Clinical Study Protocol

An Open-Label, Randomized, Multicenter Study to Evaluate the Safety, Efficacy, Pharmacokinetics and Physician Satisfaction of Two Different Doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium 0.8% Solution When Used as an Aid in the Determination of Ureteral Patency.

PVP-19IC01
NCT# 04228445

Version 2.0

Amendment 1

Development Phase:	3
Investigational Product:	3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium (Indigo Carmine 0.8% Injection, USP)
Indication:	Determination of ureteral patency
Sponsor:	Provepharm Inc. 100 Springhouse Drive, Suite 105 Collegeville PA 19426 Telephone: 1-610-601-8600
IND Number	137856
Protocol Date:	01 July 2019
Administrative Change 1 Date:	18 October 2019
Amendment 1 Date:	10 December 2019

Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines on Good Clinical Practice (ICH E6 GCP) and regulatory requirements as applicable.

CONFIDENTIAL INFORMATION

Sponsor Signature Page

An Open-Label, Randomized, Multicenter Study to Evaluate the Safety, Efficacy, Pharmacokinetics and Physician Satisfaction of Two Different Doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium 0.8% Solution When Used as an Aid in the Determination of Ureteral Patency.

Sponsor

[redacted]
Director, Clinical Operations
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Signature and Date

Medical Monitor

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Gagnon Consulting, LLC
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Signature and Date

ABBREVIATIONS

λ_z	The Apparent Plasma Terminal Phase Rate Constant
AAGL	American Association of Gynecological Laparoscopists
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BP	Blood Pressure
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
Clast	Concentration at Last Quantifiable Time Point
Cmax	Maximum Plasma Concentration
CONSORT	Consolidated Standards of Reporting Trials
CRF	Clinical Report Form
CS	Clinically Significant
CSR	Clinical Study Report
CV	Curriculum Vitae
e.g.	For Example
ECG	Electrocardiogram
EDC	Electronic Data Capture
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FPFV	First Patient First Visit

GCP	Good Clinical Practices
GEE	Generalized Estimating Equation
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IC	Indigo Carmine
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IM	Intramuscular
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent to Treat
IV	Intravenous
LH	Laparoscopic Hysterectomy
m^2	Meters Squared
MDRD	Modification of Diet in Renal Disease
MEDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
N	Number
N/A	Not Applicable
NCS	Not Clinically Significant

NIMP	Non-Investigation Medicinal Product
PH	Proportional Hazard Assumption
PK	Pharmacokinetics
PSAS	Physician Satisfaction Agreement Scale
PV	Pharmacovigilance
QS	Quantity Sufficient
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SmPC	Summary of Product Characteristics
SoA	Schedule of Assessments
$t_{½z}$	The Terminal Half-Life
TAH	Total Abdominal Hysterectomy
Tlast	Time to Reach Last Quantifiable Time Point
Tmax	Time to Reach Maximum Plasma Concentration
TTV	Time from Drug Administration to Visualization
TVH	Total Vaginal Hysterectomy
US	United States
USP	United States Pharmacopoeia
UOVS	Ureteral Orifice Visualization Scale
VS	Vital Signs
WBC	White Blood Cell
WOCBP	Woman of Childbearing Potential

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1 PROTOCOL SUMMARY

1.1. Protocol Synopsis

Title	An Open-Label, Randomized, Multicenter Study to Evaluate the Safety, Efficacy, Pharmacokinetics and Physician Satisfaction of two different doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium Injection 0.8% solution when used as an aid in the determination of ureteral patency**.**
Phase	3
Objectives	**Primary Objective** To determine whether the use of Indigo Carmine 0.8% Injection, USP solution for injection (IC) provides a visualization advantage compared to saline when used as an aid in the determination of ureteral patency. Visualization will be measured by a 3-point Ureteral Orifice Visualization Scale (UOVS): 1 = non-visualization 2 = inadequate/equivocal visualization 3 = adequate/unequivocal visualization of the urine jet stream after administration of test agent **Secondary Objectives** 1. To evaluate the safety profile of IC when used as an aid in the determination of ureteral patency. 2. To describe the time to visualization (TTV) of ureteral jets during urological and gynecological surgical procedures 3. To determine physicians' overall satisfaction with the IC treatment by assessing the proportion of surgeons who agree using the 5-point Physician Satisfaction Agreement Scale (PSAS) with the statement: *"Compared to the saline treatment, my ability to assess ureter patency was improved after the addition of IC."* 1 = Strongly Agree 2 = Agree 3 = Neither Agree nor Disagree 4 = Disagree 5 = Strongly Disagree A surgeon's evaluation is considered satisfactory if the rating is either a 1 (strongly agree) or 2 (agree). 4. To determine the IC pharmacokinetic profile in a subset of subjects from 2 investigational sites.

	5. An exploratory comparison will be performed to assess the difference between the IC high dose vs IC low dose. Surgeons will be blinded to the dose of IC.
Methodology	This is an open-label, randomized, multicenter study to evaluate the efficacy, safety, and pharmacokinetics of two doses (2.5 mL and 5.0 mL) of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium commonly referred to as Indigo Carmine (IC) 0.8% Injection, USP solution for injection when used as an aid in the determination of ureteral patency. Study will enroll up to 116 subjects from approximately 10 study centers in the United States. Subjects scheduled for urological or gynecological surgical procedures, age 18 to 85 years inclusive, will be screened for participation. Screening will occur within 30 days before study drug administration (Day of Surgery). After signing the informed consent, medical history, physical examination, baseline laboratory testing, pregnancy testing, 12-lead ECG, and vital sign measurements will be completed during the screening visit. On the day of surgery (Day 1) subjects will be evaluated for eligibility for randomization. Eligible subjects will be randomized in a 1:1 ratio to receive a dose of either IC high dose (5 mL) or IC low dose (2.5 mL). All randomized subjects will serve as his/her own control by receiving a dose of normal saline prior to receiving the randomized IC dose. The surgeon will be blinded to the IC dose a subject receives. Time of injection of saline and IC will be captured. Assessment will be recorded by videography. To evaluate the efficacy outcomes, each subject will first be injected intravenously with 5 mL 0.9% saline. The ureteral orifices/flow will be observed for up to 10 mins or until adequate/unequivocal visualization has occurred (whichever occurs first). The time period that will be captured on video is from injection up to 10 minutes or until adequate/unequivocal visualization of both ureter orifices. If both ureters cannot be visualized simultaneously, then alternating 15-30 second images of each ureter or ureteral orifice will be obtained. The surgeon will rate his/her ability to visualize the ureteral jet stream indicating ureteral patency for each ureter according to the scale above. The process will be repeated in the same patient for the IC dose. Hence, each subject will have 4 assessments for the ureter patency measurement. After the procedure, the surgeon performing the procedure will provide his/her overall satisfactory assessment by rating his/her agreement with the statement “Compared to the saline treatment, my ability to assess ureteral patency was improved after the addition of IC” using the PSAS: 1=Strongly Agree, 2=Agree, 3=Neither Agree nor Disagree, 4=Disagree, 5=Strongly Disagree.

	The videos will be sent to a central imaging group who will pool and blind the videos. Videos will then be assessed by a blinded central reviewer for assessment of ureteral patency using the same 3-point UOVS. In a subset of subjects from 2 sites (approximately 16 subjects), subjects will be consented to participate in the pharmacokinetic (PK) portion of the study. Once consented, 13 blood samples will be taken from each subject at the scheduled timepoints post IC treatment for PK analysis. Urine and stool samples will also be collected for analysis in this PK group at specified time points. All treated subjects will have a follow-up visit 7 to 30 days (± 2 days) after the procedure. A final telephone follow-up call will occur on Day 30 (± 2 days) in subjects who have the follow-up visit before Day 28. Safety assessments will include monitoring of AEs during and post the procedure, clinical laboratory tests, 12-Lead ECG, and vital sign measurements.
Study Drug	Indigo Carmine 0.8% Injection, USP (Provepharm supplied) 5 mL Pre-filled 0.9% Saline Syringes (Provepharm supplied)
Number of Subjects	Up to 116 subjects will be enrolled from approximately 10 sites, including approximately 16 subjects to participate in PK/metabolite analysis at 2 sites
Length of participation	Each subject participation is expected to be up to 60 days, to include: • Up to 30-day screening • Day of procedure • 30-day follow-up
Dose, Dosage Form, and Route of Administration	Each ampule of IC single-dose ampule contains 40 mg indigo carmine in 5 mL water for injection QS Each subject will receive: - 5 mL 0.9% normal saline injected over 1 minute via IV administration and - 2.5 mL or 5 mL IC injected over 1 minute via IV administration (dose will be blinded except to unblinded administrator)
Sample Size	The study plans to randomize 96 subjects ; 48 subjects to 2.5 mL IC and 48 subjects to 5 mL IC. This sample size calculation was determined based on two-group Chi-square test comparing proportions in 3 categories at 0.05 significance level. The sample size does not account for dropouts, protocol deviations, withdrawal of consent, etc. Up to an additional 20% (about 20) subjects

