Provepharm Inc.

STATISTICAL ANALYSIS PLAN

Protocol Number:	**PVP-19IC01**
Protocol 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 0.8% Solution When Used as an Aid in the Determination of Ureteral Patency**
Date of Original Protocol	**01 July 2019**
Administrative Change 1 Date:	**18 October 2019**
Amendment 1	**10 December 2019**
Amendment 2	**02 September 2019**
Date of Original SAP:	**11 September 2019**
SAP Amendment 1 Date:	**02 September 2020**
SAP Amendment 2 Date:	**26 June 2021**

NCT#: 04228445

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LIST OF ABBREVIATIONS AND PHRASES

Abbreviation	Definition
AE	adverse event
AEOSI	Adverse event of special interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
CMH	Cochran-Mantel-Haenszel
CP	Conditional Power
CV	Coefficient of Variation
DMC	Data Monitoring Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
ES	Effect Size
GEE	Generalized Estimating Equation
IC	Indigo Carmine
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IM	Intramuscular
IVRS/IWRS	Interactive Voice Response System/Interactive Web Response System
ITT	Intent to Treat
IV	Intravenous
Kg	Kilogram
m^2	Meters Squared
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent to Treat
mL	Milliliter
OR	Odds Ratio

Abbreviation	Definition
PP	Per Protocol
PH	Proportional Hazards
PK	Pharmacokinetic
PSAS	Physician Satisfaction Agreement Scale
PVP	Provepharm, Inc.
RBC	Red Blood Cell
RGM	Ratio of Geometric Means
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
TEAE	Treatment Emergent Adverse Event
TTV	Time from Drug Administration to Visualization of Blue Color
µg	Microgram
µL	Microliter
UOVS	Ureter Orifice Visualization Scale
VS	Vital Signs
WBC	White Blood Cell count

1. INTRODUCTION AND SCOPE

Protocol PVP-19IC01 is an Open-Label, Phase 3, Randomized, Multicenter Study to Evaluate the Safety, Efficacy, Pharmacokinetics and Physician Satisfaction of two different doses of Indigo Carmine Injection 0.8% solution when used as an aid in the determination of ureteral patency.

This Statistical Analysis Plan (SAP) is intended to provide a more technical and detailed elaboration of the principal statistical features stated in the protocols. The objective of the SAP is to reasonably assure that the statistical methodologies to be used for analysis are complete and accurate.

In the development of this SAP, the following documents were used:

- Protocol PVP-19IC01: Amendment 02, 02 September 2020
- PVP-19IC01 eCRF: Version 31. (draft), 28 August 2020

The principles in the following guidance documents are followed in preparation of this SAP:

- ICH E3 (1995): Structure and Content of Clinical Study Reports
- ICH E6 (1996): Guideline for Good Clinical Practice
- ICH E9 (1998): Statistical Principles for Clinical Trials

In the event that a discrepancy is found between the descriptions in the statistical section of the protocol and this document, the description in this document supersedes the descriptions in the statistical section of the protocol.

2. OVERVIEW OF STUDY OBJECTIVES AND ASSESSMENTS

The primary objective of this study is 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 5-point conspicuity score as follows:

1 = No jet observed

2 = Weak jet, little color contrast

3 = Color contrast or significant jet flow

4 = Strong jet flow with good color contrast

5 = Strong jet flow with striking contrast in color

Secondary objectives are to assess:

1. To evaluate the safety profile of IC when used as an aid in the determination of ureteral patency.
2. 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).
3. To determine proportion of responders. To determine proportion of subjects meeting the responder definition in conspicuity score following IC treatment based on the blinded central reviewer's assessment. A subject is a responder when there is >=1 point difference in the conspicuity score following the IC vs saline treatment (IC – Saline >=1) and the conspicuity score following the IC treatment is (3, 4, or 5). The responder criteria will be assessed separately for each ureter for each subject.

4. To describe the time to visualization (TTV) of blue color in the ureteral jets flow following IC treatment when used as a visualization aid during urological and gynecological surgical procedures
5. To determine the IC pharmacokinetic profile in a subset of subjects from 2 investigational sites.
6. An exploratory comparison will be done on the difference between the IC high dose vs IC low dose. Surgeons will be blinded to the dose of IC.

The study will enroll approximately 116 subjects from approximately 10 – 20 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. Any procedure done for the purpose of the surgery before the date of signing the informed consent may be used as long as it was within 30 days of the scheduled surgery date.

On the day of surgery (Day 1) the subject will be evaluated for eligibility for randomization. Eligible subjects will be randomized to receive a dose of either IC high dose (5 mL) or IC low dose (2.5 mL). Each randomized subject 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.

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 and video recorded for 10 mins. The time period that will be captured on video is from injection to 10 minutes post injection. If both ureters cannot be visualized simultaneously, then alternating 15-30 second images of each ureter or ureteral orifice will be obtained. The process will be repeated in the same patient for the IC dose. Hence, each subject will have 2 videos and each will be about 10 minutes in length.

The videos will be sent to a central imaging group that will pool and blind the videos. Videos will then be assessed by 2 blinded central reviewers for assessment of ureteral patency using the 5-point conspicuity score criteria. The 2 independent central reviewers will provide 2 conspicuity scores (1 for left ureter and 1 for right ureter) for each video. The consistency between the two reviewers will be checked; the two reviewers will be considered consistent if their scores for the corresponding ureter is (+/-) 1 point of a given video. In this case, the average score across the two reviewers will be the final score for efficacy analysis. Otherwise, a third reviewer (a judicator) will step in and will review this video. The scores from this third reviewer will be the final score for this video for efficacy analysis.

After the procedure, the surgeon performing the procedure will provide his/her overall satisfaction 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.

In addition, after the procedure the surgeon will also review the videos of his/her patients and will provide the conspicuity scores for each ureter after each treatment using the same 5-point conspicuity score criteria. The surgeons score will be utilized for conspicuity score concordance analysis.

In a subset of subjects from 2 sites (about 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 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. The first post-op stool will also be collected for analysis in this PK group.

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.

Protocol Schedule of Assessment is included here for the convenience of review.

Table 1: Schedule of Assessments

	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 if Follow-up visit 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], Urine[7], and Stool[8] collection		X		
Bowel Prep[9] (PK group)	X (Day -1)			
Surgery		X		
Surgeon satisfaction with IC treatment		X		
0.9% saline injection		X		
0.8% IC injection		X		
Video filming of ureteral jet flow with saline and with IC		X		
Surgeon conspicuity score of urine Jet flow		X[10]		
Urine Pregnancy Test[11]		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 approximately 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. The first post-op stool will be collected and sent for analysis for IC and/or its breakdown products.
9. Bowel prep will be required preoperatively (Day -1) for Subjects in the PK/Breakdown product collection patients.
10. The Surgeon should review the videos post-surgery to perform their conspicuity scoring of the urine jet flows. Scoring should not be done during surgery
11. Urine Pregnancy Tests will be done on day of surgery on all women of childbearing potential.

