CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216264Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA Multidisciplinary Review and Evaluation

Application Type	NDA
Application Number	216264
Priority or Standard	Standard
Submit Date	September 9, 2021
Received Date	September 9, 2021
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Division/Office	Division of Imaging and Radiation Medicine, Office of Specialty Medicine
Review Completion Date	July 6, 2022
Established/Proper Name	Indigotindisulfonate Sodium
Trade Name	Bludigo
Pharmacologic Class	Diagnostic Dye
Applicant	Provepharm SAS
Dosage Form	Injection, Solution, 8 mg/mL
Applicant Proposed Dosing Regimen	(b) (4) 5 mL
Applicant Proposed Indication/Population	Indigo carmine is indicated for use as a visualization aid (b) (4) of the integrity (b) (4) of the ureters (b) (4) urological and gynecological open, robotic, or endoscopic surgical procedures.
Applicant Proposed SNOMED CT Indication Disease Term for Each Proposed Indication	45475000: Indigo carmine stain (substance)
Recommendation on Regulatory Action	Approval
Recommended Indication/Population	BLUDIGO is a diagnostic dye indicated for use as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures.
Recommended SNOMED CT Indication Disease Term for Each Indication	45475000: Indigo carmine stain (substance)
Recommended Dosing Regimen	5 mL as a single intravenous dose (b) (4)

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OB = Office of Biostatistics
OCP = Office of Clinical Pharmacology
OPDP = Office of Prescription Drug Promotion
OPQ = Office of Pharmaceutical Quality
OSE = Office of Surveillance and Epidemiology
OSI = Office of Scientific Investigations

Glossary

AAGL	American Association of Gynecologic Laparoscopists
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AV	atrioventricular
BLA	biologics license application
BMI	body mass index
BP	blood pressure
CFR	Code of Federal Regulations
CI	confidence interval
CMC	chemistry, manufacturing, and controls
CRF	case report form
CSR	clinical study report
DBP	diastolic blood pressure
DMEPA	Division of Medication Error Prevention and Analysis
DPV	Division of Pharmacovigilance
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
EOS	end of study
FAERS	FDA adverse event reporting system
FDA	Food and Drug Administration
GCP	good clinical practice
GEE	generalized estimating equation
GI	gastrointestinal
HR	heart rate
IC	indigo carmine
ICH	International Conference on Harmonization
IM	intramuscular
IND	Investigational New Drug
iPSP	initial Pediatric Study Plan
IRB	institutional review board
IV	intravenous
MA	Marketing Authorization
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
NDA	new drug application
NDC	national drug code

(b) (4)

OCP	Office of Clinical Pharmacology
OECD	Organization for Economic Co-operation and Development
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PeRC	Pediatric Review Committee
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PREA	Pediatric Research Equity Act
PSAS	Physician Satisfaction Agreement Scale
PT	preferred term
SAE	serious adverse event
SAP	statistical analysis plan
SAS	safety analysis set
SBP	systolic blood pressure
TEAE	treatment-emergent adverse event
TTV	time to visualization
UOVS	ureteral orifice visualization scale
USP	United States pharmacopeia
WHO ICTRP	World Health Organization-International Clinical Trial Registry Platform

1. Executive Summary

1.1. Product Introduction

Indigo carmine (IC) was introduced into clinical practice in 1903 (Voelcker 1903). The drug substance in IC is indigotindisulfonate sodium (chemical name: disodium 3,3'-dioxo-[Δ $^{2,2'}$-biindoline]-5,5'-disulfonate), a dark blue solid which is soluble in water. The drug product is a dark blue sterile solution of indigotindisulfonate sodium in (b) (4) with citric acid and sodium citrate as needed to adjust pH. IC is supplied as a 5 mL volume, 8 mg/mL strength, in a sealed brown glass ampule to protect it from light. Both the drug substance and the drug product are the subject of a United States pharmacopeia (USP) monograph and were marketed in the United States as an unapproved product.

IC was originally used as a test of renal function (Lacy et al. 1955). The lack of absorption of indigo carmine by cells (Jung and Kiesslich 1999) accounts for its use in studies assessing renal function. IC's deep blue color has been used intraoperatively to aid in visualization of tissues and structures. IC is approved for marketing in Europe (Marketing Authorization (MA) number PL 43956/0001 (SERB Laboratories 2015)) for the following indication: Indigo carmine is indicated for intra-operative detection of suspected ureteral injuries during abdominal and pelvic surgery. Currently, IC is used as a dye during surgery as an aid to visualize vessels, tissues, and urinary fistulae, and to visualize ureteral efflux via cystoscopy at the end of urological or gynecological surgery performed by robotic, endoscopic, or open surgical methods. Of note, clinical use of IC was interrupted in 2014 because of drug shortage in the United States (U.S.) (Barbieri 2014).