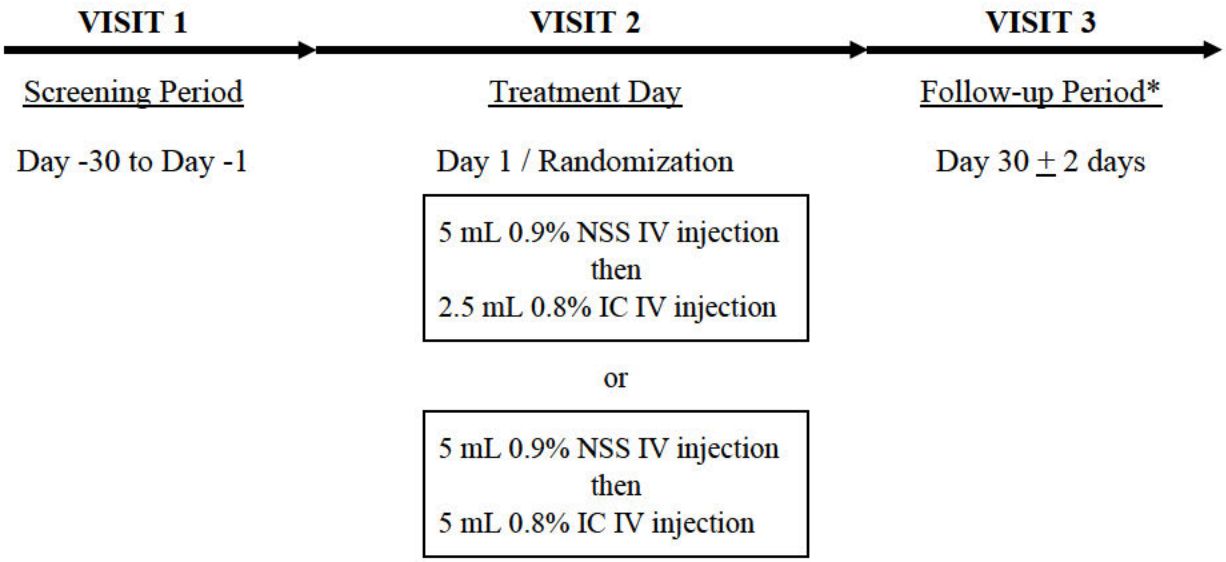
	may be enrolled to account for protocol deviations, withdrawal of consent, etc., if necessary. A subgroup of subjects at 2 sites (Approximate N=16, at least 8 from each IC dose level) will be evaluated for pharmacokinetics/metabolites.
Inclusion Criteria	I01. Subjects between ≥ 18 and ≤ 85 years old I02. Subjects who signed written, IRB approved, informed consent form I03. Subjects scheduled for urological or gynecological surgical procedures in which the patency of the ureter must be assessed by the surgeon during the procedure
Exclusion Criteria	E01. Subjects with stage 4 or 5 Chronic Kidney Failure as evidenced by a GFR ≤30 mL/min/1.73m^2 (using the MDRD) or need for dialysis in the near future, or having only 1 kidney E02. Subjects with known severe hypersensitivity reactions to IC or other dyes including contrast agents E03. Known history of drug or alcohol abuse within 6 months prior to the time of screening visit E04. Subjects, as assessed by the Investigator, with conditions/concomitant diseases precluding their safe participation in this study (e.g. major systemic diseases); E05. Unable to meet specific protocol requirements (e.g., scheduled visits) or subject is uncooperative or has a condition that could lead to non-compliance with the study procedures E06. Subject is the Investigator or any Sub-Investigator, research assistant, pharmacist, study coordinator, other staff or relative thereof directly involved in the conduct of the protocol; E07. Subjects with life expectancy ≤ 6 months; E08. Requirement for concomitant treatment that could bias primary evaluation. E09. Subjects who are pregnant or breast-feeding.
Efficacy Endpoints	**Primary Efficacy Endpoint** Surgeon's assessment of each ureter's patency by using the UOVS: 1 = Not visualized – I cannot see the ureteral jet flow 2 = Inadequately visualized or equivocal – I am less than completely confident that the ureter is patent 3 = Adequately visualized or unequivocal – I am completely confident that the ureter is patent

	Secondary Efficacy Endpoints 1. Time (minutes) to visualization (TTV) of the ureteral jets after each study drug administration 2. Physician's overall satisfaction with the IC treatment as an aid for the assessment of ureter patency **Other Endpoints** 1. UOVS scores assessed by the blinded central reviewer 2. Concordance of the surgeons' UOVS scores and the blinded central reviewer's UOVS score.
Safety Endpoints	**Safety Endpoints include** 1. Incidence of treatment emergent adverse events 2. Changes in safety laboratory tests and vital signs 3. Changes in clinically significant abnormal 12-ECG
PK Samples	The following PK samples will be collected from approximately 16 subjects from 2 study centers: 1. Plasma samples will be collected prior to and post IC injection at 2, 5, 7, 10, 15, 20, 30, and 40 mins, then at 1, 2, 3, and 4 hours after IC administration. 2. Urine collection will occur by a voided sample within 1 hour prior to the surgery and post IC injection for the following time periods 0-2 hours (including any urine drained during surgery), 2-6 hours, and 6-12 hours. 3. The first post-op stool will be collected and sent for analysis for IC and/or its breakdown products.
PK Parameters	The pharmacokinetic (PK) analysis will include at least the following parameters: 1. AUC_{0-t}, , $AUC_{0-\infty}$ 2. AUC_{extr}, $AUC_{\%extr}$, 3. C_{max}, C_{last}, T_{max}, and T_{last} 4. λ_z: the apparent plasma terminal phase rate constant. 5. $t_{½z}$: the terminal half-life, where possible, calculated as $0.693/ \lambda_z$. 6. Total excretion in urine and in stool
PK Analysis	PK samples will be prepared to analyze for plasma concentration of IC and any major metabolites. Non-compartmental analysis model will be used to calculate PK parameters using the observed concentrations and actual sampling times. All bioanalytical analyses will be completed to GLP

	standards using validated assays. The Bioanalytical Report and PK Report will be provided as appendices of the final study report.
Videography	Subjects will have video recordings of the ureteral orifices beginning with the injection of 5 mL saline and ending up to 10 minutes after the injection of IC or once both ureters have been adequately visualized. If both ureters cannot be visualized and recorded simultaneously then the camera will be switched between ureters every 15-30 seconds until adequate/unequivocal visualization or 10 minutes have passed. Once adequate/unequivocal visualization of a ureter has been obtained, then the camera may remain focused on the other ureter.
Procedure Summary	1. Subjects meeting all the inclusion and none of the exclusion criteria will be randomized to receive either 2.5 or 5 mL of IC injection. 2. The videography will be completed during the ureteral patency check. 3. The subject will be injected with 5 mL of 0.9% saline for injection intravenously over 1 minute, noting the time of injection, and the surgeon will assess the patency of the ureters by identifying the efflux of urine from the ureteral orifices for up to 10 minutes for each injection. a. If both ureteral jets cannot be assessed on camera simultaneously, then the camera will be switched between ureteral orifices every 15-30 seconds for the 10-minute observation period or until adequate/unequivocal visualization is observed. Then camera may remain focused on the other ureteral orifice until adequate/unequivocal visualization is seen or the completion of the 10-minute observation period has lapsed. b. If visualization of the ureter is adequate/unequivocal and the time is less than 10 minutes, then the time of visualization is to be noted for each ureter and the video may be stopped. 4. The surgeon will document the UOVS score assessing the patency of the ureters following the saline injection. 5. After the normal saline observation period of up to 10 minutes, a blinded dose of IC (2.5 or 5 mL, based on randomization) will be administered over 1 minute intravenously by the non-surgeon unblinded administrator, noting the exact time of injection. 6. Again, the surgeon will assess the patency of the ureters by identifying the efflux of blue urine from each ureteral orifice for up to 10 minutes using the same procedure as described above. 7. Once identified, the surgeon will note the time of identification of efflux of the IV IC injection.

	8. The surgeon will document the UOVS score assessing the patency of the ureters following the IC injection. 9. Subjects participating in the PK arm, at pre-determined time points will have blood drawn. All urine will be collected in separate containers for designated periods. Stool will be collected for the first bowel movement post-surgery in this same subgroup. 10. The surgeon will document his/her overall satisfaction by completing overall Physician Satisfaction Assessment for each subject. 11. Subjects will be followed for 30 days (± 2 days) for adverse events. 12. The videos will be sent to a central imaging group who will pool and blind the videos. The videos will then be assessed by a blinded central reviewer for ureteral patency using the same UOVS. The concordance in the UOVS scores between the surgeon's assessment and the central blinded reader will be evaluated.
Statistical Analysis Methodology	The statistical analysis for this study will be carried out using SAS® 9.3 or later and described in a detailed statistical analysis plan (SAP), which will be finalized prior to first patient first visit. Any deviations from the planned analysis as described in the SAP will be justified and recorded in the final clinical study report (CSR).