2.1. Study Endpoints

2.1.1. Efficacy Endpoints

The primary efficacy endpoint is conspicuity score after each treatment provided by the central readers:

1 = No jet observed

2 = Weak jet, little color contrast

3 = Color contrast or significant jet flow

4 = Strong jet flow with good color contrast

5 = Strong jet flow with striking contrast in color

The secondary efficacy endpoints include:

1. Proportion of physicians who agree that compared to saline, IC treatment improves visualization as an aid for the assessment of ureteral patency
2. To determine proportion of IC treatment responders per the blinded central reviewers' assessment. A subject is a responder to the IC treatment when there is >=1 point difference in the conspicuity scores between the IC and saline treatment (IC – Saline >=1) and the conspicuity score following the IC treatment is (3, 4, or 5). The responder criteria will be assessed separately for each ureter for each subject.
3. Time (minutes) from start of drug administration to visualization (TTV) of the blue color in the ureteral jets flow after IC administration

Other efficacy endpoints will include:

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

2.1.2. Safety Endpoints

The safety endpoints 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

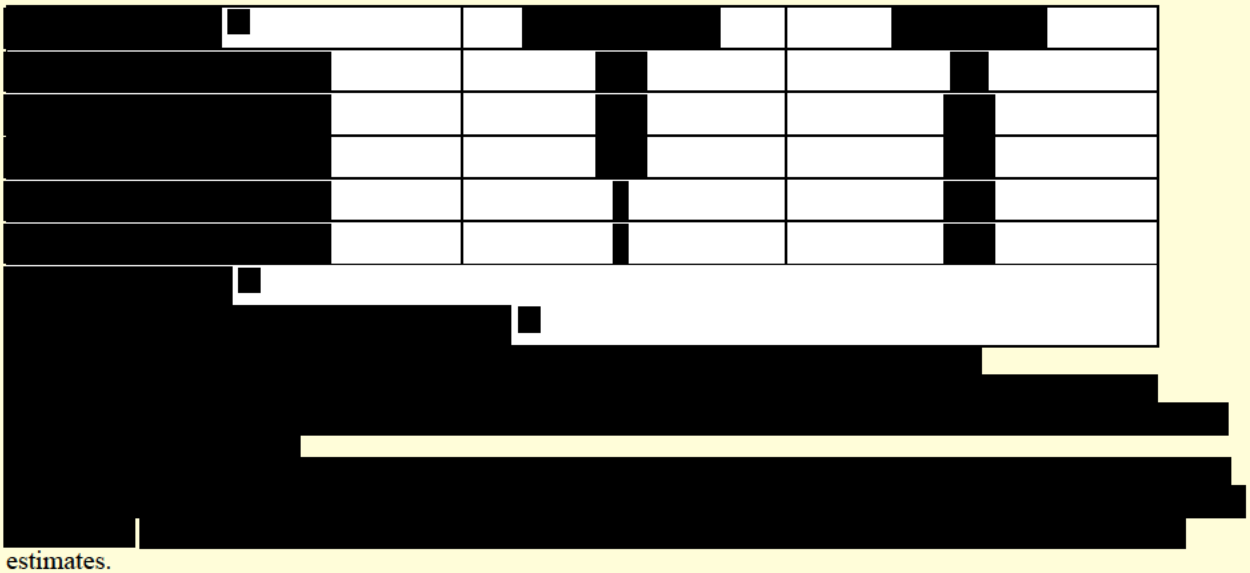
3. GENERAL CONSIDERATIONS

3.1. Sample Size Determination

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

It is therefore expected that IC will be more effective than Saline in providing visualization when used as an aid in the determination of ureteral patency. The total sample size of 116 patients, therefore, was estimated based on empirical assumptions [redacted] Therefore, the total sample size for this study will stay the same (approximately 116 patients) as planned before the protocol amendment.

Table 2: [redacted]



estimates.

3.2. Interim Analysis

A formal interim analysis is planned in Protocol Amendment 2. Patient enrollment was paused while the conspicuity score criteria was developed. A total of 21 patients were randomized and treated at the time of enrollment pause. Since the study total sample size of approximately 116 patients to be enrolled will remain unchanged for the protocol amendment, and that the 21 subjects enrolled prior to 5-point conspicuity score is developed will be excluded from the final efficacy analysis (see Section 3.3) , the timing of this planned interim analysis will be based on the enrollment of the remaining approximately 95 subjects. That is, this formal interim analysis is to be conducted when approximately 50% of the remaining patients are randomized and treated (approximately 47-48 patients). The objective of this interim analysis is to confirm the observed treatment effect size on the conspicuity score from the test dataset. The interim analysis will only analyze the conspicuity score provided by the central reviewers.

Since randomization to the IC dose level will not be unblinded during the interim analysis, the overall IC treatment effect will be analyzed using the proportional odds model; the model will include the main effect of treatment (IC vs Saline) and ureter (left vs right) and control for the repeated measures within a subject. This model is identical to the reduced model (Model 2) in the primary analysis of the primary endpoint described in **Section 5.1**.

Conditional Power (CP) will be calculated based on estimated odds ratio and corresponding standard error in log scale from the interim analysis data based on equation from Lan and Wittes

(1988) as summarized by professor Gary Kock in 2003 at Drug Information Association (DIA) 10th Annual Biostatistics Workshop in Japan, August 29, 2003 in Tokyo, Japan[1].

The prediction of the conditional power for a statistically significant result from the final analysis (at the two-sided 0.05 level) given the observed difference between treatments at a formal interim analysis can be based on the estimated treatment effect (equation 2); 2-sided 95% confidence intervals for the CP will also be calculated.

2. Estimated conditional power if the observed treatment difference at the interim analysis applies to the remainder of the study

$$ProbNorm\left\{\left[\sqrt{\frac{(1-P)}{P}}+\sqrt{\frac{P}{(1-P)}}\right]Z-\frac{Z_\alpha}{\sqrt{1-P}}\right\}$$

where $Z = (\hat{T}/SE)$

3. 100(1-γ) confidence interval for conditional power on the basis of $\hat{T}$ and SE

$$ProbNorm\left\{\sqrt{\frac{(1-P)}{P}}\times(Z\pm Z_\gamma)+\sqrt{\frac{P}{(1-P)}}\times Z-\frac{Z_\alpha}{\sqrt{(1-P)}}\right\}$$

Where *T*_hat = estimate of treatment effect at interim and SE = standard error of the treatment effect. In this study, T-hat will be the estimate of odds ratio of treatment and SE will be the standard error of the estimate, both are in the log scale. P=proportion of study completed (=0.5), alpha (α)=0.05/2, gamma (γ)=0.05

Following Mehta et al (2011, page 6)[2] adaptive approach the interim analysis results will be classified into three zones based on the estimated CP and decision to be made in each zone is specified as follows.

[1] Page 8 "Statistical Considerations for the Conduct and Reporting of Interim Analyses". (GGK 8/11/2003)

[2] Adaptive increase in sample size when interim results are promising: A practical guide with examples. Cyrus R. Mehta and Stuart J. Pocock. Statist. Med. 2011, 30 3267--3284

Table 3: Interim Analysis Conditional Power and Decision Principle

Conditional Power	Decision	Rationale
CP <0.20	Stop the study because the evidence for unfavorable efficacy is sufficiently convincing that no additional efficacy data is necessary.	An unfavorable zone: the conditional power (CP) is fairly low, suggesting that the final result is not likely to achieve significance, hence, the study has fairly low probability to reject the null hypothesis. This zone is defined to be CP < 40%
0.2 <=CP <0.4	No sample size re-estimate because the evidence for unfavorable efficacy. Complete the study per current protocol.	
0.4 <=CP <=0.8	Re-estimate sample size, study continues to complete the enrollment per re-estimated sample size	A Promising zone: the conditional power is in between 40% to 80%, suggesting that the interim analysis result is promising, but the study has to have an increased sample size to have at least 80% power to reject the null hypothesis
CP > 0.8	No sample size re-estimate. Complete the study per current protocol.	A favorable zone: the conditional power is fairly high, suggesting that the interim result is as good or better than the expectation, the study has fairly high probability to reject the null hypothesis as is. This zone is defined to be CP > 80%.

To protect the data integrity, an independent statistician/programmer who is not involved with the study will be responsible to perform the analysis. A Data Monitoring Committee (DMC) will be formed to review the interim analysis results. All members of the DMC will not be involved with the conduct of the study. To perform the interim analysis, the independent statistician will receive the conspicuity score assessed by the central reviewers and the randomization code for each video/subject. It is worthy to point out that randomization is only pertained to the treatment of IC vs Saline but the dose of IC will **not** be revealed for interim analysis.

The DMC may choose to share with the study team the aggregated results/data (e.g., summary tables) but the individual subject data will not be shared with the study team. All documentations for the interim analysis, including but not limited to analysis data, associated programs, summary tables and/or listings, and DMC communications, will be securely stored away until the study database is locked.

3.3. Analysis Population

The following analysis sets will be identified for this study.

