 is confirmed safe and well tolerated up to CCI in the phase 1 study. Since a large safety margin is confirmed, 3 dose levels of CCI, CCI and CCI were proposed as initial doses in this phase 2 study by selecting CCI as the central dose and using a common ratio of "3" to differentiate between doses and to determine the optimal dose.

4.3 End of Study Definition

The study start is defined as the date the first subject signs informed consent. The end of the study is defined as the last visit or scheduled procedure shown in the Schedule of Assessments for the last subject in the study.

5 STUDY POPULATION

All screening assessments must be completed and reviewed to confirm the potential subject 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

Subject 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 authorization for US study sites) must be obtained from the subject prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. Subject is scheduled to undergo laparoscopic/minimally invasive colorectal surgery.
3. Subject will need visualization of the ureter(s).
4. Subject is considered an adult (≥ 18 years of age) according to local regulation at the time of signing the informed consent form (ICF).
5. Female subject is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1 of the following conditions apply:
   a. Not a woman of childbearing potential (WOCBP; see [Appendix 12.3 Contraception Requirements])
   b. WOCBP who agrees to follow the contraceptive guidance (see [Appendix 12.3 Contraception Requirements]) from the time of informed consent through at least 30 days after final study treatment administration.
6. Female subject must agree not to breastfeed starting at screening and throughout the study period.
7. Female subject must not donate ova starting at first dose of IP and throughout the study period and for 30 days after final study treatment administration.
8. Male subject with female partner(s) of childbearing potential (including breastfeeding partner) must agree to use contraception (see [Appendix 12.3 Contraception

Requirements]) throughout the treatment period and for 30 days after final study treatment administration.
9. Male subject must not donate sperm during the treatment period and for 30 days after final study treatment administration.
10. Male subject with pregnant partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy throughout the study period and for 30 days after final study treatment administration.
11. Subject agrees not to participate in another interventional study while participating in the present study.

Subjects enrolled after optimal dose determination:

12. Subject has any of the following values at screening:
    - Body mass index > 25
    - Estimated glomerular filtration rate (eGFR) ≥ 15 mL/min/1.73 m^2 and < 60. Subjects with an eGFR ≥ 60 mL/min/1.73 m^2 may be considered after discussion with the medical monitor.

Waivers to the inclusion criteria will **NOT** be allowed.

5.2 Exclusion Criteria

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

1. Subject is anticipated to require ureteral stenting during surgery.
2. Subject has a history of known retroperitoneal fibrosis.
3. Subject has an active urinary tract infection.
4. Subject has received any investigational therapy within 28 days or 5 half-lives, whichever is longer, prior to screening.
5. Subject has any condition that, in the investigator's opinion, makes the subject unsuitable for study participation.
6. Subject has a known or suspected hypersensitivity to ASP5354, ICG or any components of the formulation used.
7. Subject has had previous exposure to ASP5354.
8. Subject has moderate to severe cardiac disease that limits daily functioning (New York Heart Association Class III-IV) or other medical conditions that the investigator feels would impact safety or study compliance.
9. Subject has a mean resting heart rate ≤ 45 bpm or ≥ 115 bpm, mean systolic blood pressure (SBP) ≥ 160 mmHg or mean diastolic blood pressure (DBP) ≥ 100 mmHg on day -1. If the mean blood pressure exceeds the limits above, repeat readings can be taken. Subject who has adequately controlled blood pressure is eligible.
10. Subject has a mean corrected QT interval (Triplicate ECG) using Fridericia's formula (QTcF) > 430 msec (for male subjects) and > 450 msec (for female subjects) on day -1. If the mean QTcF exceeds the limits above, the mean of 1 additional triplicate ECG may be taken.
11. Subject has any of the following screening laboratory values:
    - Hemoglobin ≤ 9 g/dL

- Absolute neutrophil count ≤ 1500/μL
- Platelet count ≤ 100000/μL
- eGFR < 60 mL/min/1.73 m^2 (Not applicable to subjects enrolled after optimal dose determination.)
- Serum bilirubin ≥ 2 × upper limit of normal (ULN)
- Aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase ≥ 2.5 × ULN
- Alanine aminotransferase (ALT) or serum glutamic pyruvic transaminase ≥ 2.5 × ULN

12. Subject has taken ICG or other NIR-F imaging agents within 48 hours prior to study treatment administration.
13. Subject has taken diuretics or inhibitors of renal transporters defined by FDA (see [Appendix 12.6 List of Excluded Concomitant Medications]) within 48 hours prior to study treatment administration.
14. Subject has used any illicit drugs, unless legally prescribed and is not being abused (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) within 1 month prior to day -1.
15. Subject has a history of alcohol abuse. Subject should not have consumed any alcohol within 48 hours of surgery.

Waivers to the exclusion criteria will **NOT** be allowed.

5.3 Restrictions During the Study

Not applicable.

5.4 Screen Failures

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

For screen failures, the demographic data, date of signing the ICF, inclusion and exclusion criteria, 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

Rescreening is not allowed. However, in case that the results of screening assessments that do not meet the parameters required by the eligibility criteria (e.g., clinical laboratory tests, vital signs, physical examination, ECGs, etc) may be repeated once within the screening period without the need to register the subject as a screen failure.

6 INVESTIGATIONAL PRODUCT(S)

6.1 Investigational Product Administered

The assigned doses of ASP5354 will be administered as a single dose once the surgical area of interest is in view.

Table 4 Investigational Product(s)

Name	ASP5354
Use	Test Product
Dosage Formulation	Solution for injection
Physical Description	Clear deep green solution
Unit Dose Strength	CCI
Packaging and Labeling	ASP5354 solution will be provided in 10-mL amber glass vials and stored according to the instructions on the label
Route of Administration	Intravenous bolus infusion
Administration Instruction	Administered as a single iv bolus administration under fasting conditions, once the surgical area of interest is in view
IP or Non-IP	IP
Sourcing	Provided by sponsor

IP: investigational product

ASP5354 solution for injection should be stored between -25° and -15°C. ASP5354 vials must be allowed to thaw for at least 1.5 hours at controlled room temperature or may be thawed overnight (not more than 12 hours) under refrigerated conditions (approximately 2°to 8°C). ASP5354 vials must be used for preparation of administration within 4 hours after they are removed from the freezer or refrigerator, respectively. After thawing, the vials should be gently rotated several times to ensure a uniform concentration and to avoid foaming (the concentration in the vials is not uniform after thawing). The thawed ASP5354 is filtered and further diluted with Dextrose 5% in water. The diluted ASP5354 shall be stored at controlled room temperature (20° to 25°C) prior to administration and must be administered within (not more than) 4 hours after penetration of vials.

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

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Packaging and Labeling

All IP used in this study will be prepared, packaged and labeled under the responsibility of qualified personnel at Astellas Pharma Inc. (API), Astellas US Technologies (AUST) or sponsor's designee in accordance with API, AUST or sponsor's designee standard operating procedures (SOPs), 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 vial will bear a label conforming to regulatory guidelines, GMP and local laws and regulations that identifies the contents as an investigational drug.

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

6.2.2 Handling, Storage and Accountability

1. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all IP received and any discrepancies are reported and resolved before use of the IP.
2. Only subjects enrolled in the study may receive IP and only authorized study site personnel may supply or administer IP. Only IP with appropriate expiry/retest dating may be dispensed.
3. All IP 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.
4. The investigator, institution or the head of the medical institution (where applicable) is responsible for accountability, reconciliation and record maintenance (i.e., receipt, reconciliation and final disposition records).
5. Further guidance and instruction on final disposition of used and unused IP is provided in the pharmacy manual.