1.2. Study Schema



- Subjects who have an onsite visit between Days 7 and 27 may have safety follow-up procedures completed at that time

1.3. Schedule of Assessments (SoA)

	Screening period Day -30 to -1[1]	Randomization/ Treatment Day 1		Follow-up Visit Day 7 to Day 32 or Early Termination[2]	Telephone follow-up when Follow-up visit occurred prior to Day 28
Informed Consent	X				
Inclusion / Exclusion Criteria	X	X			
Medical, surgical history, demography, medication review	X				
Physical Examination	X			X	
Height	X				
Weight	X				
Vital Signs	X	X[3]		X	
12 Lead ECG	X			X	
Randomization		X			
Concomitant Medications	X	X		X	X
AE/SAE		X		X	X
Blood Safety Labs[4]	X			X[5]	
PK Blood[6]		X			
PK Urine[7]		X			
Bowel Prep[8]	X (Day -1)				
Stool collection for assay[9] (PK group)		X			
Surgery		X			
0.9% saline injection		X			
0.8% IC injection		X			
Video filming of ureteral jet flow with saline and with IC		X			
Urine Pregnancy Test[10]		X			

[1] Bloodwork/ECG completed as part of the pre-operatively work-up (within 30 days of surgery) will be acceptable for study use and will not have to be repeated for study participation.
[2] Subjects who have onsite visit between Day 7 and 27 may have safety follow-up completed with an additional telephone call between Day 28-32.
[3] Continuous monitoring, including heart rate and rhythm, will be required during the procedure and in the immediate post-operative period, vital signs will be collected for the study at the following specific time points: immediately prior to each injection and 5 minutes after each injection and thereafter approximately every 15 minutes through 1 hour following the procedure
[4] Blood Safety Labs: hematology = White blood cell count (WBC), Red blood cell count (RBC), Hemoglobin, Hematocrit, platelets, differential blood count (Neutrophils, lymphocytes, monocytes, eosinophils, basophils). Serum chemistry = total bilirubin, AST, ALT, alkaline phosphatase (ALP), creatinine, blood urea nitrogen, sodium, chloride, potassium, bicarbonate, phosphorus, calcium, glucose, albumin.
[5] Must be performed at the same laboratory as the initial bloodwork.
[6] For approximately 16 Subjects at 2 planned sites blood plasma collection will occur prior to and post IC injection at 2, 5, 7, 10, 15, 20, 30, and 40 mins, then at 1, 2, 3, 4 hours after IC administration.
[7] For a total of 16 Subjects at 2 planned sites urine collection will occur by a voided sample within 1 hour prior to the surgery and post IC injection for the following time periods 0-2 hours (including any urine drained during surgery), 2-6 hours, and 6-12 hours.
[8] Bowel prep will be required preoperatively (Day -1) for Subjects in the PK/Breakdown product collection patients.
[9] The first post-op stool will be collected and sent for analysis for IC and/or its breakdown products.
[10] Urine Pregnancy Tests will be done on day of surgery on all women of childbearing potential.

2. INTRODUCTION

Indigo carmine (IC) was introduced into clinical practice in 1903 by Voelcker and Joseph (Voelcker 1903) and was originally used as a test of renal function (Lacy 1955). In the 100+ years since then, indigo carmine has been used as a vital dye during surgery to identify vessels, tissues, and fistulae, and to help visualize ureteral urine ejection jets after urological or gynecological surgery. All these indications rely on IC's deep blue color to identify tissues and structures. Indigo carmine is a contrast stain that produces a very vivid coloration and is not absorbed by cells (Jung 1999).

It is currently marketed as an unapproved drug product in the United States (US) but is approved in the United Kingdom, France, Germany, Belgium, Luxemburg, and the Netherlands. The intention of this development program is to obtain an approval for indigo carmine when used as an aid in the determination of ureteral patency.

2.1. Study Rationale

Gynecologic and urologic surgery may lead to injury of the ureters and bladder because of the close anatomic locations of these structures to other genitourinary structures. Although gynecologic and urologic surgeons are highly trained in surgical techniques to reduce the occurrence of complications, urinary tract injuries still occur in many surgical procedures. The current estimates of urinary tract injuries with all types of gynecologic surgery range from 0.2 to 15 per 1000 cases. Two of the largest reported series of hysterectomies suggest that urinary tract injuries are more common with laparoscopic hysterectomy (LH) than with abdominal (total abdominal hysterectomy (TAH)) or total vaginal hysterectomy (TVH) (AAGL 2012). Clinical experience and published literature of case studies suggest that intraoperative detection and repair of urinary tract injuries significantly reduces morbidity and improves outcomes after such complications of gynecologic surgery (Chi 2016, Cohen 2018).

Most studies of laparoscopic surgery with known risk for lower urinary tract injury have included an evaluation for the recognition of urinary tract injuries using intraoperative cystoscopy. These studies have demonstrated that many urinary tract injuries are not recognized at the time of hysterectomy when specific measures are not taken to confirm ureteral patency and absence of bladder injury (AAGL 2012). In a study by Gilmour et al (Gilmour 2006) less than 50% of cases of ureteral injuries were detected intraoperatively when intraoperative cystoscopy was not performed. For bladder injuries, less than 25% of cases were detected intraoperatively when intraoperative cystoscopy was not performed. When intraoperative cystoscopy was performed at the time of laparoscopic hysterectomy (LH), 90-100% of ureteral injures and 80% of bladder injuries were detected intraoperatively. The current literature suggests that routine intraoperative cystoscopy at the time of LH is cost-effective, beneficial, and is of low risk to the patient (Ibeanu 2009).

The "gold standard" for detecting ureteral patency during gynecological and urological surgery is use of a blue dye, typically indigo carmine. However, due to drug shortages in the supply of indigo carmine to the US, some hospitals have utilized inferior alternative agents such as methylene blue, preoperative oral phenazo-pyridine and sterile water or a 10% dextrose solution (Barbieri 2014). The advantages of using indigo carmine compared to these other products is its excellent safety profile and the fact that it is not absorbed by cells and is readily excreted giving surgeons a rapid indication of ureteral patency.

2.2. Background

Indigo carmine (IC) has a long history of use in intraoperative cystoscopy as the standard of care following surgeries with high risk of injury to the urinary tract such as in hysterectomies, pelvic organ prolapses, and anti-incontinence operations (Grimes 2017). In the US, approximately 600,000 hysterectomies, 226,000 prolapse repairs, and 135,000 stress incontinence operations are performed annually (Vakili 2005; Gilmour 2006). There have been very few adverse events reported in the literature with the use of IC use across applications.

Cystoscopy is a procedure that allows for visual examination of the lower urinary tract in both males and females. Under direct visualization, the cystoscope is inserted into the urethra and advanced up to the bladder. A rigid or flexible cystoscope allows complete visual inspection of the urethra, bladder and ureteral orifices. Intraoperative injuries to the bladder and urethra can be seen, which then enables immediate repair. Ligation of the ureter manifests as failure of urine to be propelled into the bladder. Failure to identify a "jet" of urine emanating from the ureteral orifice intraoperatively suggests a ureteral injury.

Identification of ureteral patency is done by visualizing ureteral ejection of blue dye after the intravenous injection of 5 mL of indigo carmine. Many clinicians also administer 5 mg of furosemide intravenously to hasten the excretion of the indigo carmine. If the ureter is patent, ejection of indigo carmine usually occurs 5 to 10 minutes after the intravenous infusion. Failure to see the dye within 20 to 30 minutes mandates further investigation by the surgeon. An intraoperative intravenous pyelogram or retrograde ureteropyelogram, and/or ureteral catheter placement is usually performed to verify ureteral integrity in these cases. Visualization of urinary jets may be seen without prior indigo carmine injection and indicates ureteral integrity. In many cases, the visualization of urinary jets is equivocal when indigo carmine or other agents to enhance detection of urinary jets is not administered

Since indigo carmine is not metabolized, following IV administration it is quickly excreted by the kidneys. This allows for the urine to be sufficiently colored blue within approximately 10 minutes (AAGL 2012).

3. OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

To determine whether the use of Indigo Carmine 0.8% Injection, USP solution for injection (IC) provides a visualization advantage compared to saline when used as an aid in the determination of ureteral patency. Visualization will be measured by a 3-point Ureteral Orifice Visualization Scale (UOVS):

1 = non-visualization

2 = inadequate/equivocal visualization

3 = adequate/unequivocal visualization of the urine jet stream after administration of test agent

3.1.2. Secondary Objectives

1. To evaluate the safety profile of IC when used as an aid in the determination of ureteral patency.
2. To describe the time to visualization (TTV) of ureteral jets during urological and gynecological surgical procedures.
3. To determine physician's overall satisfaction with the IC treatment by assessing the proportion of surgeons who agree with the statement "Compared to the saline treatment, my ability to assess ureteral patency was improved after the addition of IC" using the 5-point Physician Satisfaction Agreement Scale (PSAS):

   1 = Strongly Agree

   2 = Agree

   3 = Neither Agree nor Disagree

   4 = Disagree

   5 = Strongly Disagree

   A physician evaluation is considered satisfactory if the rating is either a 1 (strongly agree) or 2 (agree).
4. To determine the IC pharmacokinetic profile from approximately 16 subjects from 2 investigational sites.
5. An exploratory comparison will be performed to assess the difference between the IC high dose vs IC low dose. Surgeons will be blinded to the dose of IC.

3.2. Endpoints

3.2.1. Efficacy Endpoints

The primary efficacy endpoint is the surgeon's assessment of ureteral patency by UOVS:

1 = Not visualized – I cannot see the ureteral jet flow

2 = Inadequately visualized or equivocal – I am less than completely confident that the ureter is patent

3 = Adequately visualized or unequivocal – I am completely confident that the ureter is patent

The secondary efficacy endpoints include:

1. Time (minutes) to visualization (TTV) of the ureteral jets after each study drug administration
2. Proportion of physicians who agree that compared to saline, IC treatment improves visualization as an aid for the assessment of ureteral patency

Other efficacy endpoints will include:

1. UOVS scores assessed by the blinded central reviewer
2. Concordance of the UOVS scores between the surgeons' assessments and the blinded central reviewer assessments.

3.2.2. Safety Endpoints

The safety endpoints will include:

1. Treatment emergent adverse events
2. Proportion of subjects with clinically important changes in clinical safety laboratory tests after treatment
3. Proportion of subjects with clinically important changes in vital signs after treatment
4. Proportion of subjects with clinically important changes in ECG after treatment

4. STUDY DESIGN

4.1. Overall Design

This is a phase 3, prospective, multicenter, parallel-group study to evaluate the safety, efficacy, pharmacokinetics and physician satisfaction of Indigo Carmine injection 0.8% solution when used as an aid in the determination of ureteral patency.