Enrolled Analysis Set: All subjects who sign the ICF for the study are considered to have enrolled.

Intent-to-Treat (ITT) Analysis Set: The ITT Analysis Set will include all randomized subjects. This dataset may also be referenced as the 'Randomized Set'. The ITT subjects may or may not receive randomized treatment. All ITT subjects will be included in the randomized treatment group even if a dosing error occurred. See additional discussion in Section 3.7.

Safety Analysis Set: The safety set will include all ITT Analysis Set subjects who are treated with any study drug (Saline or IC) and will be used for safety and tolerability assessments. In the safety analysis set all subjects will be included in the actual treatment group received if a dosing error occurred. See additional discussion in Section 3.7.

Efficacy Analysis Set: The efficacy analysis set includes all Safety Analysis Set subjects who have a surgical procedure to assess ureteral patency and who have received both study drugs (Saline and IC dose) and a video approximately 10 minutes in length is available after each treatment. **The 21 patients who were randomized and treated prior to the development of the conspicuity score will be excluded from the efficacy population.** This will be the primary population for efficacy assessments. This is also referenced as the modified intent-to-treat (mITT) Analysis Set. All efficacy evaluations will be based on the mITT population. In the mITT analysis set all subjects will be included in the actual treatment group received if a dosing error occurred. See additional discussion in Section 3.7.

Per Protocol (PP) Analysis Set: The PP set includes 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. In the PP analysis set all subjects will be included in the actual treatment group received if a dosing error occurred. See additional discussion in Section 3.7.

3.4. Test Hypothesis and *P* Value Justification

The study has a planned interim analysis to review the effect size assumption used to determine the sample size. To control for the overall alpha for the primary efficacy analysis, an alpha of 0.0001 will be used for the interim analysis, and alpha of 0.0499 will be used for the final analysis. The details of the interim analysis, including scope, method, and decision rules, will be described in the Data Monitoring Committee charter.

In the final analysis, there are two null hypotheses for the primary efficacy endpoint conspicuity.

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 0.0499, the two p-values will be ordered from large to small. When both nominal p-values are less or equal to 0.0499, 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 the larger nominal p-value is greater than 0.0499 but the smaller p-value is less or equal to 0.025, the null hypothesis associated with the larger p-value is accepted and the null hypothesis associated with the smaller nominal p-value will be rejected. If the smaller nominal p-value is greater 0.025, the null hypothesis associated with this p-value is also accepted. The Overall Type I error control will apply to the primary endpoint conspicuity only.

3.5. Procedures for Handling Missing Data

Missing or partial data associated with efficacy assessments will be discussed specifically within each section. No missing data imputation will be performed for safety parameters. However, AEs with missing severity assessments will be tabulated as "severe," and AEs with missing relationship assessments will be tabulated as "related" for the purpose of analysis; and the missing data will be presented in data listing as is.

3.6. Analysis Center

This is a multicenter study; however investigative center will not be included in the analysis model as a covariate due to expected small sample size per center, additionally, the primary efficacy conspicuity score data will come from central readers whereas the conspicuity score from the local readers (surgeons) will be used for confirmatory analysis. Potential center effect will be assessed if data suggested.

3.7. Treatment Group vs Randomization Group

All qualified subjects will be randomized to either a dose of 2.5 mL IC (referred to as the Low dose) or a dose of 5.0 mL IC (referred to as the High dose). All randomized subjects will receive a dose of normal saline before receiving the randomized IC dose.

Hence, there are 2 randomization groups, namely IC High Dose and IC Low Dose and 3 treatment groups (IC High dose, IC Low dose, and Saline) in this study.

If a dosing error occurred (e.g., a subject was randomized to the IC High dose but actually received IC Low dose, and vice versa), the subject will be included in the actual treatment group received for efficacy, PK, and safety analyses; this will be an important protocol deviation and the incident will be clearly documented in the study report and in the database.

Data presentation for study population, physician overall satisfaction, safety lab, and 12-ECG will be based on the 2 randomization groups; whereas the data presentation for conspicuity, TTV, adverse events, and vital signs will be based on the 3 treatment groups. A Study Overall group may be included in certain data presentations (e.g., demographics) if data warrant. The Saline group will include all subjects who had saline dose, hence, the number of subjects in this treatment group will expect to be twice the size of IC Low dose and IC High dose. If data warrants, the Saline group may also be split by IC dose group. That is, subjects received the IC Low dose vs subjects received the IC High dose.

3.8. Definitions and Derived Variables

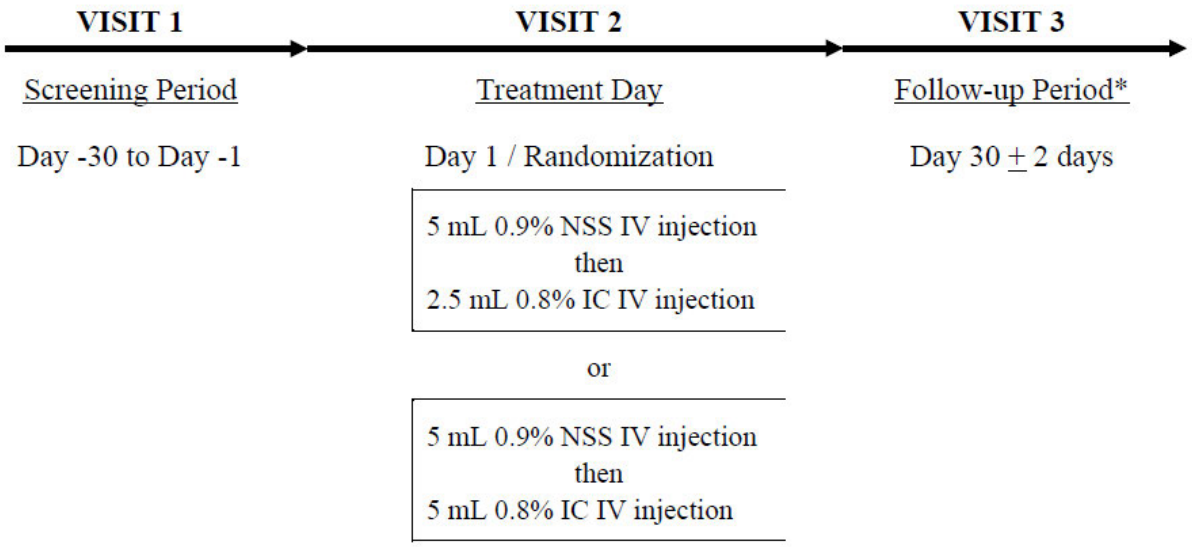
3.8.1. Study Period Definitions

The study duration for each subject is divided into 3 periods

1. Screening Period (Visit 1): The duration from signing informed consent until before receiving the first dose of study drug will be described as the screening period. **The last measurement taken prior to receiving the first dose of study drug (saline injection) is the Baseline measurement. Hence, Baseline measure could be either a scheduled assessment or an unscheduled assessment.**
2. Treatment period (Visit 2): Study day 1 (procedure day) is the treatment period. Although the actual date/time for some PK samples may occur on study day 2 or day 3, all PK analysis will be labelled as treatment period because PK period starts on Study Day 1.
3. Post treatment follow-up period (Visit 3): this period starts the day after procedure through end of study. **Subjects who completed Visit 3 final follow-up are considered complete the study.**

A study scheme is provided in Figure 1.

Figure 1: Study Scheme



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

3.8.2. IC Treatment Responders

A responder (Yes/No) variable will be derived for each subject by comparing the conspicuity score following IC treatment vs the conspicuity score after saline treatment. When there is >=1 point improvement (IC – Saline) in the conspicuity score and the conspicuity score following the IC treatment is (3, 4, or 5) for a subject, this subject is considered a responder of the IC treatment. The responder assessment will be performed for each ureter separately.