History of IC and the Evolution of Dose Used in Clinical Practice

IC has been widely used as a visualization aid in urology. The first clinical use of IC, was to visualize cystoscopically the urine stream emerging from the ureter (Voelcker 1903). IC was preferred because it is excreted primarily by the kidney and causes dark blue discoloration of urine within 10 min of injection. The use of 4% solution of IC was modified to a 0.4% solution, because the more dilute solution was eliminated more rapidly than the 4% solution. The 0.4% solution in a volume of 20 mL was injected intramuscularly (IM) into the gluteal region (80 mg of indigo carmine), with the onset of blue-colored urine in 5 to 10 minutes.

The administration of 4 mL of a 4% solution of IC injected IM was used to cystoscopically determine the functional capacity of kidneys (Thomas 1909b; Thomas 1909a). The transition to intravenous (IV) injection of IC occurred around 1922 (Harpster 1922); appearance of the dye within 10 to 15 minutes was deemed to be consistent with normally functioning kidneys.

Closed suprapubic prostatectomy for benign prostatic hypertrophy (Harris 1929) was performed only when there was ureteric excretion following 6 mL of 0.8% IC IV or 4 mL of 4% IC IM pre-operatively. Of note, IC was used in this context to determine the adequacy of renal function. The currently used volume of 5 mL of 0.8% IC solution administered IV to test renal

function has been used since 1944 (Douglass and Ransom 1944) and has been recommended by the American Association of Gynecologic Laparoscopists (AAGL) (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012) for the cystoscopic evaluation of ureteral integrity. The historical developments in the use of IC are listed in Table 1 below.

Table 1. Summary of Key Developments in the Administration Route, Dose, and Strength of IC

Year	Author	Route of Administration	Dose	Concentration
1903	Voelcker and Joseph	Intramuscular	4 mL	4% suspension of indigo carmine
1904	Voelcker and Joseph	Intramuscular	20 mL	0.4% solution of indigo carmine
1909	Thomas	Intramuscular	4 mL	4% solution of indigo carmine
1911		Intramuscular	20 mL	0.4% solution of indigo carmine
1922	Harpster	Intravenous	20 mL	5 indigo carmine tablets dissolved in 100 mL of sterile water
1929	Harris	Intravenous	6 mL	0.8% solution of indigo carmine
		Intramuscular	4 mL	4% solution of indigo carmine
1935	Dukes	Intravenous	10 mL	0.4% solution of indigo carmine
1944	Douglass[a]	Intravenous	5 mL	0.8% indigo carmine solution
2010	Barikmo[b]	Intravenous	2.5 mL	0.8% indigo carmine solution

Source: (Voelcker 1903; Voelcker 1904; Thomas 1909b; Thomas 1909a; Thomas 1911; Harpster 1922; Harris 1929; Dukes 1935; Douglass and Ransom 1944; Barikmo et al. 2010)
[a] The dose of 5 mL of 0.8% indigo carmine solution is the current standard for use in cystoscopy.
[b] This study, which used 2.5 mL of 0.8% indigo carmine solution, suggests that a smaller dosage may also provide adequate results.
Abbreviations: IC, indigo carmine

On September 9, 2021, the Applicant submitted new drug application (NDA) 216264 for IC under the 505(b)(2) pathway. The drug was developed under Investigational New Drug (IND) 137856 starting in December 2017.

1.2. Conclusions on the Substantial Evidence of Effectiveness

IC is classified as a diagnostic dye. Evidence submitted with this application demonstrates the efficacy of IC as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecologic surgery. The main support for efficacy was derived from one adequate and well-controlled Phase 3 Study (PVP-19IC01) along with pharmacokinetic data from a Phase 1 Study (PVP-19IC02) and supportive evidence from the scientific literature.

PVP-19IC01 was a randomized, intrapatient controlled, blinded to dose, multicenter, parallel-group study conducted at 7 study sites to evaluate the safety and efficacy of two dose levels (2.5 mL and 5 mL) of IC 0.8% solution for injection when used as an aid in the determination of ureteral patency. Each patient underwent cystoscopy and received 5 mL of 0.9% sodium chloride injection followed by the randomized IC dose. A total of 62 subjects were randomized to the 5 mL group and 59 subjects to the 2.5 mL group. Of these 121 randomized subjects, 60 patients received 5 mL and 58 patients received 2.5 mL of IC respectively. The primary efficacy endpoint was the urine efflux conspicuity score assessed by two blinded, independent, central reviewers using a 5-point ordinal scale. Both high dose and low dose IC injections showed

improved visualization of urine efflux when compared to saline injection. Following administration of IC, approximately 68% of patients in either dose group achieved a score of 4.5 or greater based on the 5-point ordinal scale. The 95% confidence interval (CI) lower limit for the odds ratios between the IC and saline treatments was 7.4, indicating that the odds to observe a score of 5 was at least 6 times greater after IC injection than saline injection. In addition, 92% of subjects were rated as responders with ≥1 -point improvement in the conspicuity score with IC versus saline for either the left or right ureter. Blue color in the ureter efflux was observed in 89% of ureters following high dose and 86% of ureters following low dose IC injections respectively without any statistical difference between the two dose groups.