The study is unblinded for the primary comparison of saline vs IC due to the inherent properties of IC; each subject serves as his/her own control. However, the surgeon is blinded to the IC dose received for assessing any effect on the determination of the ureteral patency. The subject is blinded to the randomized dose of IC treatment. The central rater is blinded to the treatment and the IC dose when reviewing and rating the video. The preparer and administrator of IC will be unblinded.

The study will be comprised of 3 periods:

- **Screening Period (up-to 30 days prior to surgery)**
  - To coincide with the pre-operative testing period
- **Randomization / Dosing (Day 1)**
  - Single doses of saline and IC will be administered on the day of dosing (i.e., the day of procedure)
- **30-day Safety Follow-up Period**
  - Safety follow-up procedures to coincide with postoperative follow-up

4.2. Scientific Rationale for Study Design

A recent review of the literature found that there were no prospective studies that directly demonstrated that IC produces an additive advantage as a visualization aid in determining ureteral patency in urological and gynecologic surgeries. This clinical study will demonstrate that the addition of IC is additive in the identification of ureteral patency. A comparison of the use of the dye to an injection of 0.9% saline would allow physicians to directly compare the procedure without dye to the same procedure with the use of dye.

Additionally, timing of the visualization of the dye is also broadly described in the literature. This study is designed to determine the mean time needed for the dye to be identified by the physician during a procedure. This is important as excretion of the dye is thought to be concentration dependent, making the timing of the visualization of the dye in the bladder related to the dose administered (Oravisto, 1957).

4.3. Justification for Dose

The 5 mL dose is the IV dose most frequently mentioned in the literature (AAGL, 2012; Gill, 2001; Harris, 1997; Jabs, 2001; Jelovsek, 2007; Lee, 1996; O'Brien, 1990; Pettit, 1994; Ribiero, 1999; Speights, 2000) and is the recommended dose in the label of the marketed unapproved US product and the approved non-US product (American Regent Package insert, 2017; Serb SmPC, 2015).

[redacted]

4.4. End of Study Definition

A subject is considered to have completed the study if he/she has completed all phases of the study including the follow-up safety procedures and 30-day safety check. The end of study participation date for a subject is the date of the final safety follow-up visit.

The end of the study date for the study is defined as the date of last scheduled procedure shown in the Schedule of Assessments from the last subject in the trial.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Subjects are eligible to be included in the study only if **all** the following criteria apply:

I01. Subjects between ≥ 18 and ≤ 85 years old

I02. Subjects who signed written, IRB approved, informed consent form

I03. Subjects scheduled for urological or gynecological surgical procedures in which the patency of the ureter must be assessed by the surgeon during the procedure

5.2. Exclusion Criteria

Subjects are excluded from the study if **any** of the following criteria apply:

E01. Subjects with stage 4 or 5 Chronic Kidney Failure as evidenced by a GFR ≤30 mL/min/1.73m^2 (using the MDRD) or need for dialysis in the near future or having only 1 kidney

E02. Subjects with known severe hypersensitivity reactions to IC or other dyes, including contrast dyes

E03. Known history of drug or alcohol abuse within 6 months prior to the time of screening visit

E04. Subjects, as assessed by the Investigator, with conditions/concomitant diseases precluding their safe participation in this study (e.g. major systemic diseases)

E05. Unable to meet specific protocol requirements (e.g., scheduled visits) or subject is uncooperative or has a condition that could lead to non-compliance with the study procedures

E06. Subject is the Investigator or any Sub-Investigator, research assistant, pharmacist, study coordinator, other staff or relative thereof directly involved in the conduct of the protocol

E07. Subjects with life expectancy ≤ 6 months

E08. Requirement for concomitant treatment that could bias primary evaluation.

E09. Subjects who are pregnant or breast-feeding

5.3. Screen Failures

"Enrolled" means a subject's, or their legally acceptable representative's, agreement to participate in a clinical study following completion of the informed consent process and the subject has been randomized to receive study drug. Potential subjects who are screened for the purpose of determining eligibility for the study, but fail to meet all inclusion/exclusion criteria to participate in the study, or who are randomized but did not have a surgical procedure to assess ureteral patency are considered screen failures, unless otherwise specified by the protocol.

Screen failures include subjects who consent to participate in the clinical study but are not subsequently dosed with IC. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details and eligibility criteria. Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened. Subjects may not be enrolled more than once nor receive study drug more than once.

6. STUDY DRUG

Study drug is defined as any investigational drug(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study subject according to the study protocol.

For this study, it is the administration of 5 mL of 0.9% saline IV followed by the blinded dose of 0.8% IC of either 2.5 mL or 5 mL.

6.1. Study Drug(s) Administered

ARM Name	Control (open label)	Low Dose (blinded)	High Dose (blinded)
Drug Name	0.9% Saline	Indigo Carmine	Indigo Carmine
Type	Drug	Drug	Drug
Dose Formulation	Pre-filled Syringe	Ampule	Ampule
Unit Dose Strength(s)	0.9% saline for injection	40 mg/ 5mL	40 mg/5mL
Dosage Level(s)	5 mL	2.5 mL	5 mL
Route of Administration	IV injection over 1 minute	IV injection over 1 minute	IV injection over 1 minute
Use	placebo-comparator	experimental	experimental
IMP and NIMP	NIMP	IMP	IMP
Sourcing	Provided centrally by the Sponsor	Provided centrally by the Sponsor	Provided centrally by the Sponsor
Packaging and Labeling	N/A	Indigo Carmine 40 mg/5 mL solution for injection is supplied in 5 mL ampules	Indigo Carmine 40 mg/5 mL solution for injection is supplied in 5 mL ampules.

6.2. Preparation/Handling/Storage/Accountability

1. Both 0.9% Saline and IC are to be administered as IV injections. The IC is not to be diluted. The start and stop time for each injection is to be captured down to the second.
2. IC is to be stored at room temperature.
3. IC is packaged in 5 mL single dose ampules in type 1 brown glass. Each subject will be assigned 1 ampule.
4. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study drug received and any discrepancies are reported and resolved before use of the study drug.
5. Only subjects enrolled in the study may receive study drug and only authorized site staff may supply or administer study drug. All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.
6. The investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).
7. Further guidance and information for the final disposition of unused study drugs are provided in the Study Reference Manual.

6.3. Measures to Minimize Bias: Randomization and Blinding

Randomization	All subjects will be centrally assigned to randomized study drug using the randomization feature of the electronic data capture (EDC) system. Before the study is initiated, each site will be provided access and training on the enrollment and randomization procedures. **Study drug will be dispensed during the procedure visit. One vial should be used per subject.**
Blinded IC dose using an unblinded administrator	Subjects will be randomly assigned in a [1:1] ratio to the IC dose. Investigators will remain blinded to each subject's assigned IC dose throughout the course of the study. In order to maintain this blind, an unblinded administrator will draw up and administer the IC. Only the dose of IC is blinded as all subjects receive both 0.9% saline and IC. An unblinded witness will confirm the dose administered. **There will be no dose unblinding permitted at the site level as all subjects will receive both 0.9% saline and IC.** In the event of a Quality Assurance audit, the auditor(s) will be allowed access to unblinded study drug records at the site(s) to verify that randomization/dispensing has been done accurately.

6.4. Study Drug Compliance

The study drug will be administered during the surgical procedure; therefore, the subjects will receive study drug directly from the unblinded administrator, under medical supervision. The date and time of each dose (a dose of saline followed by a dose of IC treatment) administered in the clinic will be recorded in the source documents and recorded in the CRF. The randomized dose of IC treatment will be provided by the EDC system to the designated unblinded site staff (i.e. pharmacist, coordinator, anesthesiologist, and or operating room/procedure nurse).

6.5 Concomitant Therapy

Any medication or vaccine (including over the counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Total Daily Dosage information

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy. Prior therapy includes medications a subject received within 30 days of study enrollment.

6.6 Prohibited Concomitant Medications/Foods/Supplements

Prohibited medications, foods, and/or supplements are anything that could discolor urine. The length of time required from last dose to study randomization is at the discretion of the investigator and should be based on the known duration of the effect on urine coloration and documented in the source documentation. Examples of prohibited medications are:

Blue/Green	**Red**	**Orange/Bright Yellow**	**Brown/Black**
methylene blue	warfarin	multivitamins	metronidazole
amitriptyline	rifampin	isoniazid	nitrofurantoin
cimetidine	phenazopyridine	sulfasalazine	chloroquine
indomethacin	ibuprofen	riboflavin (vitamin B2)	primaquine
zaleplon	dantron		furazolidone
methocarbamol	pheninidione		cascara
metoclopramide	nefopam		levadopa
triamterene	clofazimine		phenytoin

7. DISCONTINUATION OF STUDY DRUG AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1. Subject Discontinuation/Withdrawal from the Study

- A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon for this study.

- If a subject is discontinued from the study after the subject is randomized but before receiving the study drug, the subject will be classified as randomized not treated; reason for discontinuation will be collected and no other study procedures will be performed.

- If a subject is discontinued after the subject has received study drug, at the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted, as shown in the Schedule of Assessments (SoA). See SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

- If the subject withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

- If a subject withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

7.2. Lost to Follow up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled follow-up visits and is unable to be contacted by the study site.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local

equivalent methods). These contact attempts should be documented in the subject's medical record.

- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled as part of Section 10.1.9.

8. STUDY ASSESSMENTS AND PROCEDURES

The study is set up in 3 phases. There is a subset of subjects who will participate in the PK portion of the study, which will be described in Section 8.4.