3.8.3. Time to Visualization (TTV) of Blue Color in the Ureteral Jets After IC Administration

Each randomized subject is expected to receive 1 dose of saline and 1 dose of IC; time of each injection, including start time and completion time, will be recorded as HHMMSS (hour, minute, and second). **TTV event is assessed only after IC treatment within 10 minutes after the injection start.** Time when this event occurred after IC injection will be captured as HHMMSS. Therefore, TTV following each injection will be derived as follows.

TTV (minutes) = (time when adequate visualization occurs - start time of injection) / 60

If the adequate visualization of blue color in the ureter jet flow at an ureteral orifice following IC injection for a subject did not occur for any reason, this subject will be censored for this analysis. The censored time will be set to 10 minutes.

TTV will be derived for each subject following IC injection for each ureter separately.

3.8.3.1. Missing Data Imputation Rules for TTV

In order to calculate TTV, the injection start time and time when blue color is adequate visualized in the ureter jet flow must be present. Although the clinical team will make the best effort to eliminate missing or partial time, mistakes could occur. The following rules will be implemented should a subject had missing or partial injection time or time when blue color in the ureter jet flow is adequately visualized.

1. **When the injection start time is missing or partial**

   1) If injection start time is missing but the completion time is present, the missing start time will be set to be the same as the injection completion, and vice versa.

   2) If the hour and minutes are non-missing but the seconds are missing for injection start time, the first second (01) will be assigned to the injection time.

   3) Otherwise the median elapsed time (defined as time between surgery start time and injection start time) in the study across all subjects who did not have missing data will be used to impute the missing time. That is, the missing injection start time will be set as (Surgery start time + median elapsed time).

The rules will be implemented based on this order.

2. **When event occurred but the time is missing or partial**

A subject is considered to have had this event when the conspicuity score is 4 or higher per central reader score for this ureter. When a subject has this event but the time when the event occurred is missing, the following rules will be applied.

1) If the time is partial (i.e., only the seconds are missing), the first second (01) will be assigned.

2) Is the time is missing, the subject will not be censored for this endpoint. The missing time, however, will be assigned to 10 minutes after the injection start time.

3) This event will be considered as an important protocol deviation. The subject will be excluded from the efficacy PP analysis set. A sensitivity analysis may be performed for the TTV endpoint based on the efficacy PP analysis set.

If a subject had issue(s) with a ureter initially this subject will be censored for this analysis regardless if the issue(s) is or is not fixed during the procedure.

3.8.4. Physician Satisfaction Agreement Scale

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.

Overall Satisfaction =Yes if PSAS is 1 or 2, otherwise

Overall Satisfaction = No

Physician who did not provide rating (missing data) will be grouped in the 'No' group for overall satisfaction analysis.

3.8.5. Concordance in Conspicuity Assessments between the Surgeons and the Blinded Central Readers

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. In addition, each qualified subject must have 2 kidneys, resulting 4 observations of conspicuity score per subject from the surgery and four observations from the blinded central readers.

The following notation will be adapted for each conspicuity score for a given subject:

- CS_{SL} is the conspicuity score from the left ureter after saline injection
- CS_{SR} is the conspicuity score from the right ureter after saline injection
- CS_{IL} is the conspicuity score from the left ureter after IC injection
- CS_{IR} is the conspicuity score from the right ureter after IC injection

After the videography is read by the central blinded reader, each subject will then have 4 pairs of conspicuity scores. For example, CS_{SL} from the surgeon and the CS_{SL} from the central reader. Because the conspicuity has value of 1, 2, 3, 4, or 5, the difference in each pair of conspicuity scores between the surgeon and the blinded central readers will be derived and it will have a possible value ranging from (-4 to +4). Based on the difference in each pair of conspicuity score, a new dichotomized concordance response variable will be derived as

- Concordance Response = Yes if the difference in conspicuity score is within +/- 1 point. That is, the difference is ranged from (-1 to +1), inclusive.
- Concordance Response = No if the difference in conspicuity is more than 1 point

4. STUDY POPULATION SUMMARIES

All study population summaries will include 2 randomization groups plus an 'Overall' column to display the summary statistics across all subjects.

Study population data listing by individual subject will be prepared for all study population endpoints, including the observed value and derived variables.

4.1. Disposition

A summary table (**Table 14.1.1.1**) will provide frequency counts for subject disposition, including all enrolled subjects, randomized subjects, treated subjects (Saline or IC), subjects who completed study, subjects who did not complete study, and reason for not receiving Saline injection or IC injection, and reasons for study discontinuation.

Primary reason for early termination of study will include

1) Adverse Event
2) Study Non-compliance
3) Lost to Follow-Up
4) Physician Decision
5) Sponsor Decision
6) Subject Decision
7) Other

If a subject who receives a dose of normal saline injection and fails to receive a dose of IC, the subject will be considered to have discontinued study drug treatment. Reason for treatment discontinuation will be captured and tabulated. Moreover, the disposition table will identify number (%) subjects included for safety analysis set, efficacy analysis set (mITT), and efficacy per protocol (PP) analysis set.

Another summary table (**Table 14.1.1.2**) will be produced to provide number of subjects in the safety, mITT, and efficacy PP analysis set by investigator and will display the center with the highest enrollment first.

Disposition summary will be based on the ITT analysis set.

4.2. Demographics and Baseline Characteristics

The demographic summary (**Table 14.1.2.1**) will include descriptive statistics for age, age group (age <65, age >=65, >=75 years), sex, race, ethnicity, weight, height, and body mass index (BMI) at baseline for overall and by treatment group.

Baseline characteristics and patient population characteristics will also include the following study surgery information:

1) Surgery duration (hours), method of surgery (cystoscopic, robotic, open, laparoscopic, other), and surgeon's specialty
2) Requiring (yes/no) fluid to be instilled into the bladder
    a. If Yes, the amount and type of fluids
3) Type, amount, and timing (relative to the start time of saline injection) of hydration
4) Use of any diuretics during the procedure (yes/no)

Demographics and baseline characteristics will be tabulated for the safety analysis set with no formal inferential tests. The same demographics table will also be prepared for the mITT analysis set (**Table 14.1.2.2**).

4.3. Medical History

All medical history data captured will be mapped using MedDRA (version 22). Subjects with medical history by SOC and preferred term will be tabulated by randomization group and study overall (**Table 14.1.3**) based on the safety analysis set.

4.4. Protocol Deviations

All protocol deviations will be identified and will be classified as either an 'Important Protocol Deviation' or 'Protocol Deviation'.

Important Protocol Deviation: An Important Protocol Deviation is a protocol deviation that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. Examples may include:

- Failure to meet all entry criteria;
- Non-compliant with study drug treatment regimen;
- Did not receive randomized treatment due to dosing error;
- Use of prohibited concomitant medications;

Protocol Deviation: Any alteration/modification, divergence or departure from the IRB-approved protocol. A protocol deviation is an unanticipated or unintentional divergence or departure from the expected conduct of an approved study that is not consistent with the current research protocol, or consent document.

All protocol deviations will be tabulated by protocol type, protocol deviation category for each treatment group and study overall (**Table 14.1.4**) based on the safety analysis set.

4.5. Treatment Compliance

Doses of study medication (Saline and IC) will be administered to the study subjects under the observation of study personnel while confined to the study site. The exact time of administration of study medication will be documented within each subject's eCRF.

No formal Compliance data will be tabulated for the study report.

4.6. Prior and Concomitant Medications

All prior and concomitant medications will be mapped using WHO drug (version September 2019). Subjects with prior and concomitant medications will be tabulated by medication class and drug standardized name. The medication class will be the WHO Drug ATC level 3 text if available, otherwise, ATC level 2 text will be the medication class (**Table 14.1.5.1**). The prior and concomitant medications summary will be based on the safety analysis set.

All efforts will be made to ensure the capture of onset/stop dates of a medication. If at the end there are missing dates in the concomitant medication dataset, the following rules will be implemented for the purpose of analysis.