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

6.3 Randomization and Blinding

This is an open-label study; however, enrollment, randomization and dispensation of IP will be performed via the interactive response technology (IRT) system. Prior to initiation of treatment, study site personnel will obtain the randomization number and treatment assignment from the IRT system. Specific IRT procedures will be described in the respective study manual.

6.4 Investigational Product Compliance

IP will be administered intravenously at the study site by study site personnel. The exact date and time (in minutes) of IP administration will be documented.

6.5 Previous and Concomitant Treatment (Medication and Nonmedication Therapy)

6.5.1 Previous Medication

The investigator must record the use of all previous (from day -28 or informed consent date, whichever is later, prior to visit 2) treatment in the eCRF, both medication and nonmedication treatments.

6.5.2 Concomitant Medication

The investigator must record the use of all concomitant (any medication between visit 2 and follow-up [visit 3]) treatment in the eCRF, both medication and nonmedication treatments.

6.5.3 Prohibited Medication

The following concomitant medications will not be allowed from 48 hours prior to the IP administration until the completion of the surgical procedure:

- ICG unless used for anastomotic evaluation
- Other NIR-F imaging agents
- Diuretics
- Inhibitors for renal transporters defined by FDA (see [Appendix 12.6 List of Excluded Concomitant Medications])

A list of excluded concomitant medications is provided in [Appendix 12.6 List of Excluded Concomitant Medications].

6.5.4 Alternative Therapy for Visualization

If the investigator judges that anatomical visualization of ASP5354 is not sufficient for the subject at 30 minutes after administration, the investigator can proceed with surgery using other modalities for visualization.

6.6 Dose Modification

Since this is a single dose study, dose modification is not applicable.

6.7 Criteria for Continuation of Treatment

Not applicable, as ASP5354 is a single dose anatomical imaging agent and has no therapeutic indications.

7 STUDY PROCEDURES AND ASSESSMENTS

7.1 Efficacy Assessments

7.1.1 Primary Efficacy Assessments

7.1.1.1 Anatomical Visualization of the Index Ureter(s) Using ASP5354

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

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

If the anatomical visualization of the index ureter(s) was not assessed, "Not Done" will be entered in the eCRF. However, in the only case that the anatomical visualization of the index ureter(s) was not assessed at 30 minutes after ASP5354 administration, the answer will be imputed as follows:

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

7.1.2 Exploratory Assessments

7.1.2.1 Intensity of Fluorescence

The intensity of fluorescence will be qualitatively assessed during surgery by the investigator, by direct examination of the ureter. A Likert Scale (0 = None, 1 = Mild, 2 = Moderate and 3 = Strong) will be used to determine the intensity of fluorescence at each time point. The contralateral ureter will also be visualized when feasible, per the investigators judgment, at similar time points.

7.1.2.2 Duration of Ureter Visualization

Intraoperative ureter fluorescence visualization will be assessed at approximately 10, 20, 30, 45 and 60 minutes postadministration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments (end of surgery). The duration of visualization of the index ureter(s) will be based on the result from the first positive visualized time point to the last positive visualized time point. Images will be captured on a bright field, NIR-F and overlay view (if available). The contralateral ureter will also be visualized when feasible, per the investigators judgment, at similar time points.

7.1.2.3 Signal Background Ratio

Fluorescence images will be captured and recorded during the entire surgery and archived. Images will be captured on a bright field, NIR-F and overlay view (if available). SBR will be calculated using fluorescence images at predose, approximately 10, 20, 30, 45 and 60 minutes postadministration of ASP5354, and then every 30 minutes thereafter and/or at the last time point before removal of visualization instruments (end of surgery), as follows [Hoogstins et al, 2019]:

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

Additional detail will be provided in the Imaging Charter.

7.1.2.4 Binary Questions (Yes/No) for the Benefit of Visualization by ASP5354 during Surgery

The investigator will be asked the following questions:

- Was the location of the index ureter(s) as expected (Yes or No)?
- Did visualization of the index ureter(s) occur with white light at 30 minutes after ASP5354 administration (Yes or No)?
- Did visualization of the index ureter(s) occur with white light at the end of surgery (Yes or No)?
- Was NIR-F superior to white light in terms of visualization of the index ureter(s) (Yes or No)?
- Did the location of the ureter visualized by NIR-F alter the operative plan (Yes or No)?
- Were any ureter anomalies or abnormalities observed (Yes or No)?

7.1.2.5 Ability to Visualize the Contralateral Ureter When Amenable to Visualization

The contralateral ureter will be assessed using the same binary questions for anatomical visualization and Likert Scale, when feasible, per the investigator's judgment, at similar time points.

7.2 Safety Assessments

Study procedures and their timing are summarized in the Schedule of Assessments [Table 1]. Protocol waivers or exemptions are not allowed.

Procedures conducted as part of a subject's routine 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].

7.2.1 Adverse Events

Should subjects develop a visible adverse event (AE) of the skin, such as rash or discoloration, photographs of the skin should be obtained, if possible, starting as soon as possible after first presentation of the AE. A calibrated color card and ruler for scale will be included in the photograph as a reference when taking the picture so that the skin color can be assessed.

Should subjects develop a hypersensitivity reaction, an additional blood sample for determination of histamine and tryptase concentrations should be taken as soon as possible after the onset of the hypersensitivity reaction.

See [Section 7.3 Adverse Events and Other Safety Aspects**]** for information regarding AE collection and data handling.

7.2.2 Laboratory Assessments

Laboratory assessments will be performed at a local laboratory. Blood samples will be collected via a peripherally or centrally placed iv cannula, an intra-arterial cannula or by direct venipuncture in a suitable vein.

Blood samples for hematology (complete blood count) and serum chemistry and urine samples for urinalysis will be collected as indicated in the Schedule of Assessments [Table 1]. The clinical laboratory tests to be performed in the study are listed in [Appendix 12.7 Laboratory Assessments].

Drugs of abuse (e.g., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and alcohol tests will be performed on samples collected as indicated in the Schedule of Assessments [Table 1]. Drugs of abuse and alcohol tests will be performed according to the study site's preferred method.

Pregnancy tests will be performed on samples collected as indicated in the Schedule of Assessments [Table 1]. Pregnancy tests (WOCBP only) will be performed on serum samples.

The investigator or subinvestigator must review the laboratory report and document this review.

Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator or subinvestigator who is a qualified physician.

7.2.3 Vital Signs, Height and Weight

Blood pressure (SBP and DBP), pulse and respiratory rate measurements will be taken as indicated in the Schedule of Assessments [Table 1]. All vital signs will be measured with the subject in the sitting or supine position.

Height and weight will be measured using standard institution practice and equipment and recorded as indicated in the Schedules of Assessments [Table 1].

7.2.4 Physical Examination

Physical examinations will be performed as indicated in the Schedule of Assessments [Table 1] and whenever there is a medical indication.

A full physical examination will include the following body systems: general appearance, skin, lymphatic, head and neck, ears, nose and throat, chest and lungs, cardiovascular, abdomen, extremities, musculoskeletal and neuromuscular.

On day 1 (pre- and postoperatively) and at the follow-up visit, a symptom-directed physical examination will be performed.

7.2.5 Electrocardiogram

Routine (12-lead) ECGs will be taken as indicated in the Schedule of Assessments [Table 1]. Routine 12-lead ECGs will be taken after the subject has been resting in the supine position. Routine 12-lead ECGs will be taken in triplicate.