The U.S. Food and Drug Administration (FDA) statistical team performed analyses to assess inter-reader agreement in the urine efflux conspicuity scores for IC versus saline between Reviewer 1 and Reviewer 2. Statistical tests accounting for ureter and dose suggests that there is a significant reviewer effect (nominal p-value<0.001), namely, scoring by reviewer 1 and reviewer 2 are different. To explore this further, the FDA statistical team conducted a responder analysis and found that the reviewer disagreement in conspicuity scores is less with count of units whose scores exceed a threshold compared to actual numerical scores from the reviewers (Full details are provided in Section 8.3). Most importantly, while there was limited agreement between the two readers in the conspicuity scores of urine efflux, the scores were significantly higher after IC injection when compared to saline injection.

In addition, two prospective studies from the scientific literature provide additional supportive evidence of the clinical utility of IC in the visualization of ureteral efflux to evaluate the integrity of the ureters (Vakili et al. 2005; Ibeanu et al. 2009). The dose of IC used in the studies was not cited in the two publications.

In summary, the Applicant has submitted adequate data that provides substantial evidence of effectiveness for IC as an adjunct for the cystoscopic visualization of urinary flow from the ureteric orifices during urogynecology surgeries for timely diagnosis of iatrogenic ureteral compromise.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Indigo carmine (referred to as IC or Bludigo) is a diagnostic dye indicated for use as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures. Indigo carmine is excreted by the kidneys following intravenous (IV) administration, allowing the urine to be sufficiently colored blue within 10 minutes to aid cystoscopic visual examination of the integrity of the urinary tract. A cystoscope equipped with a lens is inserted into the urethra and advanced up to the bladder. IC, following IV administration, colors the urine blue and enhances the visualization of urine efflux from the ureteral orifices into the bladder. Sluggish or absent efflux of dye from ureteral orifices indicates a potential lack of patency of the ureter or loss of renal function.

In this new drug application, data supporting the efficacy and safety of IC in the indicated patient population are derived primarily from the Phase 3 Study PVP-19IC001 (Study 01). Supporting data are provided by a Phase 1 Study (PVP-20IC02, Study 02) for pharmacokinetics and safety conducted in healthy volunteers, and evidence including safety data from the scientific literature. For the efficacy analysis of Study 01, 121 patients were enrolled, and 118 patients treated. For the safety analysis set (SAS) there were a total of 126 subjects from Study 01 and Study 02. The primary efficacy end point for the Phase 3 study was the urine efflux conspicuity score provided by two central reviewers after independent, blinded viewing of the approximately 10-minute video clip obtained during surgery. The conspicuity score was assessed on a 5-point ordinal scale (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). Following administration of IC, the majority of all observations (approximately 68%) in either dose group achieved a score of 4.5 or higher. In contrast, following administration of saline, only two patients (1%) achieved a score of 4.5 or higher, with 28% of the patients receiving a score of 3.5 and 25% receiving a score of 1. Of note, 32% patients in the 5 mL group and 31% in the 2.5 mL group had a score below 4. In contrast, 98% of patients in the saline group had a score below 4. Both the high dose and low dose IC injections were statistically significantly better than saline injection ($p<0.0001$) in improving visualization of urine efflux. However, the level of concordance between the independent reviewers was suboptimal. The 95% confidence interval (CI) lower limit for the odds ratios between the IC and saline treatments was 7.4 or higher. No statistically significant difference was observed between the two IC doses. In addition, contrary to the abundant scientific literature support for the efficacy of the 5 mL dose, (b) (4)

Regarding safety, no adverse events were observed in the period after saline injection and before IC injection. No deaths occurred and none of the serious adverse events observed were deemed attributable to IC. The most common adverse reactions reported in the two dose groups combined were constipation (5%) and dysuria (4%). Other reactions with lower incidence included nausea, vomiting, abdominal pain, pyrexia,

and alanine aminotransferase (ALT) increase. The occurrence of these reactions was similar in the 2.5 mL dose and 5 mL dose groups. Adverse reactions of special interest observed in the study included hypotension, severe elevation in blood pressure, bradycardia, tachycardia, and hypersensitivity reactions all of which had an incidence of <2%. The characteristics of certain cardiovascular events with onset close to the time of IC injection suggest that the events are caused by pharmacologic effects of the drug. The uncommon occurrence of these adverse events (AEs) and the small total number of subjects (N=118) exposed to IC do not allow at present a full assessment of the incidence and severity of the events by dose group. In addition, a review by OSE/DPV that included the FDA adverse event reporting system (FAERS) database and the scientific literature, identified several reports of cardiovascular serious adverse events (SAEs) and hypersensitivity reactions and one death as a result of myocardial infarction leading to sinus arrhythmia, hypotension, and ventricular tachycardia. To mitigate the risk of these uncommon adverse reactions, the prescribing information (PI) contains -the following warnings.