Concomitant meds will include medications with end-date that on or after the surgery date when present or if ongoing is checked. When ongoing is not checked and the end-date is partial the following rules will be used to determine if a medication is concomitant.

a) EXCLUDE a medication if the year is before the surgery
b) EXCLUDE a medication if the year is the same year but the month is before the surgery;
c) INCLUDE a medication if the year is the same year as the surgery but the month is missing

When end-date is completely missing, the med will be accounted as concomitant medication.

Concomitant other procedures will be mapped using MedDRA version 22. All other procedures performed during the study will be tabulated by system organ class and preferred term (**Table 14.1.5.2**).

5. EFFICACY ANALYSIS

Efficacy data will be displayed by treatment group except PSAS and TTV.

All efficacy analyses will be based on the Efficacy analysis set (mITT Population). Although the PP analysis set is defined in Section 3.3 efficacy analysis based on the PP analysis set will not be routinely provided. That is, when number of mITT subjects excluded from the efficacy PP analysis set is more than 10% of the mITT analysis set, then efficacy analysis will also be performed based on the efficacy PP analysis set to evaluate the consistency and the robustness of the efficacy conclusion drawn from the mITT analysis set.

Efficacy data listing by individual subject will be prepared for all efficacy endpoints, including the observed value and derived variables.

5.1. Conspicuity Score from the Central Readers

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 conspicuity endpoint. The recording videography will be read by the surgeon (who is unblinded for treatment and blinded for IC dose) and by two center readers who is blinded to the treatment (Saline vs IC) and IC dose. The score from the central readers will be the source for the primary endpoint analysis and the conspicuity score provided by the surgeons will be utilized for concordance analysis (see Section 5.5 for details).

There will be 2 central reviewers to watch each video after the videos are blinded. The ratings are acceptable for consistency if the scores from the two independent reviewers for a ureter under the same treatment are within (+/-) 1 point. In this case, the average score across the two reviewers will be the final score from the central reader. Otherwise, a third independent reviewer (a judicator) will review the same video and this judicator's score will be the final score from the central reader.

As indicated earlier in Section 3.7 the summary table for this endpoint will include 3 treatment groups, namely IC High Dose, IC Low Dose, and Saline Group. It is expected that the sample size (n) for the Saline group will be twice the size of the sample size for the IC Hight dose and IC Low Dose.

The treatment effect on the conspicuity score will be evaluated using a Generalized Estimating Equation (GEE) for Repeated Measures to control for the intra-subject correlation by fitting a proportional odds model to evaluate the treatment effect. A full model (**Model 1**) will provide the chi-square tests (Score Statistics for Type 3 GEE Analysis) for the effects from treatment (IC High dose / IC Low dose, Saline), ureter (left side ureter vs right side ureter), and treatment*ureter interaction on the response based on multi-normal distribution for an ordinal

response. The model estimates the cumulative logits of better outcome (conspicuity=5) to the poor outcome (conspicuity=1). Since it is expected that the interaction effect will not be statistically significant (p >0.10), a reduced model (**Model 2**) will also be used in which the interaction effect will be dropped. The goodness of fit of the models (Full model vs the Reduced model) 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 (treatment, ureter, treatment*ureter interaction in full model) will be determined based on the Score Statistics for Type 3 GEE Analysis.

For the primary endpoint analysis, GEE models will be fitted separately for each comparison, namely IC Hight dose vs Saline, and IC Low dose vs Saline, and IC High dose vs IC Low dose.

An example of SAS syntax is showing below for this analysis, in which the conspicuity is treated as an ordinal response variable and a proportional odds model is used model the better response compared to the poorer response. Stokes et al. (2000)[3] recommended that the null hypothesis of treatment effect would be based on the score statistics for Type 3 GEE analysis, whereas the parameter will be the GEE parameter estimates with the empirical standard error because the Z and the Wald statistic generally produce more liberal p-value than the score statistic.

In this example, the treatment is a binary code; IC High dose=1 and Saline=0. For analysis of IC Low dose vs Saline, IC Low dose=1 and Saline=0; when comparing IC High dose vs IC Low dose, IC High dose=1 and IC Low dose=0.

```
PROC GENMOD DATA=respH descending;
      CLASS USUBJID URETER(ref='Right') TRT (ref='Saline');
      MODEL conspicuity = trt ureter trt*ureter                    (Model 1)
                        /LINK=clogit DIST=mult TYPE3;
      REPEATED subject = Usubjid / type=ind;
      ESTIMATE 'OR: IC vs Saline' trt 1 -1/ exp;
      ods output Estimates=OR1 GEEEmpPEst=est1 Type3=pval1;
Run;

PROC GENMOD DATA=respH descending;
      CLASS USUBJID URETER(ref='Right') TRT (ref='Saline');
      MODEL conspicuity = trt ureter                               (Model 2)
                        /Link=clogit DIST=mult TYPE3;
      REPEATED SUBJECT = Usubjid / type=ind;
      ESTIMATE 'OR: IC vs Saline' trt 1 -1/ exp;
      ods output Estimates=OR2 GEEEmpPEst=est2 Type3=pval2;
Run;
```

Where:

[3] Stokes ME, David CS, Koch GG (2000). Categorical Data Analysis using the SAS System 2nd Edition (page 485).

1. Dataset respH include subjects from IC High dose only.
2. The 'descending' option indicates that the ordered values are listed in descending order.
3. Conspicuity score (1, 2, 3, 4, or 5)
4. 'TRT' is Treatment (coded to IC or Saline)
5. 'URETER' is coded to 'Left' or 'Right'
6. 'USUBJID' is the unique Subject Identification
7. REPEATED statement controls the intra-subject correlation due to repeated measures. In this example, independent correlation matrix is used.
8. Three output datasets:
    a. OR=estimated odds ratio (IC /Saline)
    b. EST= Analysis of GEE Parameter Estimates based on Empirical Standard Error Estimates
    c. PVAL= P-value for each effect in the model per Score Statistics For Type 3 GEE Analysis

The analysis summary table (**Table 14.2.1.1**) will include distribution of conspicuity score in each treatment group by ureter (left vs right and overall), p-value based on score statistics for Type 3 GEE analysis, odds ratio estimates and standard error, and 95% confidence intervals for the odds ratio. Three treatment group comparisons will be provided, namely difference between IC High dose vs Saline, IC Low dose vs Saline and IC High dose vs IC Low dose (an exploratory analysis).

The summary table will display the nominal p-values from each comparison (High dose vs Saline, Low dose vs Saline, and High dose vs Low dose). However, the statistical significance of the normal nominal p-value between the IC High doe vs Saline and between the IC Low dose vs Saline will follow the rule defined in Section 3.4 in order to control for family-wide type I error for the primary endpoint.

5.1.1. Missing Data Handling Procedure

When conspicuity assessment cannot be performed, conspicuity score will be missing. Reason for each Not Assessed cases will be documented in the database.

- For the primary analysis (Table 14.2.1.1), missing data will not be imputed. Subjects will be included in the input dataset as is. This analysis will be referred to as the Observed Data Analysis.
- However, the impact of missing data will be evaluated by performing 3 sensitivity analyses. The sensitivity analysis will use the same model specified for the primary analysis; nominal p-values from each sensitivity analysis will be reported as. Those 3 sensitivity analyses are described below:
    - Sensitivity Analysis 1 – This analysis will include all MITT subjects who have no missing data. That is, all subjects in this analysis will have 4 conspicuity scores as planned (Table 14.2.1.2).

- Sensitivity Analysis 2 – This analysis will exclude all subjects who are not in the PP population and subjects who have ≥ 1 missing data. This would represent the best outcome for the study since all potential confounded issues will be removed (Table 14.2.1.3).
- Sensitivity Analysis 3 – This analysis will impute the missing data that creates the worst scenario for the study (Table 14.2.1.4).
  - Missing data for Saline Treatment: Assign missing value for saline treatment to be the best value from this patient if scores are available. If no score is available for this patient, then assign the best score of 5 to missing data.
  - Missing score for IC Treatment: Assign missing value for IC treatment to the worst score the patient had if scores are available. If none is available, assign the worst score of '1' to missing data.