The investigator will review, sign and date the ECG after recording to ensure subject safety. The time of the ECG, the interval measurements, as well as an overall conclusion, will be documented. This overall conclusion will be recorded as normal, abnormal not clinically significant or abnormal clinically significant in the eCRF. Any clinically significant ECG abnormalities should be recorded as an AE.

The anonymized ECG recordings will be printed, signed and saved in the source. Alternatively, per time point, the ECGs can be stored electronically and reviewed in a timely manner by the investigator.

7.2.6 Order of Assessments

The following order should be followed when more than 1 assessment is required at a time point:

1. 12-lead ECG
2. Vital signs
3. Blood or urine collection for pharmacokinetics
4. Blood sampling for clinical laboratory tests

7.3 Adverse Events and Other Safety Aspects

The definitions of an AE or SAE can be found in [Appendix 12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up or Reporting].

The investigator and medically qualified designee(s) are responsible for detecting, documenting and recording events that met the definition of an AE or SAE.

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

In order to identify any events that may be associated with study procedures and could lead to a change in the conduct of the study, Astellas collects AEs even if the subject has not received IP. AE collection begins after the signing of the ICF and will be collected through to the last clinical study protocol-defined assessment or when the subject is determined to be a screen failure.

7.3.2 Method of Detecting Adverse Events and Serious Adverse Events

The methods of recording, evaluating and assessing seriousness, causality and severity of AEs and SAEs are described in [Appendix 12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up or Reporting]. Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

An AE with a change in severity is recorded as a new AE.

7.3.3 Follow-up of Adverse Events

All AEs occurring during or after the subject has discontinued the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized by the investigator.

If after the protocol-defined AE collection period (see [Section 7.3.1 Time Period for Collecting Adverse Event and Serious Adverse Event Information]), an AE progresses to an SAE, or the investigator learns of any (S)AE (serious adverse event or adverse event) including death, where he/she considers there is reasonable possibility it is related to the IP or study participation, the investigator must promptly notify the sponsor.

7.3.4 Reporting of Serious Adverse Events

Prompt notification by the investigator to the sponsor of an SAE is essential, so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study intervention under clinical investigation are met.

In the case of an SAE, the investigator must contact the sponsor by fax or email immediately (within 24 hours of awareness).

Procedures for reporting SAEs to the sponsor are described in [Section 12.4.5 Reporting Procedures for Serious Adverse Events].

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

Green discoloration of the urine will not be considered an AE as this is an expected, known effect of short duration without any expected untoward clinical symptoms; however, this still needs to be recorded.

Under this protocol, the following event will not be considered as an (S)AE:

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

7.3.6 Special Situations

Certain special situations observed in association with the IP, such as incorrect administration (e.g., wrong dose of IP or background therapy) are collected in the eCRF, as protocol deviations per [Section 10.3 Major Protocol Deviations] 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 12.4.5 Reporting Procedures for Serious Adverse Events] and the details of the associated special situation are to be included in the clinical description on the SAE worksheet.

The special situations are:

- Pregnancy
- 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 12.4.6 Reporting Procedures for Special Situations].

7.3.7 Supply of New Information Affecting the Conduct of the Study

When new information becomes available that is necessary for conducting the study properly, 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 subjects, who will be required to sign an updated ICF in order to continue in the study.

7.3.8 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 (CA) or IRB/IEC, where applicable, in order to protect subjects 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.

7.3.9 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 subject'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 subjects. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify the relevant CA 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.

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 and Timed Procedure Schedule [Table 2].

Blood samples will be collected via a peripherally or centrally placed iv cannula, an intra-arterial 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 the IP. Blood sampling, processing, storage and shipment instructions are provided in a Sample Handling and Processing 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.

7.4.2 Analysis of ASP5354 in Urine

Urine point samples for the analysis of ASP5354 in urine will be collected from the urine that collects in the catheter tubing at the time points indicated in the Schedule of Assessments [Table 1] and Sample Collection and Timed Procedure Schedule [Table 2]. The urine sample for the amount of ASP5354 excreted in urine during surgery will be collected from the urine in the catheter bag after the end of surgery.

A urinary catheter will be inserted depending on the surgery procedure. While the catheter is in place, urine will be collected in a catheter bag. At the time of point urine collection, the catheter bag will be disconnected and the tubing will be allowed to fill with fresh urine. The point of urine collection will be obtained from the urine that collects in the catheter tubing. Aliquots of at least 0.2 mL will be collected in polypropylene vials for the urine point samples. The timing of urine point collections during the period of catheterization is detailed in Table 2. Total urine volume of urine in the catheter bag will be calculated by dividing the weight of urine in the catheter bag by predefined density. Urine sample for pharmacokinetics of the IP and possible metabolite(s) (if applicable) will be collected from the urine in the catheter bag.

Urine sampling, calculation, processing, storage and shipment instructions are provided in a Sample Handling and Processing 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 clinical study report.

7.5 Pharmacodynamics

Pharmacodynamic variables will include the primary endpoint (anatomical visualization of the index ureter) and exploratory efficacy endpoints (Likert Scale, visualization duration and SBR [Section 7.1 Efficacy Assessments]).

7.6 Electronic Clinical Outcome Assessment

Not applicable.

7.7 Other Assessments

7.7.1 Green Discoloration of Urine

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

7.8 Total Amount of Blood

The total amount of blood drawn for each subject will vary depending on the length of the surgery. At any time during the study, if any laboratory abnormalities are found for a subject, additional blood may be drawn for safety monitoring. The average expected blood draw is shown in Table 5.

Table 5 Blood Sampling

Sample Type	Number of Samples	Sample Volume (mL)	Total Volume (mL)
Serum chemistry	4	8.5	34.0
Hematology	4	4.0	16.0
ASP5354 pharmacokinetic profiling	4[a]	4.0	16.0
Total			**66.0**

a The number of samples for pharmacokinetic profiling will depend on the duration of the surgery. E.g., if surgery lasts for 120 minutes, samples will be taken pre-dose, 10 minutes postdose, either 30 or 60 minutes postdose and at the end of surgery.

8 DISCONTINUATION

8.1 Discontinuation of Individual Subject(s) From Study Treatment

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

8.2 Discontinuation of Individual Subject(s) From Study

A discontinuation from the study is defined as a subject who enrolled in the study and for whom the study is permanently discontinued for any reason.

The subject 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 subject's involvement in the study at any time if the subject's clinical condition warrants it.

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

A subject must discontinue the study for any of the following reasons:

- Subject requests to stop the study.
- Any clinical AE, laboratory abnormality or intercurrent illness that, in the opinion of the investigator, indicates to continue in the study is not in the best interest of the subject.
- Subject signs informed consent, but surgery is performed without administration of ASP5354.

8.2.1 Lost to Follow-up

Every reasonable effort is to be made to contact any subject lost to follow-up during the course of the study to complete study-related assessments and record outstanding data.

8.3 Discontinuation of the Study Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the sponsor.

8.4 Discontinuation of the Study

The sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause, including optimal dose determination, provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If the sponsor terminates the study for safety reasons, the sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

9 STATISTICAL METHODOLOGY

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before first subject screened. Changes from the planned analyses in the final SAP that impact the statistical analyses will be justified in the clinical study report.

In general, continuous data will be summarized with descriptive statistics (number of subjects, mean, SD, minimum, median and maximum), frequency and percentage for categorical data. For analysis by dose group, unless specified otherwise, subjects are grouped by the dose received for those randomized before determination of optimal dose, and a separate group for additional subjects enrolled after determination of optimal dose.