Cardiovascular Reactions: Severe or life-threatening cardiovascular reactions including cardiac arrest, arrhythmia, asystole, second degree atrioventricular block, hypotension, elevation in blood pressure, bradycardia, and tachycardia have been reported generally within 60 minutes following administration of indigotindisulfonate sodium injection products and required urgent intervention.

Indigotindisulfonate may cause vasoconstriction by interference with vasodilation mediated by nitric oxide dependent mechanisms and by direct vasoconstriction. Indigotindisulfonate may also cause hypotension. Patients with hypertension, heart rate and conduction disorders, or medications causing bradycardia may be at increased risk for elevated blood pressure, hypotension, and bradycardia.

Closely monitor blood pressure and cardiac rhythm during and following the injection of Bludigo. Interrupt administration if reactions are observed.

Hypersensitivity reactions: Serious anaphylactic reactions with hypotension, dyspnea, bronchospasm, urticaria, or erythema have been reported with the use of indigotindisulfonate sodium injection. Bludigo is contraindicated in patients with known hypersensitivity to indigotindisulfonate. Monitor patients for anaphylactic reactions and have emergency equipment and trained personnel readily available.

The Applicant will conduct a clinical trial under a postmarketing commitment (PMC) (b) (4) dose of IC (b) (4) .

The Applicant will conduct a second PMC study to further evaluate the efficacy of IC in patients with renal insufficiency. Given the favorable benefit to risk profile of IC, approval of this NDA for 5 mL dose of IC is recommended with appropriate labeling changes and a clinical and pharmacologic PMC as detailed above.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Accidental injury to the ureters and urinary bladder is a rare but serious complication of abdominopelvic surgeries particularly in minimally invasive laparoscopic procedures with limited field of view of the surgical field. Early recognition, i.e., immediately after the surgical procedure, of a compromise to the integrity of the ureter is critical for timely remediation to improve patient outcome from such injuries. • The use of cystoscopy following gynecological surgeries for hysterectomy, pelvic organ prolapse, and incontinence has been recommended by a number of professional associations, including the American Urogynecology Society (Cohen et al. 2018). In 2012, the American Association of Gynecologic Laparoscopists (AAGL) Practice Committee reported that the risk for lower urinary tract injuries during laparoscopic hysterectomy may be as high as 3% and that most of these injuries are typically detected with intraoperative cystoscopy and recommended that surgeons consider routine use of cystoscopic evaluation following laparoscopic total hysterectomy. • IC has a long history of use as an adjunct in intraoperative cystoscopy. There is more than 100 years of clinical experience with IC administered during cystoscopy that is reported in the scientific literature and provides supportive evidence of its safety and efficacy when administered via the IV route for its use as a visualization aid of the ureteral urinary efflux.	• Iatrogenic ureteral injury is estimated to occur with all types of gynecologic surgery and ranges from 0.2 to 2.6 per 100 cases. Timely intraoperative recognition and immediate repair of urinary tract injuries reduces morbidity and improves outcome from such complications (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012; Cohen et al. 2018). • Several studies have evaluated the utility of routine cystoscopy in diagnosing ureteral injury intra-operatively (Wiskind and Thompson 1995; Gilmour et al. 2006; Gustilo-Ashby et al. 2006; Kratz et al. 2012).
Current Treatment Options	• Options for intra-operative localization of the ureters and visualization of the efflux of urine from the ureters include the use of intra-operative ureteral stents and off-label uses of dyes that cause urine discoloration e.g., methylene blue or fluorescein or use of unapproved dyes (Doyle et al. 2015; Lea et al. 2018). • Visualization may not be adequate with saline injection.	• IC has advantages over other products and techniques that include its history of long-term clinical experience reported in the scientific literature. IC is not absorbed by cells (Jung and Kiesslich 1999) and is readily excreted via kidneys, coloring the urine and allowing surgeons to assess ureteral patency cystoscopically at