5.2. PSAS with IC Treatment

Each subject will have 1 PSAS score. Ratings in the PSAS with the IC treatment will be tabulated (**Table 14.2.2**) by treatment (IC High dose, IC Low dose, and overall (pooled across high dose and low dose) with number (%) subjects in each rating category and overall satisfactory.

Two-sided 95% confidence intervals on proportion of physicians who are satisfied (with ratings 1 or 2) will be derived using the Wilson confidence limits for the binomial proportion. This is also known as score confidence limits. The Wilson interval has been shown to have better performance than the Wald interval and the exact (Clopper-Pearson) interval (SAS Users' manual).

A binominal 1-way proportion approach will be used to examine that ≥ 50% of the physicians are satisfied with the IC treatment (a superiority test) as an aid for the assessment of ureter patency comparing to the saline treatment. Below is an example of SAS syntax to implement this analysis.

```
DATA psas;
      Set PSASscore;
      If score in (1, 2) then psasC='1Yes'; else psasC='2No';
Run;
PROC SORT data=psas; BY trt psasC;
PROC FREQ DATA=psas ORDER=DATA;
   TABLES psasC / BINOMIAL(SUP P=0.45 MARGIN=0.05 CL=WILSON) alpha=.05;
   BY trt;
   ODS OUTPUT BinomialCLs=bCL
              BinomialSup=pval
      ;
run;
```

This syntax makes the 'Yes' response to be the first category so the binomial test will examine the proportion of response in the Yes category. The null proportion of 0.45 and margin of 0.05 makes the point estimate of superiority limit to be 0.50 (superiority limit = the null proportion + margin). The 'TRT' is coded to 'High', 'Low' or 'All'. 'All' includes subjects from both IC High and IC Low doses.

An exploratory analysis to examine any difference in PSAS with the use of IC High dose vs the IC Low dose will be performed via a Cochran-Mantel-Haenszel (CMH) test for 'Row Mean Scores Differ'. Below is an example of SAS syntax to implement this analysis

```
PROC FREQ DATA=psas;
   TABLES dose*psas / noprint CMH2 alpha=.05;
   ODS OUTPUT CMH=pval
      ;
run;
```

where 'dose' is coded to Low or Hight; the 'PSAS' is the satisfaction rating score (1, 2, 3, 4, or 5).

Normal p-value for each analysis will be reported as is without controlling for multiple comparisons.

5.3. IC Treatment Responders

In this study each subject is served as his/her control when the IC treatment is given as a visualization aid when determining ureter patency, IC treatment responder, hence, could be defined based on the conspicuity score following IC and saline.

To determine proportion of subjects meeting the responder definition in conspicuity score following IC treatment the blinded central reviewer's assessment will be used. A subject is a responder when there is >=1 point improvement in the conspicuity scores following the IC vs saline treatment (IC – Saline >=1) and the conspicuity score following the IC treatment is (3, 4, or 5). The responder criteria will be assessed separately for each ureter for each subject.

Since the responder definition will be defined for each ureter per subject, when a subject has missing data, the corresponding responder variable cannot be defined. The primary analysis will exclude all subjects that the responder definition cannot be derived due to missing data; this will be referred to as the Observed Analysis.

To assess the impact of missing data, a sensitivity analysis will be conducted. In this sensitivity analysis, the responder variable will be set to 'No' for those that cannot be derived due to missing data. This analysis will represent the worst-case scenario for this analysis.

Summary tables (Tables 14.2.3.1 and 14.2.3.2) will provide number (%) subjects meeting the responder definition in the following categories: overall (left or right), left ureter, right ureter, or both ureters for each IC dose and overall; 95% confidence intervals based on normal approximation will also be provided, along with the difference in proportion of responders between the IC high dose vs IC low dose.

5.4. Time to Visualization

In this study each subject will contribute 2 TTV observations, one for left and one for right ureter. Each time to event observation will be considered an independent observation.

As indicated earlier in Section 3.7 the summary table for this endpoint will include 2 treatment groups, namely IC High Dose, IC Low Dose. Time (minutes) to visualization (TTV) of blue color in the ureter jet flow will be evaluated using the Kaplan-Meier Survival Analysis approach. Descriptive summary, including number of subjects (%) subjects censored; time to event percent-tiles (25%, 50%, and 75%) and 95% confidence intervals and Kaplan-Meier mean (SE) will be estimated for each ureter. The following is an example SAS syntax for this analysis.

```
ods output CensoredSummary=Ncensor
          ProductLimitEstimates=PL
          Means=KMmeans
          Quartiles=quart
          Survivalplot=SurvivalPlotData
             ;

PROC LIFETEST DATA=ttv METHOD=PL ATRISK
       PLOTS=survival(ATRISK(ATRISKTICK)= 0 2 4 6 8 10);
             TIME aval*Cnsr(1);
             *---- stratified test ---*;
             STRATA ureter / GROUP=trt;
             ;
run;
where TRT is coded to HighDose vs LowDose
```

In additional, KM-survival analysis descriptive summary will also be provided for the overall. In this analysis, number of observations will not be the same as number of subjects.

Using data 'SurvivalPlotData' Kaplan-Meier survival curves will be presented for each treatment group along with number of subjects at risk at 0, 2, 4, 6, 8, and 10 minutes. The KM-survival plots will be prepared for each ureter and overall.

If data warrants, a Cox Proportional hazards (PH) model will be fitted to estimate hazards ratios (HR) between IC High dose vs IC Low dose using a stratified Cox PH model that includes main effect of treatment and stratified by ureter (left vs right). The HR estimate and 95% CI for the HR will be based on the Score Statistics. The p-value for treatment effect will be based on the Score Statistics because the p-value from the score statistics produced by PROC PHREG will be the same as the stratified log-rank test produced by PROC LIFETEST. In order to obtain HR 95% CI using the score statistics, an external SAS macro developed by based on Lin (2016)[4] will be used (because the current SAS 9.4 does not have this computation option). Note the option of TIES=DISCRETE should be specified for SAS procedure PROC PHREG.

A summary table (**Table 14.2.4**) will include the following.

- number (%) subjects with and without (censored) event from the left ureter and from the right ureter. This will allow each subject to be accounted once per treatment,
- Kaplan-Meier product limit estimates of quartiles of time to first event (25%, 50%, and 75% tiles and 95% CIs) and
- KM means (SE) of time to first event
- Hazard ratio (IC High/IC Low) and 95% Cis and p-value based on score test.

To compute the KM means and its standard error, in case a subject is censored at 10 minutes the option **TIMELIM** will be set to the maximum observed time in minutes when SAS procedure PROC LIFETEST is used to perform KM survival analysis. Hence, the calculated mean survival time will be the area under the Kaplan-Meier survival curve from 0 to this maximum observed time.

Below is an example of SAS syntax to estimate the difference between treatment groups IC High dose vs IC Lower dose using the external SAS macro HazardRatio. **This parameter will not applicable for Saline treatment.**

```
DATA CoxHS;
      set ttv;
            If CNSR=0 THEN EVENTYN=1;   *-- YES ---*;
            If CNSR=1 THEN EVENTYN=0;   *-- NO ---*;
      TIME=AVAL;
      if trt='IC High' then Treatment=1; else Treatment=0;
run;
```

[4] Lin DY, Dai L, Cheng G, Sailer MO: On confidence intervals for the hazard ratio in randomized clinical trials. Biometrics, 75: 1098-1102, 2016. http://dlin.web.unc.edu/software/HazardRatio/

```
*---- use the macro to get Score HR 95% CI ***************;
%HazardRatio (Data = CoxHS, Result=HR HL, Time = Time, Status =
EventYN, Ties=DISCRETE, Stratum = Ureter, Treatment = Treatment, Alpha
= 0.05, Accuracy = 0.00001);

data Result1;
      set HR_HL; if method='Score';
      HR=exp(estimate);
      HR Lower=exp(Lower);
      HR_Upper=exp(Upper);
Run;
```

5.5. Concordance Assessments

The concordance between the surgeon's conspicuity and the blinded reader's conspicuity will be assessed in three ways.