- **Screening** (up to 30 days before the procedure date)
    - Any procedure that is completed as part of the standard of care for the surgery is not required to be repeated during screening for participation in the study.
    - These procedures may pre-date the Informed Consent Process.
    - Once consented, the subject will be entered into the EDC system
- **Randomization/Treatment** (Day 1/Day of Surgery/Treatment)
    - After a subject is confirmed to be eligible, the subject will be randomized via the EDC system. Randomization will be a 1:1 ratio to be assigned to low dose (2.5 mL IC) or high dose (5 mL IC).
    - **Videography** will occur by cystoscopy, robotically, or, if an open surgical procedure, by use of a video camera. Recording will occur from immediately prior to the start of the 0.9% saline injection until 10 minutes after the completion of the IC injection or until adequate visualization/unequivocal has occurred for both ureters, whichever comes first. See Section 8.1.3 for additional details.
    - **Dosing Step by Step**

1. The subject will be injected with 5 mL of 0.9% saline for injection intravenously over 1 minute, noting the time of injection, and the surgeon will assess the patency of the ureters by identifying the efflux of urine from the ureteral orifices for up to 10 minutes post injection.
    a. If both ureteral jets cannot be assessed on camera simultaneously, then the camera will be switched between ureteral orifices every 15-30 seconds for the 10-minute observation period or until adequate/unequivocal visualization is observed. Then the camera may remain focused on the other ureteral orifice until adequate/unequivocal visualization is seen or the completion of the 10-minute observation period has lapsed.
    b. If visualization of the ureter is adequate/unequivocal and the time is less than 10 minutes, then the time of visualization is to be noted for each ureter and the video may be stopped.
    c. The surgeon will document the UOVS score following the use of saline in the assessment of the patency of the ureters.

2. After the normal saline observation period of up to 10 minutes, IC (2.5 or 5 mL, based on randomization) will be administered intravenously over 1 minute, noting the exact time of injection.
3. Again, the surgeon will assess the patency of the ureters by identifying the efflux of blue urine from each ureteral orifice for up to 10 minutes. If /when visualization of each ureter is adequate/unequivocal then the time of visualization is to be noted for each ureter and the procedure may continue.
4. The surgeon will document the UOVS score with the use of IC in the assessment of the patency of the ureters.
5. Pharmacokinetic samples will be collected in a subgroup of subjects at each dose level at pre-determined time points during the procedure after the administration of the IC. Urine and stool will be collected at specified time points in this same subgroup.
6. The surgeon will document his/her overall satisfaction by completing one Physician Satisfaction Assessment for each subject.
7. Subjects will be followed for 30 days (± 2 days) for adverse events.

- Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study drug.
- Adherence to the study design requirements, including those specified in the SoA is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the subject's routine clinical management (e.g., blood counts) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined.

8.1. Efficacy Assessments

8.1.1. Ureter Assessment

Visualization and video recording of the ureteral orifices or the jet flow will be captured through time until flow is observed or for 10 minutes post-completion of each injection. To capture this information the following will be recorded:

- Type of surgical procedure/visualization
  - Cystoscopic
  - Robotic
  - Open
- Time each injection started
- Time each injection completed
- Time of visualization of the ureter jet flow at the ureteral orifice.
- For any procedure requiring that fluid be instilled into the bladder, the amount of and type will be captured.
- Type, amount, and timing of hydration and use of any diuretics during the procedure will be recorded including dosing times.
- Each ureteral orifice will be assessed independently using UOVS after each injection, once the urine jet flow through the ureter or at the ureteral orifice is observed, or for 10 minutes, whichever is shorter, following the completion of the injection.
  - **3-point UOVS**

    1 = Not visualized – I cannot see the ureteral jet flow

    2 = Inadequately visualized or equivocal – I am less than completely confident that the ureter is patent

    3 = Adequately visualized or unequivocal – I am completely confident that the ureter is patent

8.1.2. Physician Satisfaction Agreement Scale (PSAS)

After the completion of the procedure, the surgeon will be asked to rate the experience of using IC for each patient using the PSAS:

“Compared to the use of saline treatment, my ability to assess ureteral patency was improved after the addition of IC”

1 = Strongly Agree

2 = Agree

3 = Neither Agree nor Disagree

4 = Disagree

5 = Strongly disagree

The surgeon is considered satisfied with the IC treatment if his/her rating is either a 1 (Strongly Agree) or a 2 (Agree); otherwise, the surgeon is considered unsatisfied with the IC treatment.

8.1.3. Videography specifics

Video recording will be conducted via cystoscopy, robotically or directly with a video camera for an open procedure such as open radical prostatectomy. Acquisition parameters will be provided to the study sites that detail the video equipment requirements, procedure and views. Sites will also receive training materials to assure alignment and consistency of the video acquisition.

Recording will begin prior to the saline injection and continue uninterrupted until 10 minutes after the IC injection or until both ureters have been adequately visualized, whichever comes first. If both ureteral orifices cannot be recorded simultaneously in the same field of view, the camera will alternate between the ureters approximately every 15-30 seconds until both have been adequately visualized. Once adequate visualization has been confirmed with one ureter, the camera may remain fixed on the other ureter until patency is verified or the 10-minute observation period is complete.

The videos will be submitted electronically to a central imaging group, for anonymization and to confirm adequate image quality and adherence to the acquisition parameters. De-identified videos will be provided to a blinded central reviewer for patency assessment using the same 3-point UOVS used by the surgeon. The TTV and response to the 5-point PSAS will not be included in the central reviewer assessments. Central reviewer data will be compiled and transferred at pre-defined intervals for evaluation of concordance with the surgeon's assessment.

8.2. Safety Assessments

Planned time points for all safety assessments are provided in the SoA.

8.2.1. Physical Examinations

A complete physical examination will include, at a minimum, assessments of the Cardiovascular, Respiratory, Gastrointestinal, Urological/Gynecological (as appropriate) and Neurological systems. Height and weight will also be measured and recorded. These will be repeated at the follow-up visit except for height and weight. Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2. Vital Signs

During screening and follow-up visits body temperature, pulse rate, respiratory rate, and blood pressure will be assessed. Blood pressure and pulse measurements should be preceded by at least

5 minutes of rest for the subject in a quiet setting without distractions (e.g., television, cell phones).

Continuous monitoring, including heart rate and rhythm, will be required during the procedure and in the immediate post-operative period, vital signs will be collected for the study at the following specific time points: immediately prior to each injection and 5 minutes after each injection and thereafter approximately every 15 minutes through 1 hour following the procedure. Any clinically significant changes to vital signs or heart rhythm will be reported as an adverse event.

Clinically significant vital sign changes are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

8.2.3. Electrocardiograms

12-lead ECG will be obtained using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals as part of the screening and follow-up visit. Initial ECGs will be assessed by an investigator, as normal or abnormal finding. Abnormal findings will be rated as clinically significant (CS) or not clinically significant (NCS). Follow-up ECGs will be compared to the original ECG for changes and if the changes are CS or NCS.

Any clinically significant rhythm changes during surgery will require ECG follow-up or rhythm strip for self-limiting episodes during surgery. Clinically significant changes in ECG or heart rhythm will be captured as an adverse event.

8.2.4. Clinical Safety Laboratory Assessments

Laboratory assessments will be done at the local level. The follow-up labs are to be done at the same laboratory as the screening labs.

The following safety labs are to be obtained:

- Hematology: white blood cell count (WBC), red blood cell count (RBC), hemoglobin, hematocrit, platelets, differential blood count (neutrophils, lymphocytes, monocytes, eosinophils, basophils).
- Serum chemistry: total bilirubin, AST, ALT, alkaline phosphatase (ALP), creatinine, blood urea nitrogen, sodium, potassium, chloride, bicarbonate, phosphorus, calcium, glucose, albumin.

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study drug should be repeated until the values

return to normal or baseline or are no longer considered clinically significant by the investigator or medical monitor.

- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in subject management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification), then the results must be recorded in the CRF.

8.2.5 Follow-up for Subjects in whom Ureteral Patency was Not Visualized or Poorly Visualized After IC Treatment

Subjects in whom ureteral patency was not visualized or was poorly visualized after IC treatment will be treated with appropriate follow-up as per the discretion of the surgeon. The information regarding follow-up will be collected in the eCRF and narratives will be provided in the clinical study report (CSR).

8.3. Adverse Events and Serious Adverse Events

AEs will be reported by the subject (or, when appropriate, by a caregiver, surrogate, or the subject's legally authorized representative). See Section 10.2 for Adverse Events: Definitions and Reporting.

Adverse events that occur during the surgical procedure will be reported by the investigator or surgical staff designee.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, or considered related to the study drug or study procedures.

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the start of the saline injection until the follow-up visit.

Medical occurrences that begin before the start of study drug but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.2.3. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after

a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study drug or study participation, the investigator must promptly notify the sponsor.

8.3.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AE and SAE and the procedures for completing and transmitting SAE reports are provided in Section 10.2.

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.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.2). Further information on follow-up procedures is provided in Section 10.2.

8.3.4. Regulatory Reporting Requirements for SAEs

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

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

For all studies, safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it in the Site Regulatory binder and will notify the IRB, if appropriate according to local requirements.

8.3.5. Pregnancy

Details of all pregnancies in female subjects or female partners of male subjects will be collected after the start of study drug and until study end visit/follow-up telephone call.

If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy.

Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.4. Pharmacokinetics

PK samples of plasma, urine, and stool will be collected from approximately 16 subjects at 2 participating sites who have agreed to participate and have been trained in the specific requirements for the study.

8.4.1. Plasma samples

- Approximately 2 mL of plasma (4 ml of whole blood) will be collected for each measurement of plasma concentrations of indigo carmine prior to and post injection at 2, 5, 7, 10, 15, 20, 30, and 40 mins, then at 1, 2, 3, and 4 hours
- Samples will be used to evaluate the PK of IC and its breakdown products. Each plasma sample will be divided into 2 aliquots (1 each for PK and a back-up). Samples collected for analyses of IC (plasma) concentration may also be used to evaluate safety or efficacy aspects related to concerns arising during or after the study.