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Because of the long history of clinical use, IC has become the standard dye for assessment of ureteral patency during gynecological and urological surgery to visualize the efflux of urine from the ureteral orifices via cystoscopy. Shortages in the supply of IC in the U.S. during 2014 resulted in challenges to practitioners (Barbieri 2014). • Use of ureteral stent is helpful to delineate the ureters to prevent accidental compromise of the ureter during urologic and nonurological surgeries (Rodriguez and Payne 2001; Park et al. 2012).	the end of the surgical procedure. • Though the incidence is small, ureteral stents themselves can injure the ureters directly or by increasing the pliability of the ureter (Kuno et al. 1998). • See Table 2 for additional details.
Benefit	• Data from the Phase 3 study demonstrates that IC is effective in aiding cystoscopic visualization of urine efflux. • Conspicuity scores by central reviewers and by operating surgeons indicate that indigo carmine injection provided better visualization when compared to saline. Central reviewers scored approximately 68% of each IC dose group as a 5 or 4.5 while only 1% achieved a similar score after saline injection. • For either the left or right ureter, 92% of patients showed a ≥1-point improvement in the conspicuity score with indigo carmine compared to saline (indigo carmine – saline ≥1) and a conspicuity score with indigo carmine ≥3 (color contrast or significant jet flow). • No statistically significant differences were observed between the two indigo carmine doses (2.5 mL and 5 mL) in exploratory analyses. • The efficacy of the 5 mL dose of IC is supported by long-term clinical use reported in the scientific literature (b) (4) (b) (4) (b) (4) However, no supportive data for this proposal are provided. Further study is needed to evaluate the optimal dose of IC for patients with various levels of renal insufficiency.	• Considering the incidence of ureteral injuries ranging from 0.2 to 2.6 per 100 cases in patients undergoing urogynecological surgeries, the intra-operative recognition of ureteral injury can help improve surgical outcome (Chi et al. 2016; Cohen et al. 2018). • Results from Study PVP-19IC01 indicate that the primary efficacy endpoint was achieved in all patients and was generally similar among subgroups (e.g., age, sex, race, type of surgery, surgeon specialty) in the clinical trial. • Additional support for IC's effectiveness for this indication is obtained from the scientific literature. • (b) (4) . – Historically, the most used dose of IC in a similar clinical context is 5 mL. The 5 mL dose is the only recommended dose in the labeling of the unapproved product marketed by American

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• The level of agreement in the conspicuity scores between the two independent, blinded central reviewers was suboptimal.	Regent in the U.S. under NDC#0517-0375-05 (American Regent 2022). The 5 mL dose is also listed in the labeling of the non-U.S.-approved product (SERB Laboratories 2015; American Regent 2017) – The dose recommended in the practice guidelines of the American Association of Gynecological Laparoscopists (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012) is 5mL • [redacted (b) (4)] • In addition, the Applicant will conduct a [redacted (b) (4)] study to further evaluate [redacted (b) (4)] in patients with various levels of renal insufficiency.
Risk and Risk Management	• The safety population consisted of patients from the two studies (Study PVP-19IC01 and Study PVP-20IC02). • No deaths, SAEs, or study withdrawals related to IC were reported in studies PVP-19IC01 and PVP-20IC02. • IC induced changes in heart rate (bradycardia) and blood pressure (high or low) and atrioventricular block considered potentially serious and life threatening are reported in the FAERS data base and in the scientific literature. • IC-induced transient, spurious changes in the pulse oximetry readings are reported because of drug interference with the SpO2 measurement process. In general, these spurious changes are not considered serious and are typically very transient and self-limited. • Exploratory analyses of data from the Phase 3 study show IC-induced	• The acute and serious cardiovascular and hypersensitivity reactions to indigo carmine require monitoring and urgent intervention (Cotter et al. 2022). • Accordingly, warnings and precautions and adverse reactions information were placed in the PI to mitigate risk as noted above. • [redacted (b) (4)]

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	increases in systolic blood pressure (SBP) in the 5 mL group, particularly in subjects in the higher body weight range. • Following a review of FAERS data, scientific literature, VigiBase and the Applicant's Clinical Safety Data, the DPV concluded that the following adverse reactions albeit rare are attributed to the administration of IC. These include hypotension with cardiac arrest, sudden blood pressure increase, atrioventricular block second degree, hypersensitivity including anaphylactic reactions, and injection site discoloration.	

1.4. Patient Experience Data

Patient Experience Data Relevant to This Application

				Section of review where discussed, if applicable
☐	The patient experience data that were submitted as part of the application include:			
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient-reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify):		
☐	Patient experience data that were not submitted in the application, but were considered in this review:			
	☐	Input informed from participation in meetings with patient stakeholders		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Other: (Please specify):		
X	**Patient experience data were not submitted as part of this application and was not needed.**			

2. Therapeutic Context

2.1. Analysis of Condition

The AAGL Practice Committee reported that the risk of lower urinary tract injuries during laparoscopic hysterectomy may be as high as 3% and that the majority of these injuries are detected with intraoperative cystoscopy (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012). The potential for compromising the ureter during these surgical procedures typically occurs within the last 5 cm of the uretero-vesical junction. Likewise, in surgeries and procedures related to the urinary bladder it is important to identify the location of the ureteral orifices and bladder trigone to minimize complications. Based on Class II-III evidence, AAGL in their 2012 Practice Guidelines for intraoperative cystoscopy in laparoscopic hysterectomy recommends routine use of cystoscopy with intravenous administration of 5 mL of indigo carmine to evaluate ureteral integrity and function after completing surgery (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012). More recently, the American Urogynecologic Society consensus statement based on clinical evidence concluded that cystoscopy should be performed at the completion of all pelvic reconstructive surgeries, with the exception of operations solely for posterior compartment defects, in order to assess compromise of ureteral patency from inadvertent ligation of ureter in a suture during surgery (Dandolu et al. 2003; Cohen et al. 2018).