5.5.1. Treatment Effect on Conspicuity per Surgeons Review

The first approach is to evaluate the treatment effect based on the surgeons conspicuity score. The objective for this analysis is to check if the conclusion of treatment effect shown from the central readers rating can be confirmed by the surgeons reading. If the answer is a 'Yes' then one can conclude that concordance is achieved. Hence, this analysis serves as a confirmatory analysis of the primary analysis. In additional, we can also compare the magnitude of treatment effect (expressed as the odds ratio) between the primary analysis and confirmatory analysis. All subjects with ≥ 1 missing data from surgeon will be excluded from this analysis.

Procedure and summary table (**Table 14.2.5.1**) for this confirmatory analysis will follow the same GEE approach described in Section 5.1, however, the nominal p-values will be displayed as is without control for multiplicity.

5.5.2. Covariate Analysis

The second approach is to pool the conspicuity scores from both surgeon and blinded reader into a single dataset and add a new covariate (evaluator) into the GEE model; the purpose is to assess if the effect of evaluator is or is not a statistically different covariate. A summary table (**Table 14.2.5.2**) will include the results from the following covariate analysis. All subjects with ≥ 1 missing data from either surgeon or central reader will be excluded from this analysis.

To perform this analysis, a new variable (rater: surgeon vs central reader) and interactions will be added to the full model (**Model 3**) and Reduced model (**Model 4**). Score statistics for Type 3 GEE analysis will be used to determine if the rater is statistically significant factor.

```
PROC GENMOD DATA=resp descending;
      CLASS usubjid ureter(ref='Right') Rater(ref='Surgeon')
             TRT(ref='Saline');
      MODEL conspicuity = trt ureter rater                          (Model 3)
                  trt*ureter rater*ureter rater*trt
                  Rater*ureter*trt
                        /LINK=clogit DIST=mult TYPE3;
      REPEATED subject = usubjid / type=ind;
Run;

PROC GENMOD DATA=resp descending;
      CLASS usubjid ureter(ref='Right') Rater(ref='Surgeon')
             TRT (ref='Saline');
      MODEL conspicuity = trt ureter rater                          (Model 4)
                        /Link=clogit DIST=mult TYPE3;
      REPEATED SUBJECT = usubjid / type=ind;
Run;
Where rater will be coded as (central vs surgeon).
```

This covariate analysis will be performed only for the comparisons between the IC treatment vs the Saline treatment. Each GEE model will be fitted separately for each comparison.

5.5.3. Dichotomization of the Difference in Conspicuity

The third approach is to dichotomize the difference in conspicuity between the surgeon and the blinded reader. The four scores from the surgeon will be paired with the four scores from the blinded central reader based on treatment and ureter for a given subject. Based on the difference in the paired conspicuity (see Section 3.8.5) a concordance response variable will be derived for each pair of the conspicuity:

a. the ratings are in agreement when the difference in score is (+1, 0, -1), otherwise
b. the ratings are not in agreement when the difference is more than +/- 1 point

The association of this concordance response variable (agree vs not agree) with treatment and ureter will also be evaluated using the GEE model based on the concept of logistic regression for dichotomized response. In case of missing data that prevent from forming a pair of observations to derive this concordance response variable, the data will be excluded from this analysis.

The score statistics will be used to determine if the treatment is statistically associated with this dichotomized response. The following is an example of SAS syntax for this analysis between the IC Hight dose vs Saline. This analysis will be performed separately for each comparison (IC High dose vs Saline, IC Low dose vs Saline, and IC High dose vs IC Low dose). All subjects with ≥ 1 missing data from either surgeon or central reader will be excluded from this analysis.

```
PROC GENMOD DATA=dischotH descending;
CLASS usubjid ureter(ref='Right') TRT(ref='Saline');
MODEL response (event='Y') = trt ureter trt*ureter                    (Model 5)
                              /LINK=LOGIT DIST=BINOMIAL TYPE3;
REPEATED subject = usubjid / type=EXCH;
ESTIMATE 'OR: IC vs Saline' trt 1 -1 / exp;
Run;

PROC GENMOD DATA= dischotH descending;
CLASS usubjid ureter(ref='Right') TRT(ref='Saline');
MODEL response (event='Y') = trt ureter                               (Model 6)
                                    /LINK=LOGIT DIST=BINOMINAL TYPE3;
REPEATED SUBJECT = usubjid / TYPE=EXCH;
ESTIMATE 'OR: IC vs Saline' trt 1 -1 / exp;
```

Where:

1. 'TRT' is coded to IC or Saline
2. 'URETER' is coded to 'Left' or 'Right'
3. 'USUBJID' I the unique Subject Identification
4. REPEATED statement controls the intra-subject correction due to repeated measures. In this example, exchangeable working correlation structure is used (TYPE=EXCH).

The analysis summary table (**Table 14.2.5.3**) will include distribution of number (%) of pairs that are in agreement vs not in agreement in each treatment group by ureter (left vs right), score statistics for Type 3 GEE analysis, odds ratio estimates, and 95% confidence intervals for the odds ratio between IC High Dose vs Saline, IC Low dose vs Saline and IC High dose vs IC Low dose (an exploratory analysis). All nominal p-values will be displayed as is without control for multiplicity.

5.6. Covariate Analysis and Subgroup Analysis

The impact of some potential covariates on the primary efficacy endpoint will be evaluated if data warrants. The potential covariates include but not limited to

1. Surgery type
2. Surgeons specialty type
3. Age and age group, sex, race group

Each variable will be assessed individually by adding the variable to the primary efficacy models described in Section 5.1.

Similarly, if data warrants, subgroup analyses defined by the above variables will be performed. All those analyses, if performed, will be exploratory/ confirmatory.

6. PHARMACOKINETICS EVALUATIONS

PK portion of the study are dropped. Hence, all previously defined PK analyses are no longer applicable.

7. SAFETY AND TOLERABILITY EVALUATIONS

Safety data summaries will be displayed by randomization group for clinical safety laboratory test and ECG; adverse events and vital signs will be tabulated by treatment groups. Safety data listing by individual subject will be prepared for all safety endpoints.

7.1. Total Exposure

All subjects are expected to receive a dose of normal saline and a dose of IC. Hence, no formal total exposure in terms of number of doses or treatment duration will be applied.

7.2. Adverse Events

Adverse events reported from Day 1 beginning at the Saline injection through the final follow-up will be considered treatment emergent adverse events (TEAEs). **Adverse event reported after Saline injection and prior to IC injection will be mapped to Saline treatment, all adverse events after IC injection, including events reported during the follow period will be mapped to IC treatment. For all AEs, if the onset date is missing/partial, the AE is TEAE and will be mapped to IC study unless the available portion of the date/time indicates otherwise.**

The Medical Dictionary for Regulatory Activities **(**Version 22**)** will be used to classify all AEs with respect to system organ class and preferred term.

The following summary tables will be produced for the TEAEs. All data summaries will provide number (%) subjects as well as total number of events in each category by treatment group (IC High, IC Low, and Overall).

1. a topline summary of TEAEs (**Table 14.3.2.1**)
2. a summary table by preferred term in descending order of total incidence (Table **14.3.2.2**)
3. a summary table by system organ class and preferred term (**Table 14.3.2.3**)
4. a detailed summary table by system organ class, preferred term and severity (**Table 14.3.2.4.1**)
5. a detailed summary table by system organ class, preferred term and relationship (**Table 14.3.2.4.2**)
6. a table of serious TEAEs by system organ class and preferred term (**Table 14.3.2.5**)
7. TEAE leading to study discontinuation (**Table 14.3.2.6**)

In addition, adverse event of special interest **(Table 14.3.2.7)** will be identified and summarized if data warrant. Adverse event of special interest (AEOSI) could include but not limit to transient elevation of blood pressure and reflex bradycardia. For example, TEAE of tachycardia, hypotension, hypertension, rash or erythema, respiratory symptoms, such as dyspnea or

bronchospasm will be flagged as AEOSI. All adverse events will be reviewed before database lock to identify all AEOSI in the study.