9.1 Sample Size

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

9.2 Analysis Sets

The allocation of subjects to analysis sets, except the pharmacokinetic analysis set (PKAS), will be determined prior to database hard-lock.

9.2.1 Full Analysis Set

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

9.2.2 Safety Analysis Set

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

9.2.3 Pharmacokinetic Analysis Set

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

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

9.3 Demographics and Baseline Characteristics

Demographics and other baseline characteristics will be summarized by descriptive statistics by dose group and overall for the FAS and SAF.

9.3.1 Subject Disposition

The number and percentage of subjects who completed and discontinued screening period and reasons for discontinuation will be presented for all subjects who signed the ICF. A similar table for study disposition will also be presented by dose group and overall for the FAS and SAF. All disposition details and dates of first and last evaluations for each subject will be listed.

9.3.2 Previous and Concomitant Treatment (Medication and Nonmedication Therapy)

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

9.3.3 Medical History

Medical history will be coded using MedDRA and will be summarized by SOC and preferred term (PT) by dose group and overall for the SAF.

Medical history for each subject will be listed.

9.3.4 Investigational Product Exposure

Since this is a single dose study, the analysis of duration of exposure is not applicable. Study drug dosing information will be listed.

9.4 Analysis of Efficacy

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

9.4.1 Analysis of Primary Endpoint

9.4.1.1 Primary Analysis

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

9.4.1.2 Secondary Analysis

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

9.4.1.3 Subgroup Analysis

No specific subgroup analysis is planned.

9.4.2 Analysis of Secondary Endpoints

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

9.4.3 Analysis of Exploratory Endpoints

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

The duration of anatomical visualization of the index ureter(s) will be summarized using descriptive statistics by dose group.

The SBR for index ureter(s) will be summarized using descriptive statistics by dose group and time point.

The binary questions for the benefit of visualization during surgery will be summarized by frequency and percentage by dose group.

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

9.5 Analysis of Safety

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

9.5.1 Adverse Events

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

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

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

AE data will be listed.

9.5.2 Laboratory Assessments

For quantitative clinical laboratory measurements, descriptive statistics will be used to summarize results and change from baseline by dose group and time point.

Shift from baseline to the postbaseline 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.

9.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline by dose group and time point.

Vital signs data will be listed.

9.5.4 Physical Examination

Not applicable.

9.5.5 Electrocardiogram

9.5.5.1 Routine 12-lead Electrocardiogram

The routine 12-lead ECG results will be summarized by dose group and time point.

Routine 12-lead ECG data and interpretations will be listed.

9.6 Analysis of Pharmacokinetics

Descriptive statistics will be used to summarize plasma and urine concentrations of ASP5354 and pharmacokinetic parameters by dose group and time point. All concentrations and pharmacokinetic parameters will be listed.

9.6.1 Estimation of Pharmacokinetic Parameters

Since the volume of urine point sample is very small, the volume of urine in the catheter bag will be used as the total urine volume. The following pharmacokinetic parameters will be calculated using the urine collection data during surgery:

- Amount of ASP5354 excreted in urine (Ae) during surgery
- Percent of drug dose excreted into urine (Ae%) during surgery

Other pharmacokinetic parameters will be estimated by a population pharmacokinetics approach using concentrations of ASP5354. All details of population analyses will be described in a separate analysis plan and a separate report will be written.

9.7 Analysis of Pharmacodynamics

Pharmacodynamic analyses will be conducted on the FAS as efficacy analyses [Section 9.4 Analysis of Efficacy].

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

9.8 Other Analyses

9.8.1 Green Discoloration of Urine

The frequency and percentage of patients who experience green discoloration of urine will be summarized by dose group. The duration of green discoloration will be summarized using descriptive statistics by dose group.

9.9 Major Protocol Deviations

Major protocol deviations as defined in [Section 10.3 Major Protocol Deviations] will be summarized for all randomized subjects by dose group, by study site and overall.

Major protocol deviation data will be listed by subject.

The major protocol deviation criteria will be uniquely identified in the summary table and listing.

9.10 Interim Analysis (and Early Discontinuation of the Study)

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

9.11 Additional Conventions

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

For the primary efficacy endpoint of anatomical visualization at 30 minutes after dosing and end of surgery, imputation will be performed when the assessment is not done at 30 minutes after dosing.

See the SAP for details of the definitions for analysis windows to be used for analyses by time point.

10 OPERATIONAL CONSIDERATIONS

10.1 Data Collection

The investigator or site designee will enter data collected using an electronic data capture system. In the interest of collecting data in the most efficient manner, the investigator or designee should record data (including clinical laboratory values, if applicable) in the eCRF within 5 days after the subject's visit.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with the source. These documents should be appropriately maintained by the study site.

The monitor should verify the data in the eCRFs with the source and confirm that there are no inconsistencies among them.

Clinical laboratory tests are performed at the local laboratory.

10.2 Demographics and Baseline Characteristics

10.2.1 Demographics

Demographic information will be collected for all subjects at screening and will include age, sex, race, ethnicity, weight, height and body mass index.

10.2.2 Medical History

Medical history will be collected at screening in order to assess inclusion/exclusion criteria.

10.2.3 Surgical Procedure and Indication

Details on surgical procedure and indication will be collected at screening in order to assess inclusion/exclusion criteria.

10.3 Major Protocol Deviations

A protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. All deviations from the protocol are to be recorded. A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited.

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and must protect the rights, safety and well-being of subjects. The investigator should not implement any deviation from, or changes of, the protocol, unless it is necessary to eliminate an immediate hazard to subjects.

A major protocol deviation is one that may potentially impact the completeness, accuracy or reliability of data contributing to the primary endpoint or affect the rights, safety or well-being of a subject. Major protocol deviations will have additional reporting requirements.

When a major deviation from the protocol is identified for an individual subject, the investigator or designee must ensure the sponsor is notified. The sponsor will follow up with

the investigator, as applicable, to assess the deviation and the possible impact to the safety and/or efficacy and/or pharmacokinetic parameters of the subject to determine subject continuation in the study.

The major protocol deviation criteria that will be summarized at the end of the study are as follows:

Protocol Deviation 1 - Entered into the study even though the subject did not satisfy entry criteria

Protocol Deviation 2 - Developed withdrawal criteria during the study and was not withdrawn

Protocol Deviation 3 - Received wrong treatment or incorrect dose

Protocol Deviation 4 - Received excluded concomitant treatment

The investigator will also assure that deviations meeting IRB/IEC and appropriate regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and appropriate regulatory authorities will be provided to the sponsor and maintained within the Trial Master File.

10.4 Study Organization

10.4.1 Visualization Review Committee

The visualization data will be assessed by the principal investigator/investigator and the sponsor's medical representative, who form the VRC. The VRC will be held at the following time points, but ad-hoc meetings may occur until the optimal dose is determined: 1) after the initial 3 subjects have completed the surgical procedures at each of the dose levels, and 2) then in increments of 3 subjects (up to a total of 15 subjects per dose level) for those who have completed the surgical procedures at each of the expanded dose levels. For further details on the VRC, refer to the VRC charter.

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12 APPENDICES

12.1 Ethical, Regulatory and Study Oversight Considerations

12.1.1 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

12.1.2 Institutional Review Board/Independent Ethics Committee/Competent Authorities

GCP requires that the protocol, any protocol amendments, IB, ICF and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IRB/IEC. The IRB/IEC will review the ethical, scientific and medical appropriateness of the study before it is conducted. IRB/IEC approval of the protocol, ICF and subject information and/or advertising, as relevant, will be obtained prior to initiation of any study-specific procedures.