2.2. Analysis of Current Treatment Options

It should be noted that currently no drug product has been approved for intra-operative use to observe efflux of urine from the ureteral orifices for assessing the integrity of the ureters during urogynecologic surgeries. Indigo carmine while unapproved has been clinically used with cystoscopy for several decades for this purpose. Other options available for the prevention and or identification of inadvertent intra-operative injuries to the ureters and urinary bladder are summarized below. Two products, phenazopyridine and methylene blue, that received FDA approval for different indications are also used off-label for identification of inadvertent intra-operative injuries to the ureters and urinary bladder. Through NDA 216264, the Applicant seeks approval to market IC for use as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures for timely detection of iatrogenic injuries. IC is currently marketed by American Regent under national drug code (NDC) 00517-0375-05 (American Regent 2022). See Table 2 below.

Table 2. Approaches for Intraoperative Identification of Damage to Ureters During Lower Pelvic Surgery

Product Name	Clinical Use	Dosing	Information on Important Safety and Tolerability Issues
Ureteral stent	Delineation of ureters during surgery.	N/A	-Presence of ureteral stent provides intra-operative guidance. -Invasive procedure with potential injury to the ureters from the stents.
Phenazopyridine	Visualize the reddish-brown discolored urine (b) (4)	100 mg or 200 mg tablet orally	-Used with (b) (4) -Potential for methemoglobinemia and acute hemolytic anemia (b) (4) .
Vitamin B – 2 (riboflavin)	Visualize the yellow discolored urine efflux with cystoscopy.	400 mg of riboflavin orally the night before surgery	Determining the optimal interval between dosing and cystoscopy can be problematic.
10% or 50% Dextrose	Used to fill the urinary bladder during cystoscopy to observe urinary efflux due to the lower viscosity of the urine.	N/A	-In one study(Narasimhulu et al. 2016) 100 mL of 50% dextrose solution was used. -Larger volume recommended for the visualization of the entire urinary bladder. -Potential for urinary tract infection particularly in high-risk patients.
Methylene blue	Visualize the blue-green discolored urine efflux with cystoscopy.	50 mg IV over 5 minutes	-May cause serotonin syndrome in patients receiving SSRIs, SNRIs, or MAOs. -The incidence of adverse reactions has limited its role.
Cystoscopy alone	Visualize ureteral efflux without the aid of a renally-excreted dye.	N/A	-Cystoscopy in abdominopelvic surgeries is routinely used to detect iatrogenic ureteral injury. -Retrospective study by Chi et al. concluded that the practice of universal cystoscopy at the time of hysterectomy for benign indications is associated with decreased incidence of delayed complications(Chi et al. 2016).

Abbreviations: G6PD, glucose 6 phosphatase deficiency; MAO, monoamine oxidase inhibitor; IV, intravenous; SNRI, selective norepinephrine reuptake inh bitor; SSRI, selective serotonin reuptake inhibitors

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

IC has been used in the U.S. as an unapproved drug for several clinical uses including for the assessment of the patency of ureters during urogynecologic surgeries.

3.2. Summary of Presubmission/Submission Regulatory Activity

NDA 216264 for indigo carmine was received on 9/10/2021, from Provepharm Inc. The application regulatory path is 505(b)(2). There are no prescription drug products or discontinued drug products listed in the Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations that contain the active ingredient indigo carmine.

The original IND 137856 application was received on October 23, 2019. A summary of other important milestones in the regulatory history related to this NDA and indigo carmine are detailed below in Table 3.