8.4.2 Urine samples

- A voided urine sample will be collected within 1 hour prior to surgery.
- Post IC urine collection will include all urine voided/drained (if using catheter):
  - 0-2 hours (this includes all urine drained during surgery)
  - 2-6 hours
  - 6-12 hours
- Samples will be used to evaluate the PK of IC and its breakdown products.

8.4.3 Stool Samples

- During the evening prior to surgery, the subject will undergo a bowel prep.
- The first post-op stool will be collected and sent for analysis for IC and/or its breakdown products.

Individual PK results will not be reported to investigative sites.

The pharmacokinetic (PK) analysis will include at least the following parameters:

1. $AUC_{0\text{-}t}$, , $AUC_{0\text{-}\infty}$
2. AUC_{extr}, $AUC_{\%extr}$
3. C_{max}, C_{last}, T_{max}, and T_{last}
4. λ_z: the apparent plasma terminal phase rate constant.

5. $t_{½Z}$: the terminal half-life, where possible, calculated as $0.693/ \lambda_{z.}$
6. Total excretion in urine and in stool

PK samples will be prepared to analyze for plasma concentration of IC and any major metabolites. Non-compartmental Analysis model will be used to calculate PK parameters using the observed concentrations and actual sampling times. All bioanalytical analyses will be completed to GLP standards using validated assay methods. The Bioanalytical Report and Pharmacokinetic Analysis Report will be provided as appendices to the final study report.

9. STATISTICAL CONSIDERATIONS

9.1. Statistical Hypotheses

It is expected that the IC treatment will improve the ureter visualization with effect size greater or equal to 0.10 comparing to the normal saline using the 3-point UOVS measurement.

9.2. Sample Size Determination

The following empirical distribution table (Table 1) is created to illustrate this effect size; sample size was determined using the two-group chi-square test comparing the difference in proportions in 3 categories (Table 2).

9.2.1. Table 1 Empirical Distribution of Proportion of Subjects in Each UOVS Categories and Effect Size between the IC Dose Group vs Saline

	% subjects by Ureteral Orifice Visualization Scale [1]			
	1	2	3	Effect Size Δ^2
Saline (π_1)	0.30	0.30	0.40	
IC Low Dose (π_2)	0.10	0.20	0.70	0.1009 [2]
IC High Dose (π_2)	0.05	0.10	0.85	0.2203 [3]

[1] Score 1 = Not visualized; 2 = Inadequately visualized or equivocal; 3 = Adequately visualized or unequivocal.
[2] Effect size between IC Low dose and Saline
[3] Effect size between IC High dose and Saline
Effect size, $\Delta^2 = \Sigma(\pi_{2j}-\pi_{1j})^2/[2(\pi_{2j}+\pi_{1j})]$

9.2.2. Table 2 Number of Subjects Per Group Using Two-group Chi-square Test Comparing Proportions in 3 Categories [1, 2]

Scenario	**1**	**2**
Test significance level, α	0.0500	0.0500
Number of categories, C	3	3
Effect size, $\Delta^2 = \Sigma(\pi_{2j}-\pi_{1j})^2/[2(\pi_{2j}+\pi_{1j})]$	0.2203	0.1009
Power (%)	80.00	80.00
n per group	22	48

[1] nQuery Advisor (version 7)
[2] Sample size estimates did not account for the repeated measures from each subject. Hence, the actual study power could be greater than 80% since each subject is expected to have 2 measurements under the same treatment and each subject is also serving as his/her own control.

The study enrollment target is to have a total of 96 subjects who are randomized and treated with study drug: 48 subjects will be randomly assigned to 2.5 mL IC and 48 subjects assigned to 5mL

IC. This sample size calculation was based on two-group Chi-square test comparing proportions in 3 categories at the 0.05 significance level. The sample size does not account for dropouts, protocol deviations, withdrawal of consent, etc. Up to an additional 20% (20) subjects may be enrolled to account for protocol deviations, withdrawal of consent, etc.

A subgroup of subjects at 2 sites (approximate N=16, 8 from each IC dose level) will be evaluated for pharmacokinetics/metabolites.

Note: "Enrolled" means a subject's, or their legally acceptable representative's, agreement to participate in a clinical study following completion of the informed consent process and the subject has been randomized to receive study drug. Potential subjects who are screened for the purpose of determining eligibility for the study, but failed to meet all inclusion/exclusion criteria to participate in the study, or who are randomized, but did not have a surgical procedure to assess ureteral patency are considered screen failures, unless otherwise specified by the protocol.

9.3. Populations for Analyses

The following populations are defined:

Population	Description
Enrolled	All subjects who sign the ICF
Intend-to-Treat (ITT) Analysis Sets	All randomized subjects will be included in the ITT Analysis set. The ITT set will include subjects randomized but not treated.
Efficacy Analysis Set	The efficacy analysis set includes all randomized subjects who have a surgical procedure to assess ureteral patency and who have received study drug (saline and IC dose). This will be the primary population for efficacy assessments.
Per Protocol Analysis Set	All subjects from the Efficacy Analysis Set who did not have any major protocol deviation that may confound the interpretation of the efficacy assessment. This Analysis Set will serve as a confirmation analysis set for efficacy analysis.
Safety Analysis Set	All randomized subjects who received at least 1 dose of study drug. Subjects will be analyzed according to the drug they actually received should the randomized IC dose be different from the actual received IC dose.
Pharmacokinetic Analysis Set	All subjects who received at least one dose of study drug, and for whom at least one PK parameter value is reported.

9.4. Statistical Analyses

The statistical analysis plan will be finalized prior to First Patient First Visit (FPFV) and it will include a more technical and detailed description of the statistical analyses. This section is a summary of the key elements of the planned statistical analyses.

9.4.1. Type 1 Error Control for the Primary Efficacy Endpoint

There are two null hypotheses for the primary efficacy endpoint UOVS.

1. there is no difference between the IC high dose and the normal saline
2. there is no difference between the IC low dose and the normal saline

Multiplicity due to the two null hypotheses will be controlled by Hochberg method. That is, to control family-wide Type I error to be less than or equal to 0.05, when both nominal p-values are less or equal to 0.05, both null hypotheses will be rejected and one will conclude that both IC dose groups are statistically different from the saline group in the examined parameter. When one of the two nominal p-values is greater than 0.05 but the second nominal p-value is less or equal to 0.025, the null hypothesis associated with the first nominal p-value is accepted and the null hypothesis associated with the second nominal p-value will be rejected. If the second nominal p-value is greater 0.025, the null hypothesis associated with the second nominal p-value is also accepted. The Overall Type I error control will apply to the primary endpoint UOVS only.

9.4.2. Treatment Effect Based on the Operating Surgeon's UOVS Scores

In this study each subject will serve as his/her own control and each subject will have repeated measures from the same treatment (1 from the left and 1 from the right ureter); therefore, each subject is expecting to contribute 4 observations for UOVS endpoint. The UOVS score provided by the surgeon will be the primary efficacy endpoint whereas the score provided by the blinded central reviewer will serve only as an estimate of concordance.

The treatment effect on the UOVS score will be evaluated using a Generalized Estimating Equation (GEE) for Repeated Measures to control for the intra-subject correlation. A full model will provide the chi-square tests for the effects from drug (IC high dose, IC low dose, Saline), site (left side ureter vs right side ureter), and drug*site interaction on the response based on multi-normal distribution for ordinal response. The model estimates the cumulative logits of better outcome (score=3) to the poor outcome (score=1). Since it is expected that the interaction effect will not statistically significant ($p > 0.10$), a reduced model will also be used in which the interaction effect will be dropped. The goodness of fit of the models will be evaluated. A REPEATED statement will be used to control for the repeated measures within a subject. The difference between the IC high dose vs saline, the IC low dose vs saline will be presented in the summary along with the estimates and 95% confidence intervals of the estimates. Effect of a source (drug, site, drug*site interaction in full model) will be determined using the Score Statistics for Type 3 GEE Analysis.

An exploratory comparison will be performed to assess the difference between IC high dose vs IC low dose and will be presented in the summary along with the estimates and 95% confidence intervals of the estimates. Effect of a source (drug, site, drug*site interaction in full model) will be determined using the Score Statistics for Type 3 GEE Analysis.

9.4.3. Treatment Effect on UOVS Based on Central Rater's Assessment

The UOVS score provided by the blinded central reviewer will be analyzed using the same GEE approach. One way to assess the consistency between the surgeon and central rater is to evaluate the effect of raters (surgeon or blinded central rater) on the response using covariate approach. In this approach a new covariate indicating evaluator (surgeon vs blinded central reviewer) will be added to the GEE model.

Another way to assess the consistency between the raters is to derive the difference in UOVS score between the surgeon and the central rater. This difference will have a value of (-2, -1, 0, +1, +2) for each of the 4 readings per subject, with 0 indicating a perfected agreement. The association of the difference in score with treatment and site will also be evaluated using the GEE model.

9.4.4. Treatment Effect on Time to Visualization

Time (minutes) to visualization (TTV) will be evaluated using the Kaplan-Meier Survival Analysis approach; time to event percent-tiles (25%, 50%, and 75%) and 95% confidence intervals will be estimated along with the log-rank test for equal survival between the groups (IC vs Saline); Cox Proportional hazards model approach may be used to estimate hazards ratios if data warrant. In this analysis the potential intra-subject correction will not be controlled. That is, each time to event observation will be treated as an independent observation. Potential covariates (such as site will be evaluated) if data warrants.

Similarly, the difference in time to visualization between the IC high dose and the IC low dose will be evaluated using the log-rank test and Cox PH model.