Any substantial amendments to the protocol will require CA and IRB/IEC approval before implementation, except for changes necessary to eliminate an immediate hazard to subjects.

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 study site and adherence to requirements of 21 Code of Federal Regulations, ICH guidelines, the IRB/IEC, EU Regulation No. 536/2014 for studies (if applicable), and all other applicable local regulations.

12.1.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or nonsubstantial amendments. Depending on the nature of the amendment, either IRB/IEC or CA approval or notification may be required. The changes will become effective only after the approval of the sponsor, investigator, IRB/IEC and appropriate regulatory authorities.

Amendments to this protocol must be signed by the sponsor and investigator. Written verification of IRB/IEC approval will be obtained before any amendment is implemented. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval, but will be submitted to the IRB/IEC for their information, if required by local regulations.

If there are changes to the ICF, written verification of IRB/IEC approval must be forwarded to the sponsor. An approved copy of the new ICF must also be forwarded to the sponsor.

12.1.4 Financial Disclosure

Investigators and subinvestigators 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.

12.1.5 Informed Consent of Subjects

12.1.5.1 Subject Information and Consent

The investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the ICF will be reviewed, signed and dated by the subject, the person who administered the ICF and any other signatories according to local requirements. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that the ICF was signed prior to any study-related procedures and that the subject received a signed copy of the ICF.

The signed ICFs will be retained by the investigator and made available (for review only) to the study monitor, auditor and appropriate regulatory authorities and other applicable individuals upon request.

12.1.5.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject verbally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue participating in the study (e.g., report of serious adverse drug reaction). The communication must be documented in the subject's medical records and whether the subject is willing to remain in the study or not must be confirmed and documented.
2. The investigator must update the subject's ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the subject on all updated ICFs throughout their participation in the study. The investigator or his/her designee must reconsent subjects with the updated ICF even if relevant information was provided verbally. The investigator or his/her representative who obtained the written informed consent and the subject should sign and date the ICF. A copy of the signed ICF will be given to the subject and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the reconsent process.

12.1.6 Source Documents

Source data must be available at the study site to document the existence of the subjects 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 subject.

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, achieved, retrieved or transmitted electronically via computerized systems (and/or other kinds 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 (eCOA) 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 subject file and be available for archiving.

12.1.7 Record Retention

The investigator will archive all study data (e.g., subject identification code list, source data eCRFs and investigator's file) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for US study sites, 2 years after approval of the NDA or discontinuation of the IND). The sponsor will notify the study site/investigator if the NDA/MAA/J-NDA is approved or if the IND/IMPD/CHIKEN TODOKE is discontinued. The investigator agrees to obtain the sponsor's agreement prior to disposal, moving or transferring of any study-related records. The sponsor will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subject’s medical records and/or study progress notes.

The documents of the VRC (minutes and SOPs and others) and the judgment committee outside the study sites (minutes and SOPs and others) shall be retained by the sponsor.

12.1.8 Subject Confidentiality and Privacy

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited unless the subject provides written consent or approval. Additional medical information may be given only after approval of the subject to the investigator or to other appropriate medical personnel responsible for the subject's well-being.

The sponsor shall not disclose any confidential information on subjects obtained during the performance of their duties in the study without justifiable reasons.

Even though any individuals involved in the study, including the study monitors and auditors, may get to know matters related to a subject's privacy due to direct access to source documents, or from other sources, they may not disclose the content to third parties.

The sponsor affirms the subject's right to protection against invasion of privacy. Only a subject identification number will identify subject data retrieved by the sponsor. However, the sponsor requires the investigator to permit the sponsor, sponsor's representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The sponsor agrees to comply and process personal data in accordance with all applicable privacy laws and regulations, including, without limitation, the Personal Information Protection Law in Japan and privacy laws in the US. If the services will involve the collection or processing of personal data (as defined by applicable data protection legislation) within the European Economic Area (EEA), then the sponsor shall serve as the controller of such data, as defined by the EU Data Protection Directive (DPD), and investigator and/or third party shall act only under the instructions of the sponsor in regard to personal data. If the sponsor is not based in the EEA, the sponsor must appoint a third party to act as its local data protection representative or arrange for a co-controller established in the EU for data protection purposes in order to comply with the DPD.

12.1.9 Arrangement for Use of Information and Publication of the Study

Information concerning the test product, patent applications, processes, unpublished scientific data, the IB and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this study, the investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

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

12.1.10 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final clinical study report 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 his/her 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 hard-lock.

12.2 Procedure for Study Quality Control

12.2.1 Study Monitoring

The sponsor or delegated contract research organization (CRO) is responsible for monitoring the study to ensure that the rights, safety and well-being of subjects are protected, the study is properly conducted in adherence to the current protocol and GCP and the study data reported by the investigator/subinvestigator are accurate, complete and verifiable with the source. The sponsor is responsible for assigning the study monitor(s) to this study for proper monitoring. They will monitor the study in accordance with planned monitoring procedures.

12.2.2 Direct Access to Source Data/Documents

The investigator and the study site must accept monitoring and auditing by the sponsor or delegated CRO, as well as inspections from the IRB/IEC and appropriate regulatory authorities. In these instances, they must provide all study-related records including source documents when they are requested by the sponsor monitors and auditors, the CRO, the IRB/IEC or appropriate regulatory authorities. The confidentiality of the subject's identity shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

12.2.3 Data Management

Data management will be coordinated by the Data Science department of the sponsor in accordance with the SOPs for data management. All study-specific processes and definitions will be documented by data management. eCRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and the WHO Drug Dictionary, respectively.

12.2.4 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.

To support quality around subject safety and reliability of study results, quality tolerance limits (QTLs) are defined and monitored. QTLs represent the acceptable variation of study data, taking into consideration the current state of medical and statistical knowledge about the variables to be analyzed as well as the statistical design of the study. It is a level point, or value associated with a parameter that should trigger an evaluation if a deviation is detected to determine if there is a possible systematic issue (i.e., a trend has occurred). The QTLs defined for this study are provided below.

Table 6 Quality Tolerance Limit

QTL #: Name and Parameter	Definition	Parameter Justification
QTL1: Sufficient data for assessment of anatomical visualization of the index ureter(s): % missing data of anatomical visualization of the index ureter at 30 minutes after dosing ASP5354 and/or end of surgery.	Number of subjects who missed assessment of anatomical visualization of the index ureter(s) at 30 minutes after dosing ASP5354 and/or end of surgery per total number of randomized subjects (%).	Since anatomical visualization of the index ureter(s) at 30 minutes after dosing ASP5354 and end of surgery is the primary endpoint to determine the optimal dose of ASP5354, missing data points would hinder evaluation of efficacy.
QTL2: Sufficient data for PK modeling simulation: % missing data of plasma and urine PK data.	Number of time points which missed both collection of plasma and urine PK and/or missed either collection of plasma or urine PK per total number of PK time points (%). Please note that the urine PK time point at end of surgery will not be included in QTL2 since this time point is optional.	Since PK data will be correlative with anatomical visualization, PK modeling simulation would be useful to consider optimal dose.

PK: pharmacokinetics; QTL: quality tolerance limit

QTL Management Activities:

- For control of risks associated with QTL1: Sufficient data for assessment of anatomical visualization of the index ureter(s), refer to [Section 7.1.1 Anatomical Visualization of the Index Ureter(s) Using ASP5354].
- For control of risks associated with QTL2: Sufficient data for assessment of anatomical visualization of the index ureter, refer to [Section 7.4.2 Analysis of ASP5354 in Urine].