Table 3. Key Regulatory Interactions for the Clinical Development of Indigo Carmine

SDN Number	Date	Subject	Summary/Comments
0001 and 0002	12/20/2017 02/20/2018	Pre-IND meeting requested by Clinpace	This meeting was to focus on the adequacy of the nonclinical and clinical package to support a planned submission of a 505(b)(2) NDA for the proposed indication.
0004	03/29/2019	Pre-IND Pediatric Response	During the meeting, the Agency recommended that Provepharm provide a written response for the iPSP.
0006	04/26/2019	EOP2 Meeting Request	Provepharm plans to submit a 505(b)(2) application was discussed.
0010-12	09/11-17/2021	iPSP submission	Submission of agreed iPSP and related documents.
0013	10/23/2019	Initial IND application submission 137856	First version of the initial IND
0013	11/21/2019	SMP	IND allowed to proceed
0020	03/19/2020	Type C Meeting WRO	Protocol revisions to PVP-19IC01
0022	04/02/2020	Type C Meeting package	PVP-19IC01 with SAP
0023	04/23/2020	Type C Meeting	The Agency agreed to the use of an ordinal scale for scoring conspicuity of urine efflux and the evaluation of inter- and intrareviewer agreement. In addition, plans for a Responder Tool, blinded, independent reviewer, and a revised SAP were discussed.
0025	09/16/2020	Protocol amendment	- 5-point conspicuity scale for urine efflux -Responder criteria and endpoint -Revised SAP

SDN Number	Date	Subject	Summary/Comments
0028	12/22/2020	New Protocol and New Investigator	PVP-20IC02 for PK study

Source: Applicant Submission 2.5 Clinical Overview Table 1-2.
Abbreviations: EOP2, end of Phase 2; IND, investigational new drug; iPSP, initial Pediatric Study Plan; NDA, new drug application; PK, pharmacokinetic; SAP, statistical analysis plan; SMP, study may proceed; WRO, written response only

The application for indigo carmine is primarily based on one adequate and well-controlled Phase 3 clinical trial PVP-19IC-01 conducted by the Applicant, with additional supporting data on safety from the Applicant-conducted Phase 1 Study PVP-20IC-02 along with scientific literature on indigo carmine. For this NDA, the Guidance for Industry titled: Applications Covered by Section 505(b)(2) (December 1999) is applicable.

During the year 2014, there was a shortage of IC in the U.S. According to the Applicant, prior to this shortage, (b) (4) The shortage in 2014 was due to the lack of raw material. The impact of the 2014 shortage of indigo carmine was felt across the U.S. and urogynecology surgeons began using alternative dyes such as methylene blue, phenazopyridine, and riboflavin to cope with the shortage (Gomella 2012; Barbieri 2014; Aguirre et al. 2015). See Table 2 above for additional discussion of these drugs.

(b) (4)

American Regent began marketing indigo carmine in July 2017 (NDC 00517-0375-05), while Akorn discontinued production of indigo carmine due to shortage of raw material. The product manufactured by American Regent has made the following claim: *"Originally employed as a kidney function test, the chief application of indigo carmine at present is localizing ureteral orifices during cystoscopy and ureteral catheterization."*

4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigation

The Office of Scientific Investigation evaluated the following aspects of the Phase 3 clinical trial.

The data for the central reviewers from the central imaging vendor, (b) (4)., related to Concordance Response Variable between Surgeon's and Central Readers' Assessment of Conspicuity (Listing 16.2.6.3) showed higher differences in interpretations between the onsite surgeons and central reviewing facility for study sites 106 and 202. Overall, the instances of nonagreement for subjects receiving IC were as follows: 31/179 (17%) for the 2.5 mL group and 22/191 (12%) for the 5 mL group.

The Summary of Clinical Efficacy stated that when there was a nonagreement between the two central reviewers, a third independent reviewer served as an adjudicator and, after reviewing the video, provided the final score for the efficacy analysis. The Office of Scientific Investigation (OSI) was requested to review the relevant data base and training manuals to summarize the number of adjudications and the scoring applied in these situations.

Listing 16.2.6.3 indicated that scores from the blinded central reviews were missing for the following study patients: for 2.5 mL, patients (b) (6) and (b) (6); for 5 mL, patients (b) (6) and (b) (6). Additionally, for patient (b) (6) the onsite surgeon scores for the left ureteral orifice were missing but blinded central reviewer scores were provided. OSI audited the source data to account for these absent entries and discrepancies.

The following is the Summary of OSI's overall assessment in the report dated 6/7/2022. Study PVP-19IC01 was audited by good clinical practice (GCP) inspections of three study sites: two clinical investigators (Drs. S.W. Robinson and L.R. Wiegand) selected as the major representative sites to audit general study conduct and the adverse event (AE) data, and an imaging contract research organization ((b) (4).) to verify the primary efficacy endpoint data. No significant GCP violations were identified at the three study sites. Study PVP-19IC01 appears to have been conducted in adequate compliance with GCP regulations and standards. The clinical data generated at the three inspected study sites appear to be acceptable in support of the Applicant's proposed indication for use.

Please refer to the clinical inspection request sent to FDA OSI/Division of Clinical Compliance Evaluation (DCCE) from the Division of Imaging and Radiation Medicine (DIRM), dated 01/11/2022, and the final Clinical Inspection Summary report from the OSI, dated 6/7/2022, for full details.