7.3. Changes in Laboratory Tests

Safety laboratory tests are scheduled to be performed during screening and first follow-up post procedure. Additional unscheduled laboratory tests would be performed as clinically indicated. Local lab will provide the results to each center.

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.

Lab results will be tabulated based on local normal ranges [**Table 14.3.3.1.1 (hematology**), **Table 14.3.3.1.2 (chemistry**)]. That is, the number of subjects with normal, low, or high lab results at screening (Baseline) and end of study will be cross-tabulated for IC High dose, IC Low dose and Study Overall without any inferential statistics. This analysis will include only the scheduled lab tests.

Number (%) subjects with abnormal lab results, either clinically significant, or not clinically significant (scheduled and unscheduled lab) determined by the investigator, will be tabulated by IC dose group and study overall without inferential statistics [**Table 14.3.3.2.1 (hematology**), **Table 14.3.3.2.2 (chemistry**)].

7.4. Changes in Vital Signs

During screening and follow-up visits body temperature, pulse rate, pulse oximeter (%) 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. Investigator will determine each vital sign as normal or abnormal, and if it is abnormal, the

investigator will also determine if the abnormal vital signs is or is not clinically significant. Any clinically significant changes to vital signs or heart rhythm will be reported as an adverse event.

Vital signs data summary will be provided by treatment groups (IC High, IC Low, and Saline). Changes in vital signs will be derived for each subject at each timepoint as

Change = Post baseline – baseline

Vital signs collected on Day 1 prior to Saline injection will be the baseline vital signs if not missing; otherwise, measurements taken at screening visit will served as the baseline.

The summary table (**Table 14.3.4.1**) will display descriptive summary of vital signs and change from baseline (sample size, mean, median, and standard deviation, range) without inferential statistics.

Number (%) subjects with abnormal vital signs, either clinically significant or not clinically significant from all vital signs assessments (scheduled and unscheduled) will be tabulated by treatment groups (IC High, IC Low, and Saline) without inferential statistics (**Table 14.3.4.2)**.

7.5. Changes in 12-Lead ECG

During screening and first post procedure follow-up visit, 12-lead ECG will be assessed. ECG interpretation will include normal, abnormal but not clinically significant; abnormal and clinically significant.

Number (%) subjects in each results interpretation category at each time point will be tabulated by randomization group (IC High, IC Low) and study overall, including shift from normal to abnormal at end of study without any inferential statistics **(Table 14.3.5)**.

Other 12-Lead ECG related parameters, including PR interval, RR interval, QRS duration, QT interval, and QTc interval, will also be collected. Those parameters will not be tabulated, but data listings will be provided.

8. TABLE OF CONTENTS OF PLANNED DATA SUMMARIES

8.1. Summary Tables

Table No.	Table Title	Analysis Set
14.1.1.1	Disposition Summary by Randomization Group	Enrolled Analysis Set
14.1.1.1	Number of Randomized Subjects by Site for each Analysis Set	ITT Analysis Set
14.1.2.1	Demographics, Baseline Characteristics, and Study Surgery Characteristics by Randomization Group	Safety Analysis Set
14.1.2.2	Demographics, Baseline Characteristics, and Study Surgery Characteristics by Randomization Group	mITT Analysis Set
14.1.3	General Medical History and Surgical History by Randomization Group	Safety Analysis Set
14.1.4	Summary of Protocol Deviations by Randomization Group	Safety Analysis Set
14.1.5	Summary of Concomitant Medications by Drug Class and Medication Preferred Name	Safety Analysis Set
14.2.1.1	Treatment Effect on Conspicuity Score Based on Central Readers Assessment - An Observed Data Analysis	mITT Analysis Set
14.2.1.2	Treatment Effect on Conspicuity Score Based on Central Readers Assessment – Sensitivity Analysis 1	mITT Analysis Set
14.2.1.3	Treatment Effect on Conspicuity Score Based on Central Readers Assessment – Sensitivity Analysis 2	The Best-Case Scenario
14.2.1.4	Treatment Effect on Conspicuity Score Based on Central Readers Assessment – Sensitivity Analysis 3	The Worst-Case Scenario
14.2.2	Physician Overall Satisfaction Assessment with IC Treatment	mITT Analysis Set
14.2.3.1	Proportion of Responders to IC Treatment Based on Central Readers Assessment - An Observed Data Analysis	mITT Analysis Set
14.2.3.2	Proportion of Responders to IC Treatment Based on Central Readers Assessment - A Sensitivity Analysis	The Worst-Case Scenario
14.2.4	Time (minutes) from the Start of Injection to Adequately Visualized Blue Color in the Ureter Jet Flow After IC Treatment	mITT Analysis Set
Repeat 14.2.1 to 14.2.4 for efficacy per Protocol set if >=10% of MITT subjects are excluded from the efficacy per Protocol analysis set		
14.2.5.1	Treatment Effect on Conspicuity Based on Surgeons Assessment	mITT Analysis Set
14.2.5.2	Analysis of Evaluator Effect as a Covariate on Conspicuity	mITT Analysis Set

Table No.	Table Title	Analysis Set
14.2.5.3	Dichotomized Concordance between Surgeons and Central Readers Assessment of Conspicuity	mITT Analysis Set
14.3.2.1	Topline Summary of Treatment Emergent Adverse Event	Safety Analysis Set
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14.3.2.3	TEAE by SOC and Preferred Term	Safety Analysis Set
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14.3.2.4.2	TEAE by SOC, Preferred Term and Relationship to Study Drug	Safety Analysis Set
14.3.2.5	All Serious TEAE by SOC and Preferred Term	Safety Analysis Set
14.3.2.6	TEAEs Leading to Study Discontinuation by SOC and Preferred Term	Safety Analysis Set
14.3.2.7	TEAE of Special Interest by Category and Preferred Term	Safety Analysis Set
14.3.3.1.1	Cross Tabulation of Hematology Test Results Relative to Normal Range at Baseline and Post Baseline	Safety Analysis Set
14.3.3.1.2	Cross Tabulation of Chemistry Test Results Relative to Normal Range at Baseline and Post Baseline	Safety Analysis Set
14.3.3.2.1	Summary of Subjects with Clinically Significant Abnormal Hematology Test at Post Baseline	Safety Analysis Set
14.3.3.2.2	Summary of Subjects with Clinically Significant Abnormal Chemistry Test at Post Baseline	Safety Analysis Set
14.3.4.1	Summary of Vital Signs Results and Change from Baseline by Timepoint	Safety Analysis Set
14.3.4.2	Summary of Subjects with Clinically Significant Abnormal Vital Signs Post Baseline	Safety Analysis Set
14.3.5	Summary of 12-Lead ECG Results by Interpretation Category and Timepoint	Safety Analysis Set

8.2. Figures

Figure No.	Figure Title	Analysis Set
14.2.2.1	Kaplan-Meier Survival Curves of Time to Visualization	mITT Analysis Set
14.2.2.2	Kaplan-Meier Survival Curves of Time to Visualization by Ureter	mITT Analysis Set

8.3. Data Listings

Listing No.	Listing Title	Analysis Set
16.2.1.1	Disposition	ITT Analysis Set
16.2.1.2	Analysis Population	ITT Analysis Set
16.2.2	Demographics and Baseline Characteristics	Safety Analysis Set
16.2.3	Medical and Surgical History	Safety Analysis Set
16.2.4	Study Drug Administration	Safety Analysis Set
16.2.5	Study Surgery	Safety Analysis Set
16.2.6	Protocol Deviations	Safety Analysis Set
16.2.7.1	Prior and Concomitant Medications	Safety Analysis Set
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Listing No.	Listing Title	Analysis Set
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16.2.12.1	Vital Signs Results	Safety Analysis Set
16.2.13	12-Lead ECG Results	Safety Analysis Set
16.2.14	Telephone Follow-up	Safety Analysis Set