12.3 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. Pregnancy test results must confirm that the subject 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

- 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 subject's medical records, medical examination or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE SUBJECTS OF CHILDBEARING POTENTIAL

Female subjects of childbearing potential are eligible for participation in the study if they agree to use 1 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 IP 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
- 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.
- Sexual abstinence
  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 test product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject. It is not necessary to use any other method of contraception when complete abstinence is elected.

[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 subjects participating in clinical studies.

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

Male subjects 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 final IP administration.[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator.
- Use a condom.
- Female partners of male subjects 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.

12.4 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up and Reporting

12.4.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a subject administered an IP, and which does not necessarily have to have a causal relationship with this treatment. 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 IP whether or not considered related to the IP.

12.4.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, biochemistry or urinalysis) or other safety assessment (e.g., vital signs, physical examination, ECGs or radiographic scans), 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 (S)AE.

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

12.4.1.2 Potential Cases of Drug-induced Liver Injury

Refer to [Appendix 12.5 Liver Safety Monitoring and Assessment] for detailed instructions on drug-induced liver injury (DILI). Abnormal values in AST and/or ALT concurrent or with abnormal elevations in TBL that meet the criteria outlined in [Appendix 12.5 Liver Safety Monitoring and Assessment], 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 12.4.5 Reporting Procedures for Serious Adverse Events].

12.4.2 Definition of Serious Adverse Events

An AE is considered "serious" if, in the view of either the investigator or sponsor, the event:

- Results in death
- Is life-threatening (An AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death; it does not include an AE that, had it occurred in a more severe form, might have caused death.)
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly, or birth defect

- Requires inpatient hospitalization (except for planned procedures as allowed per study) or leads to prolongation of hospitalization (except if prolongation of planned hospitalization is not caused by an AE)
- Other medically important events (defined in paragraph below)

Medical and scientific judgment should be exercised in deciding whether expedited 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 subject or may require intervention to prevent one of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, usually are considered serious. Examples of such events are 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.

12.4.3 Criteria for Causal Relationship to Investigational Product

A medically qualified investigator is obligated to assess the relationship between IP and each occurrence of each (S)AE. This investigator will use medical judgment as well as the reference safety information [Section 2.1.2 Summary of Key Safety Information for Investigational Product(s)] to determine the relationship. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

The investigator is requested to provide an explanation for the causality assessment for each (S)AE and must document in the medical notes that he/she has reviewed the (S)AE and has provided an assessment of causality.

Following a review of the relevant data, the causal relationship between the IP and each (S)AE 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 IP?"

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 (S)AE may have been caused by the IP (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Has the subject been administered IP?
- Plausibility (i.e., could the event been caused by the suspect drug? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and study data, etc.)
- Dechallenge/dose reduction/rechallenge:
  - Dechallenge: Did the (S)AE resolve or improve after only stopping the dose of the suspect drug without any treatment?
  - Dose reduction: Did the (S)AE resolve or improve after reducing the dose of the suspect drug?
  - Rechallenge: Did the (S)AE reoccur if the suspected drug was reintroduced after having been stopped?

- Laboratory or other test results: a specific laboratory investigation supports the assessment of the relationship between the (S)AE and the IP (e.g., based on values pretreatment, during and posttreatment).
- Available alternative explanations independent of IP exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc., and strength of the alternative explanation.
- Finally, judging which are more likely based on all the above contents, factors of reasonable possibility or confounding factors, comprehensive judgment of plausible temporal relationship between exposure to the IP and (S)AE onset and/or resolution will be provided. Did the (S)AE occur in a reasonable temporal relationship to the administration of the IP?

There may be situations in which an SAE 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 assesses causality for every event before the initial transmission of the SAE data to the sponsor. 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 his/her assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report and update the eCRF with the new information and updated causality assessment.

12.4.4 Criteria for Defining the Severity of an Adverse Event

AEs, including abnormal clinical laboratory values, will be graded using 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[a]
3 - Severe	Medically significant but not immediately life-threatening, hospitalization or prolonged hospitalization indicated; disabling; limiting self-care ADL[b]
4 - Life-threatening	Life-threatening consequences, urgent intervention indicated
5 - Death	Death related to AE

ADL: activities of daily living; AE: adverse event

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

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

12.4.5 Reporting Procedures for Serious Adverse Events

The investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the sponsor by fax or email immediately (within 24 hours of awareness).

The SAE worksheet must be signed by a medically qualified investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE 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 worksheet to:

Astellas Pharma Global Development Inc.

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, 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 should be recorded on the medical records, SAE/special situation worksheet and on the eCRF.

The following minimum information is **required**:

- International study number/study number
- Subject number, sex and age
- Date of report
- Description of the SAE (event and seriousness criteria)
- Causal relationship to the IP (including reason)
- Drug provided (if any)

The sponsor or sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., suspected unexpected serious adverse reaction [SUSAR] reporting) according to current local/regional regulatory requirements. The sponsor or sponsor's designee will submit expedited safety reports to CA and concerned ethics committee per current local regulations, and will inform the investigators 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., EMA, 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 will notify all investigators responsible for ongoing clinical studies with the test product of all SUSARs, which require submission per local requirements to the IRB/IEC.

The investigator 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 subject.

12.4.6 Reporting Procedures for Special Situations

12.4.6.1 Pregnancy

If a female subject becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing, the investigator is to report the information to the sponsor according to the timelines in **[**Section 12.4.5 Reporting Procedures for Serious Adverse Events] using the SAE worksheet as a special situation and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male subject who becomes pregnant during the study dosing period or within 30 days from the discontinuation of dosing and report the information to the sponsor according to the timelines in [Section 12.4.5 Reporting Procedures for Serious Adverse Event**s**] using the SAE worksheet as a special situation.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data, etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female subject as an AE in the eCRF or SAE per [Section 12.4.5 Reporting Procedures for Serious Adverse Events]. For (S)AEs experienced by a female partner of a male subject, (S)AEs are to be reported via the SAE worksheet.

Additional information regarding the outcome of a pregnancy when also categorized as an SAE is mentioned below:

- "Spontaneous abortion" includes miscarriage, abortion and missed abortion.
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the IP.
- If an infant dies more than 1 month after the birth, it is to be reported if a relationship between the death and intrauterine exposure to the IP is judged as *"*possible*"* by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus).

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination or other means as appropriate. (S)AEs experienced by the newborn/infant should be reported via the pregnancy reporting form. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date.

12.4.6.2 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 subject; this includes the prescribing, storing, dispensing, preparation for administration or administration of a medicinal product), overdose or "off-label use" (i.e., use outside of what is stated in the protocol) is suspected, refer to [Section 10.3 Major Protocol Deviations]. Any associated (S)AEs 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 12.4.5 Reporting Procedures for Serious Adverse Events] together with the details of the medication error, overdose and/or "off-label use."

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

12.4.6.3 Misuse/Abuse

Definition of misuse: Situations where the IP 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 IP 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 (S)AEs 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 12.4.5 Reporting Procedures for Serious Adverse Events] together with details of the misuse or abuse of the IP.

12.4.6.4 Occupational Exposure

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

12.4.6.5 (Suspicion of) Transmission of Infectious Agent

If transmission of an infectious agent associated with the IP 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 (S)AEs 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 12.4.5 Reporting Procedures for Serious Adverse Events] together with the details of the suspected transmission of infectious agent.

12.4.6.6 Suspected Drug-drug Interaction

If a drug-drug interaction associated with the IP 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 (S)AEs 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 12.4.5 Reporting Procedures for Serious Adverse Events] together with details of the suspected drug-drug interaction.