Treated subjects with missing data will be censored at 10 minutes.

9.4.5. Assessment of PSAS with IC Treatment

Ratings in the PSAS with the IC treatment will be tabulated by treatment (IC low dose, IC high dose, and study overall); 2-sided 95% confidence intervals on proportion of physicians who are satisfied (with ratings 1 or 2) will be derived using normal approximation. A binominal 1-way proportion approach will be used to examine that at least 50% of the physicians are satisfied with the IC treatment as an aid for the assessment of ureter patency comparing to the saline treatment.

Difference in PSAS with the use of IC high dose vs the IC low dose will be compared via a CMH test for mean row score.

9.4.6. Assessment of Covariates

The effect of procedure on the response will be assessed via covariate analysis. An appropriate model will be chosen for specific parameter.

9.4.7. Safety Analysis

Adverse events (AE) occurring on Day 1 post injection of the first dose of study drug and up to 32 days after the procedure are considered treatment emergent. The Medical Dictionary for Regulatory Activities (MEDRA) (Version 21 or higher) will be used to classify all AEs with respect to system organ class and preferred term. AEs will be summarized by treatment groups. Proportion of subjects with clinically important changes in clinical laboratory tests, vital signs, and 12-lead ECG before (Screening visit, baseline) and after (follow-up visit, post baseline) procedure will be tabulated by treatment and study overall. AEs occurring during continuous respiratory and cardiac monitoring during surgery (from the time of the first dose of study drug to the discontinuation of monitoring will be recorded. No formal inferential statistics will be performed for the safety parameters.

9.4.8. PK Analysis

PK parameters will be estimated using Non-compartmental analysis model based on the observed concentration; descriptive statistics for exposure, excretion in urine, and in stool by IC dose (high dose vs low dose) will be provided without inferential statistics. If data warrants difference in exposure and excretion between the IC low dose and high dose will be evaluated.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB by the investigator and reviewed and approved by the IRB before the study is initiated.

Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB
- Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2. Financial Disclosure

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

10.1.3. Informed Consent Process

The investigator or his/her representative will explain the nature of the study to the subject or his/her legally authorized representative and answer all questions regarding the study.

Subjects must be informed that their participation is voluntary. Subjects or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB or study center.

The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

A copy of the ICF(s) must be provided to the subject or the subject's legally authorized representative.

10.1.4. Institutional Review Board/Independent Ethics Committee (IRB)

As required by local regulation, the Investigator or the Sponsor must submit this clinical trial protocol to the appropriate IRB and is required to forward to the respective other party a copy of the written and dated approval/favorable opinion signed by the Chairman with IRB composition.

The clinical trial (study number, clinical trial protocol title and version number), the documents reviewed (clinical trial protocol, informed consent form, Investigator's Brochure, Investigator's curriculum vitae [CV], etc.) and the date of the review should be clearly stated on the written (IRB) approval/favorable opinion.

IMP will not be released at the study site and the Investigator will not start the study before the written and dated approval/favorable opinion is received by the Investigator and the Sponsor.

During the clinical trial, any amendment or modification to the clinical trial protocol should be submitted to the IRB before implementation, unless the change is necessary to eliminate an immediate hazard to the patients, in which case the IRB should be informed as soon as possible. It should also be informed of any event likely to affect the safety of patients or the continued conduct of the clinical trial, in particular any change in safety. All updates to the Investigator's Brochure will be sent to the IRB.

A progress report is sent to the IRB at least annually and a summary of the clinical trial's outcome at the end of the clinical trial

10.1.5. Data Protection

Subjects will be assigned a unique identifier by the sponsor. Any subject records or datasets that are transferred to the sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject who will be required to give consent for their data to be used as described in the informed consent

The Investigator should keep a separate log (Patient Master List) of patient's codes (assigned patient number), names, addresses, telephone numbers and hospital numbers (if applicable). Documents not for submission should be maintained by the Investigator in strict confidence.

The subject must be informed that his/her medical records may be examined by Study Monitors, Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

Subject race or ethnicity will be collected in this study because these data are required by several regulatory authorities (eg, on afro American population for FDA)

10.1.6. Dissemination of Clinical Study Data

The Sponsor will be responsible for preparing a CSR and to provide a summary of study results to the Investigator.

The Investigator undertakes not to make any publication or release pertaining to the study and/or results of the study prior to the Sponsor's written consent, being understood that the Sponsor will not unreasonably withhold its approval.

As the study is being conducted at multiple sites, the Sponsor agrees that, consistent with scientific standards, a primary presentation or publication of the study results based on global study outcomes shall be sought. However, if no multicenter publication is submitted, underway or planned within twelve (12) months of the completion of this study at all sites, the Investigator shall have the right to publish or present independently the results of this study in agreement with other Investigators and stakeholders. The Investigator shall provide the Sponsor with a copy of any such presentation or publication for review and comment at least 30 days in advance of any presentation or submission for publication. In addition, if requested by the Sponsor, any presentation or submission for publication shall be delayed for a limited time, not to exceed 90 days, to allow for filing of a patent application or such other justified measures as the Sponsor deems appropriate to establish and preserve its proprietary rights.

The Investigator shall not use the name(s) of the Sponsor and/or its employees in advertising or promotional material or publication without the prior written consent of the Sponsor. The Sponsor shall not use the name(s) of the Investigator and/or the collaborators in advertising or promotional material or publication without having received his/her and/or their prior written consent(s).

The Sponsor has the right at any time to publish the results of the study.

10.1.7. Data Quality Assurance

All subject data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

The investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or onsite monitoring) are provided in the Monitoring Plan.

The sponsor or designee is responsible for the data management of this study including quality checking of the data.

The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8. Source Documents

Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator’s site.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

Definition of what constitutes source data includes: Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial.

10.1.9. Study and Site Start and Closure

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

The first act of recruitment is the GO-LIVE date defined as the first site having their initiation visit and all systems available for data entry will be the study start date.

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

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

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

- Failure of the investigator to comply with the protocol, the requirements of the IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the investigator
- Discontinuation of further study drug development

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

10.1.10. Publication Policy

Any manuscript, abstract or other publication or presentation of results or information arising in connection with the study (including any ancillary studies involving trial patients) must be prepared in conjunction with the sponsor and must be submitted to the sponsor for review and comment at least 8 weeks prior to submission for publication or presentation.

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

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

10.2.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study drug, whether or not considered related to the study drug. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study drug.

Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study drug administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study drug or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

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

10.2.2. Definition of SAE

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

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization • In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. • Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

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

10.2.3. Recording and Follow-Up of AE and/or SAE

AE and SAE Recording

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

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

ADL: Activities of daily living

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

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

An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

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

Unrelated	• The AE must clearly be caused by the subject's clinical state, or the study procedure/conditions; • Definitely not related to IC; • Temporal sequence of an AE onset relative to administration of IC is not reasonable; • Another obvious cause of the AE.
Unlikely	• Time sequence is unreasonable; • There is another more likely cause for the AE.
Possibly	• Corresponds to what is known about IC; • Time sequence is reasonable; • Could have been due to another equally, likely cause.
Probably	• Is a known effect of IC • Time sequence from taking IC is reasonable; • Cannot be reasonably explained by the known characteristics of the subject's clinical state.

Follow-up of AEs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by PV to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide PV with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any new or updated SAE data to PV within 24 hours of receipt of the information via the SAE Reporting Form.

SAE Reporting to PV via Paper SAE Reporting Form

- The primary mechanism for reporting an SAE to PV will be via email of a scanned copy of the completed SAE reporting form to PV. If email is not available, facsimile transmission is acceptable to transmit this information to PV.
- In rare circumstances and in the absence of facsimile/scanning equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.

- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in Study Contact List.

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Appendix 1: Administrative Change #1 Summary of Changes

Version 1.1 Administrative Change 1

1. Addition of reference cited in document to the bibliography
2. Update of Sponsor contact information
3. Correction of mis-numbered section (6.6)
4. Clarification of timing for prohibited Medications/Foods/Supplements
5. Clarification of packaging for Indigo Carmine in section 6.2

Appendix 2: Amendment # 1 Summary of Changes

Page	Section #	Change	Reason / Rationale
Title page, page 3 and Page 10	Study Title	An Open-Label, Randomized, Multicenter Study to Evaluate the Safety, Efficacy, Pharmacokinetics and Physician Satisfaction of two different doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium ~~Indigo Carmine~~ Injection 0.8% solution when used as an aid in the determination of ureteral patency.	To include the IUPAC name in the title
Title page	Investigational Product	3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium (Indigo Carmine 0.8% Injection, USP)	To correlate the IUPAC name with the USP name of the investigational product
11	1.1 Secondary Objectives (5)	5. An exploratory comparison will be performed to assess ~~done on~~ the difference between the IC high dose vs IC low dose. Surgeons will be blinded to the dose of IC.	Clarity and readability
11	1.1 Methodology	This is an open-label, randomized, multicenter study to evaluate the efficacy, safety, and pharmacokinetics of two doses (2.5 mL and 5.0 mL) of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium commonly referred to as Indigo Carmine (IC) 0.8% Injection, USP solution for injection	To correlate the IUPAC name with the USP name of the investigational product
11	1.1 Methodology	Eligible subjects will be randomized in a 1:1 ratio to receive a dose of either IC high dose (5 mL) or IC low dose (2.5 mL).	To specify the randomization ration