12.5 Liver Safety Monitoring and Assessment

The purpose of this appendix is to provide guidance for the monitoring of 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. Any subject enrolled in a study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 × ULN or TBL > 2 × ULN should undergo detailed testing for liver enzymes (including at least ALP, ALT, AST and TBL). Testing should be repeated 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 and severe liver abnormality to inform the investigator and study team. Subjects 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	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 subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and† and* TBL > 2 × ULN or international normalized ratio (INR) > 1.5 (if INR testing is applicable/evaluated)
- 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

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and clinical laboratory tests. The study site personnel are to complete the liver abnormality case report form (LA-CRF). Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated 2 to 3 times weekly, and then weekly or less if abnormalities stabilize or the IP has been discontinued and the subject is asymptomatic.

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 a SAE. The sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to IP are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- 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 subjects, 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 subject's history, other testing may be appropriate including:
  - Acute viral hepatitis (A, B, C, D, E or other infectious agents)
  - 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.

Study Treatment Discontinuation

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

12.6 List of Excluded Concomitant Medications

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

Prohibited Medications

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

Class		**Drug**
ICG		Indocyanine green
Other NIR-F imaging agent		Methylene blue
		Other agents which emit fluorescent when excited by NIR-F spectrum
Diuretics	Loop	E.g., bumetanide, furosemide, torsemide
	Osmotic	E.g., isosorbide (also a vasodilator), glycerin, mannitol
	Thiazides	E.g., hydrochlorothiazide, indapamide, metolazone
	Potassium Sparing	E.g., eplerenone, spironolactone, triamterene
	Carbonic Anhydrase Inhibitors	E.g., acetazolamide, dichlorphenamide, methazolamide
	Xanthines	E.g., aminophylline, theophylline
Inhibitors for renal transporters defined by FDA	P-gp inhibitors	Amiodarone
		Carvedilol
		Clarithromycin
		Dronedarone
		Itraconazole
		Lapatinib
		Lopinavir and ritonavir
		Propafenone
		Quinidine
		Ranolazine, ritonavir
		Saquinavir and ritonavir
		Telaprevir
		Tipranavir and ritonavir
		Verapamil
		Simeprevir
	OAT1 and OAT3 inhibitors	P-aminohippuric acid (PAH)
		Probenecid
		Teriflunomide
	MATE1 and MATE2-K inhibitors	Cimetidine
		Dolutegravir
		Isavuconazole
		Ranolazine
		Trimethoprim
		Vandetanib

MATE: multidrug and toxin extrusion; OAT: organic anion transporter; P-gp: P-glycoprotein

12.7 Laboratory Assessments

Laboratory tests will be performed according to the Schedule of Assessments and sent to the local 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 Creatinine kinase Gamma-glutamyl transferase Glucose Inorganic phosphorus Lactate dehydrogenase Magnesium Potassium Sodium Total bilirubin Total cholesterol Total protein Triglycerides Uric acid Follicle-stimulating hormone (postmenopausal female subjects only)
	Histamine and tryptase (only in subjects 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 (optional) Casts Crystals Epithelial cells Leucocytes Erythrocytes Bacteria
Pregnancy - Serum	Human chorionic gonadotropin (female subjects only)

12.8 List of Abbreviations and Definition of Key Study Terms

List of Abbreviations

Abbreviations	Description of abbreviations
ADL	activities of daily living
AE	adverse event
Ae	amount of ASP5354 excreted in urine
Ae%	percentage of drug dose excreted into urine
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	Astellas Pharma Inc.
AST	aspartate aminotransferase
AT	aminotransferases
AUC_{0-24}	area under the concentration-time curve from time zero to 24 hours postdose
AUC_{inf}	area under the concentration-time curve from time zero to infinity
AUC_{last}	area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration
AUST	Astellas US Technologies
CA	competent authorities
CI	confidence interval
C_0	plasma concentration at time 0
CRO	contract research organization
CV	coefficient of variation
CYP	cytochrome P450
DILI	drug-induced liver injury
DBP	diastolic blood pressure
DPD	Data Protection Directive
ECG	electrocardiogram
eCOA	electronic clinical outcome assessment
eCRF	electronic case report form
EEA	European Economic Area
eGFR	estimated glomerular filtration rate
FAS	full analysis set
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GMP	Good Manufacturing Practices
HPWCs	human peripheral white blood cells
HRT	hormone replacement therapy
IB	Investigator's Brochure
ICF	informed consent form
ICG	indocyanine green
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

Abbreviations	Description of abbreviations
IEC	Independent Ethics Committee
INR	international normalized ratio
IP	investigational product
IRB	Institutional Review Board
IRT	interactive response technology
ISN	international study number
IUI	iatrogenic ureteral injury
iv	intravenous
LA-CRF	liver abnormality case report form
LC-MS/MS	liquid chromatography with tandem mass spectrometry
MATE	multidrug and toxin extrusion
max	maximum
MB	methylene blue
min	minimum
n	number of subjects
NCI-CTCAE	National Cancer Institute - common terminology criteria for adverse events
NIR-F	near-infrared fluorescence
OAT	organic anion transporter
P-gp	P-glycoprotein
PT	preferred term
QA	quality assurance
QC	quality control
QTcF	corrected QT interval using Fridericia's formula
QTL	quality tolerance limits
(S)AE	serious adverse event or adverse event
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SBP	systolic blood pressure
SBR	signal background ratio
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions
$t_{½}$	apparent terminal elimination half-life
TBL	total bilirubin
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
USM	urgent safety measure
VRC	Visualization Review Committee
V_{ss}	volume of distribution at steady state
WOCBP	women of childbearing potential

Definition of Key Study Terms

Terms	Definition of Terms
Baseline	Assessments of subjects as they enter a study before they receive any treatment.
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 subject into a study.
Randomization	The process of assigning subjects to dose groups using an element of chance to determine assignments in order to reduce bias.
Screen failure	Potential subject who signed the ICF, but did not meet 1 or more criteria required for participation in the study and was not enrolled.
Screening	A process of active consideration of potential subjects for enrollment in a study.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent form until just before the test product or comparative drug (sometimes without randomization) is given to a subject.
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.

13 ATTACHMENT 1: SUBSTANTIAL AMENDMENT 2

I. The purpose of this amendment is:

Substantial Changes
1. Add enrollment of subjects with high body mass index (BMI) and/or renal impairment after optimal dose is determined
DESCRIPTION OF CHANGE:
The study design is updated to allow the enrollment of additional subjects into the CCI arm after it is determined to be an optimal dose. This revision effects the following sections: • Study Schema (Figures 1 and 2) • Study Design Text is added to clarify that the optimal dose cohort may continue enrollment with up to a maximum of 15 subjects. Text is added to Section 9, Statistical Methodology, to clarify that there will be a separate group for the analysis of subjects enrolled after determination of optimal dose.
RATIONALE:
To obtain additional data of ASP5354 in the broadest subject population at the selected optimal dose.
2. Add inclusion criterion for BMI and/or renal impairment
DESCRIPTION OF CHANGE:
An inclusion criterion is added to enroll subjects with a BMI > 25 and/or renal impairment after the optimal dose has been determined.
RATIONALE:
The BMI criterion is added to collect further information on visualization in subjects with high BMI. The estimated glomerular filtration rate (eGFR) criterion is added to collect further information on visualization and pharmacokinetic data in subjects with eGFR < 60.