Page	Section #	Change	Reason / Rationale
12	1.1 Methodology	In a subset of subjects from 2 sites (approximately ~~about~~ 16 subjects), subjects will be consented to participate in the pharmacokinetic (PK) portion of the study	Readability
12	1.1 Study Drug	Section Title changed: Study ~~Medication~~ Drug	Consistency throughout protocol
12	1.1 Number of Subjects	Up to 116 subjects will be enrolled from approximately 10 sites, including approximately 16 subjects to participate in PK/metabolite analysis at 2 sites	Clarification and consistency
12	1.1 Sample Size	The study plans to randomize ~~A total of~~ 96 subjects ~~will be enrolled in the study~~; 48 subjects ~~randomly assigned~~ to 2.5 mL IC and 48 subjects ~~assigned~~ to 5 mL IC. This sample size calculation was determined based on two-group Chi-square test comparing proportions in 3 categories at 0.05 significance level. The sample size does not account for dropouts, protocol deviations, withdrawal of consent, etc. Up to an additional 20% (about 20) subjects may be enrolled to account for protocol deviations, withdrawal of consent, etc., if necessary. A subgroup of subjects at 2 sites (~~total~~ Approximate N=16, at least 8 from each IC dose level) will be evaluated for pharmacokinetics/metabolites	Clarification and consistency
14	1.1 Safety Endpoints (3)	3. Changes in clinically significant abnormal 12-ECG	Clarity and readability

Page	Section #	Change	Reason / Rationale
14	1.1 PK Parameters (1)	1. AUC0-t, ~~AUC0-t(90%subj),~~ AUC0-∞	Consistency with statistical analysis plan
14	PK Analysis	Non-compartmental analysis model will be used to calculate PK parameters using the observed concentrations and actual sampling times.	Clarity and accuracy
15	1.1 Procedure Summary	9. Subjects participating in the PK arm, at pre-determined time points will have blood drawn. All urine will be collected in separate containers for designated periods. Stool will be collected for ~~all~~ the first bowel movement~~s within the first 24 hours~~ post-surgery in this same subgroup.	Correction and consistency throughout protocol
18	1.3 SOA	*Deleted weight assessment during the Follow-up visit*	Post-surgery weight not required for study purposes
18	1.3 SOA Footnote # 7	For a ~~total of~~ approximately 16 Subjects at 2 planned sites blood plasma collection will occur prior to and post IC injection at 2, 5, 7, 10, 15, 20, 30, and 40 mins, then at 1, 2, 3, 4 hours after IC administration.	Clarification
18	1.3 SOA	*"IVRS/IWRS contact" row renamed to "randomization" and "X" removed from all columns except for the "Randomization/Treatment Day 1" column*	An IVRS/IWRS system will not be used for this study. Randomization will be completed via the EDC system
18	1.3 SOA "Day 2 PK" column	*Column deleted*	Based on the PK sampling schedule a separate Day 2 visit is not expected

Page	Section #	Change	Reason / Rationale
21	3.1.2 Secondary objective # 4	4. To determine the IC pharmacokinetic profile from ~~in a subset of subjects~~ approximately 16 subjects from 2 investigational sites.	Clarification
21	3.1.2 Secondary objective # 5	5. An exploratory comparison will be ~~done on~~ performed to assess the difference between the IC high dose vs IC low dose.	Clarity and readability
26	5.3 Screen Failures	Minimal information includes demography, screen failure details and~~,~~ eligibility criteria. ~~and any serious adverse event (SAE).~~	Serious Adverse Events will not be captured for subjects who are not dosed with study drug.
28	6.3 Measures to minimum Bias	**~~IVRS/IWRS~~ Randomization** All subjects will be centrally assigned to randomized study drug using ~~an Interactive Voice/Web Response System (IVRS/IWRS).~~the randomization feature of the electronic data capture (EDC) system. Before the study is initiated, each site will be provided access and training on the enrollment and randomization procedures. **Study drug will be dispensed during the procedure visit. One vial should be used per subject.**	An IVRS/IWRS system will not be used for randomization
29	6.4 Study Drug Compliance	The randomized dose of IC treatment will be provided by ~~IVRS/IWRS~~ the EDC system to the designated unblinded site staff	An IVRS/IWRS system will not be used for randomization
30	6.6	Prohibited medications, foods, and/or supplements ~~would be~~ are anything that could discolor urine.	

Page	Section #	Change	Reason / Rationale
33	8 Screening	o Once consented, the subject will be entered into ~~IVRS/IWRS~~ the EDC system. Randomization will be a 1:1 ratio to be assigned to low dose (2.5 mL IC) or high dose (5 mL IC).	An IVRS/IWRS system will not be used for randomization
33	8 Randomization/ Treatment	o After a subject is confirmed to be eligible, the subject will be randomized via ~~IVRS/IWRS~~ the EDC system.	An IVRS/IWRS system will not be used for randomization
33	8 Dosing step by step: 1	by identifying the efflux of urine from the ureteral orifices for up to 10 ~~minutes for each injection~~ post injection.	Clarity
33	8 Dosing step by step: 1c	c. The surgeon will document the UOVS score following the use of saline in the assessment of the patency of the ureters.	Addition of step for clarity of procedure
33	8 Dosing step by step: 5	Urine and stool will be collected at specified time points in this same subgroup.	Added for completeness
36	8.2.1	These will be repeated at the follow-up visit except for height and weight.	Weight is not required during the follow-up visit for study purposes
37	8.2.3	Initial ECGs will be assessed by an investigator, ~~addition to the measurements,~~ as normal or abnormal finding.	Typographical correction
38	8.2.5 Section Title	Follow-up for Subjects in whom Ureteral Patency was Not Visualized or Poorly Visualized After IC Treatment	Clarity
38	8.25	Subjects in whom ureteral patency was not visualized or was poorly visualized after IC treatment will be treated with appropriate follow-up as per the discretion of the surgeon.	Clarity

Page	Section #	Change	Reason / Rationale
38	8.3.1	All AEs and SAEs will be collected from the start of the saline injection until the follow-up visit.	to specify the timing of AE collection
39	8.3.5	Details of all pregnancies in female subjects or female partners of male subjects	To include pregnancy follow-up for pregnancies that occur during the study in female partners of male subjects
40	8.4	PK samples of plasma, urine, and stool will be collected from approximately 16 subjects	Clarification
40	8.4.1	• Approximately 2 mL of plasma (4 ml of whole blood) will be collected for each measurement of plasma concentrations	Clarity
40	8.4.3	• The first post-op stool will be collected and sent for analysis for IC and/or its breakdown products.~~; stool will be collected for 24 hours~~	Correction and consistency throughout protocol
40	8.4.3	The pharmacokinetic (PK) analysis will include at least the following parameters: 1. AUC0-t, ~~AUC0-t(90%subj),~~ AUC0-∞	Consistency with statistical analysis plan
41	8.4.3	Non-compartmental Analysis model will be used to calculate PK parameters using the observed concentrations and actual sampling times.	Clarity
42	9.2	The following empirical distribution table (Table 1) is created to illustrate this effect size; sample size was ~~is~~ determined using the two-group chi-square test comparing the difference in proportions in 3 categories (Table 2).	Accuracy and readability
43	9.2.2	A subgroup of subjects at 2 sites (~~total~~ approximate N=16, 8 from each IC dose level) will be evaluated for pharmacokinetics/metabolites.	Clarification

Page	Section #	Change	Reason / Rationale
43	9.3 Per Protocol Analysis Set	This Analysis Set will serve as a confirmation analysis set for efficacy analysis. ~~who~~	Correction of typographical error
43	9.3 Safety Analysis Set	Subjects will be analyzed according to the drug they actually received should the randomized IC dose ~~is~~ be different from the actual received IC dose.	Correction of typographical error
45	9.4.2	An exploratory comparison will be performed to assess ~~done on~~ the difference between IC high dose vs IC low dose and will be presented in the summary along with the estimates and 95% confidence intervals of the estimates.	Clarity and readability
45	9.4.3	In this approach a ~~A~~ new covariate indicating evaluator (surgeon vs blinded central reviewer) will be added to the GEE model.	Readability and clarity
45	9.44	Potential covariates (such as ~~randomization strata and~~ site will ~~also~~ be evaluated) if data warrants.	Accuracy as there are no randomization strata planned
46	9.4.6	The effect of procedure on the response ~~(randomization stratum)~~ will be assessed via covariate analysis. An appropriate model will be chosen for specific parameter.	Accuracy as there are no randomization strata planned
46	9.4.8	PK parameters will be estimated using Non-compartmental analysis model based on the observed concentration;	Clarity
56	10.2.3 Follow-up of AEs and SAEs Bullet #4	• The investigator will submit any new or updated SAE data to PV within 24 hours of receipt of the information via the SAE Reporting Form.	

Page	Section #	Change	Reason / Rationale
56	10.2.4 SAE Reporting to PV via an Electronic Data Collection Tool	*Section number and Entire table deleted*	Reporting of SAEs through an electronic data collection tool is not applicable for this study
56	10.2.3 SAE Reporting to PV via Paper SAE Reporting Form	SAE Reporting to PV via Paper SAE Reporting Form ~~CRF~~	SAE Reporting will be via a reporting form not a paper CRF.
56	10.2.3 Bullet 1	• The primary mechanism for reporting an SAE to PV will be via email of a ~~Facsimile transmission or~~ scanned copy of the completed SAE ~~paper CRF~~ reporting form to PV. If email is not available, facsimile transmission is acceptable ~~are the preferred method~~ to transmit this information to PV.	The primary method of SAE Reporting will be via a paper SAE reporting form not a paper CRF.
59	11.	20. Ribeiro S, Reich H, et al. The Value of intra-operative cystoscopy at the time of laparoscopic hysterectomy. Human Reproduction 1999; 14(7): 1727-1729	Reference added
All	N/A	*Corrections of minor typographical and spelling errors that had no effect on meaning were also made throughout the document*	N/A