Nonsubstantial Changes
1. Revise planned study period
DESCRIPTION OF CHANGE:
The completion of the planned study period is changed from *3Q2021* to *4Q2021*.
RATIONALE:
This revision is made to reflect the estimated study completion.
2. Clarify that VRC will be held until the optimal dose is determined

DESCRIPTION OF CHANGE:
Text is added to clarify that the VRC will be held to review subject data until the optimal dose is determined.
RATIONALE:
This revision is made for clarification.

3. Minor Administrative-type Changes
DESCRIPTION OF CHANGE:
Include minor administrative-type changes (e.g., typos, format, numbering and consistency throughout the protocol) and add *eGFR* to List of Abbreviations.
RATIONALE:
To provide clarifications, administrative changes and corrections to the protocol.

II. Amendment Summary of Changes:

IIA. Substantial Changes

1 Protocol Summary *1.2 Study Schema (Figure 1 Study Schema)*
WAS:
• Up to 15 subjects will be randomly assigned to receive single doses of ASP5354 at each dose level (CCI, CCI or CCI).
IS AMENDED TO:
BMI: body mass index; • Up to 15 subjects will be randomly assigned to receive single doses of ASP5354 at each dose level (CCI, CCI or CCI). **Once the optimal dose is determined, the optimal dose cohort may continue enrollment up to the maximum 15 subjects.**

1 Protocol Summary *1.2 Study Schema (Figure 2 Dose Cohort Schema)*
WAS:
• The visualization data for the subjects, at each interim time point, who completed the surgical procedure will be assessed to decide whether to: 1) expand a dose under evaluation; 2) stop a dose under evaluation in which case additional subjects would not be added to a dose level; or 3) define a dose as optimal. Visualization should occur at 30 minutes post ASP5354 administration and end of surgery and Likert Scoring (0 to 3) for each time point should be recorded.
IS AMENDED TO:
BMI: body mass index; • The visualization data for the subjects, at each interim time point, who completed the

surgical procedure will be assessed to decide whether to: 1) expand a dose under evaluation; 2) stop a dose under evaluation in which case additional subjects would not be added to a dose level; or 3) define a dose as optimal. Visualization should occur at 30 minutes post ASP5354 administration and end of surgery and Likert Scoring (0 to 3) for each time point should be recorded. **Once the optimal dose is determined, the optimal dose cohort may continue enrollment up to the maximum 15 subjects.**

1 Protocol Summary and 4 Study Design and Dose Rationale
1.1 Synopsis, 4.1 Study Design

WAS:

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

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

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

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

IS AMENDED TO:

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

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

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

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

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

1 Protocol Summary and 5 Study Population *1.1 Synopsis, 5.1 Inclusion Criteria*
ADDED:
Subjects enrolled after optimal dose determination: **12. Subject has any of the following values at screening:** ● **Body mass index > 25** ● **Estimated glomerular filtration rate (eGFR) ≥ 15 mL/min/1.73 m^2 and < 60. Subjects with an eGFR ≥ 60 mL/min/1.73 m^2 may be considered after discussion with the medical monitor.**

1 Protocol Summary and 5 Study Population *1.1 Synopsis, 5.2 Exclusion Criteria*
WAS:
Subject has any of the following screening laboratory values: ● Hemoglobin ≤ 9 g/dL ● Absolute neutrophil count ≤ 1500/μL ● Platelet count ≤ 100000/μL ● Estimated glomerular filtration rate < 60 mL/min/1.73 m^2 ● Serum bilirubin ≥ 2 × upper limit of normal (ULN) ● Aspartate aminotransferase or serum glutamic oxaloacetic transaminase ≥ 2.5 × ULN ● Alanine aminotransferase or serum glutamic pyruvic transaminase ≥ 2.5 × ULN
IS AMENDED TO:
Subject has any of the following screening laboratory values: ● Hemoglobin ≤ 9 g/dL ● Absolute neutrophil count ≤ 1500/μL ● Platelet count ≤ 100000/μL ● **eGFR**~~Estimated glomerular filtration rate~~ < 60 mL/min/1.73 m^2 **(Not applicable to subjects enrolled after optimal dose determination.)** ● Serum bilirubin ≥ 2 × upper limit of normal (ULN) ● Aspartate aminotransferase or serum glutamic oxaloacetic transaminase ≥ 2.5 × ULN ● Alanine aminotransferase or serum glutamic pyruvic transaminase ≥ 2.5 × ULN

9 Statistical Methodology
ADDED:
For analysis by dose group, unless specified otherwise, subjects are grouped by the dose received for those randomized before determination of optimal dose, and a separate group for additional subjects enrolled after determination of optimal dose.

IIB. Nonsubstantial Changes

1 Protocol Summary *1.1 Synopsis, Objectives and Endpoints*
ADDED:
Ae: amount of ASP5354 excreted in urine; Ae%: percentage of drug dose excreted into urine; ECGs: electrocardiograms; SAEs: serious adverse events; SBR: signal background ratio; TEAEs: treatment emergent adverse events

9 Statistical Methodology *9.2.1 Full Analysis Set*
WAS:
The full analysis set (FAS) will consist of all subjects who are randomized and receive ASP5354 and have at least 1 assessment of ureter visualization during surgery. This will be the primary analysis set for efficacy analyses.
IS AMENDED TO:
The full analysis set (FAS) will consist of all subjects who ~~are randomized and~~ receive ASP5354 and have at least 1 assessment of ureter visualization during surgery. This will be the primary analysis set for efficacy analyses.

9 Statistical Methodology *9.2.2 Safety Analysis Set*
WAS:
The SAF consists of all randomized subjects who receive ASP5354. The SAF will be used for all summaries of the safety data, unless otherwise specified.
IS AMENDED TO:
The SAF consists of all ~~randomized~~ subjects who receive ASP5354. The SAF will be used for all summaries of the safety data, unless otherwise specified.

1 Protocol Summary and 9 Statistical Methodology *1.1 Synopsis, 9.10 Interim Analysis (and Early Discontinuation of the Study)*
ADDED:
The VRC will be held to review subject data until the optimal dose is determined.

1 Protocol Summary and 10 Operational Considerations *10.4.1 Visualization Review Committee*
WAS:
The visualization data will be assessed by the principal investigator/investigator and the sponsor's medical representative, who form the VRC. The VRC will be held at the following time points, but ad-hoc meetings may occur: 1) after the initial 3 subjects have completed the surgical procedures at each of the dose levels, and 2) then in increments of 3 subjects (up to a

total of 15 subjects per dose level) for those who have completed the surgical procedures at each of the expanded dose levels. In addition, the VRC may meet on an ad-hoc basis. For further details on the VRC, refer to the VRC charter.
IS AMENDED TO:
The visualization data will be assessed by the principal investigator/investigator and the sponsor's medical representative, who form the VRC. The VRC will be held at the following time points, but ad-hoc meetings may occur **until the optimal dose is determined**: 1) after the initial 3 subjects have completed the surgical procedures at each of the dose levels, and 2) then in increments of 3 subjects (up to a total of 15 subjects per dose level) for those who have completed the surgical procedures at each of the expanded dose levels. ~~In addition, the VRC may meet on an ad-hoc basis.~~ For further details on the VRC, refer to the VRC charter.

12 Appendices *12.8 List of Abbreviations and Definition of Key Study Terms*	
ADDED:	
eGFR	**estimated glomerular filtration rate**

14 SPONSOR SIGNATURE

Astellas Signatories

(Electronic signatures are attached at the end of the document.)

PPD	Medical Science Oncology
PPD	Biostatistics, Data Science