4.2. Product Quality

The Office of Pharmaceutical Quality (OPQ) recommends approval without a Postmarketing Commitment based on the assessment by the Office of New Drug Products (ONDP) for drug

substance, drug product and biopharmaceutics and the Office of Pharmaceutical Manufacturing Assessment (OPMA) for process, microbiology, and facilities. An expiry of 18 months is granted for storage of the product between 20°C and 25°C (68°F to 77°F).

4.3. Clinical Microbiology

This section is not applicable to this NDA

4.4. Devices and Companion Diagnostic Issues

This section is not applicable to this NDA.

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

Indigo carmine (indigotindisulfonate sodium) is a blue dye that is rapidly excreted in the urine following intravenous administration resulting in visibly blue-colored urine. The Applicant is seeking FDA approval for the use of indigo carmine as a visualization aid in the (b) (4) of the integrity (b) (4) of the ureters (b) (4) urological and gynecological open, robotic, or endoscopic surgical procedures. The maximum clinical mass dose is 40 mg.

The Applicant did not submit pharmacology, absorption, distribution, metabolism, excretion (ADME)/pharmacokinetics (PK), general toxicology, genetic toxicology, carcinogenicity, or reproductive toxicology nonclinical study reports to support the application but did cite and provide manuscripts for the nonclinical data available in the scientific literature. Most International Conference on Harmonization (ICH) M3(R2) recommended nonclinical studies that would support the intravenous route of administration have not been performed with indigo carmine. Many studies have been performed using the oral route of administration as indigo carmine is also used as a food dye. However, based on the very low oral bioavailability of indigo carmine, nonclinical studies by the oral route of administration would not provide adequate support for the intravenous route of administration.

There is inadequate data to evaluate the safety of indigo carmine from a nonclinical perspective. Nonetheless, there is extensive clinical experience with indigo carmine.

5.2. Referenced NDAs, BLAs, DMFs

None.

5.3. Pharmacology

Nonclinical primary pharmacology data were not submitted; however, there is clinical data that the drug product is useful for the proposed indication. Nonclinical secondary pharmacology data were not submitted. Adequate nonclinical safety pharmacology studies have not been performed. Indigo carmine has alpha-adrenergic properties. Transiently increased arterial blood pressure and reflex bradycardia are known possible clinical adverse reactions of indigo carmine administration.

5.4. ADME/PK

Nonclinical ADME/PK study reports were not submitted. The plasma half-life reported in a clinical pharmacokinetic study performed by the Applicant was approximately 12 minutes. Following IV administration to humans, indigo carmine has 2 main metabolites, isatin-5-sulfonic acid and 5-sulfoanthranilic acid, that account for up to 18% of the administered dose. Indigo

carmine is primarily eliminated in the urine unchanged, (b) (4)

5.5. Toxicology

5.5.1. General Toxicology

Indigo carmine is used as a blue dye food additive (21 CFR § 74.102 for FD&C Blue # 2) and most of the referenced nonclinical scientific literature for toxicology studies used the oral route of administration. However, indigo carmine is poorly absorbed following oral administration and can be metabolized somewhat by intestinal bacterial. Therefore, data from oral toxicology studies are not useful for predicting toxicity by the intravenous route. The lone intravenous toxicology study available in the scientific literature was an LD_{50} study in rats. However, LD_{50} studies are not considered adequate for evaluating safety based on current FDA and ICH Guidances.

5.5.2. Genetic Toxicology

A number of scientific studies evaluated the mutagenic potential of indigo carmine; however, most studies were not "*standard battery*" studies as per the ICH S2B Guidance for genotoxicity studies or were "*standard battery*" studies but not performed under current recommended Organization for Economic Co-operation and Development (OECD) guidelines (June 2012). Indigo carmine was not genotoxic in any of the Ames assay studies. However, these studies either did not include the recommended *S. typhimurium* TA102 or *E. coli* WP2 *uvrA* strain or did not follow current OECD guidelines. A mouse L5187Y lymphoma toxicokinetic (TK) +/- assay reported in the scientific literature appeared to have been performed under current OECD guidelines. However, the results of this study were inconclusive as there was an increase in mutation frequency in the presence of S9 but no clear dose relationship in the positive response (an increase in mutation frequency was not observed without S9). Indigo carmine was negative in a recent mouse micronucleus assay performed under current OECD guidelines but using the oral route of administration. From the Nonclinical Reviewer's perspective, additional nonclinical genetic toxicology studies are not needed since indigo carmine: 1) is not absorbed by cells; 2) is rapidly excreted mostly unchanged in the urine; and 3) will likely be administered during a once in a lifetime surgical procedure.

5.5.3. Carcinogenicity

No animal studies have been performed by the Applicant to evaluate the carcinogenic potential of indigo carmine. The Applicant summarized findings from nonclinical scientific literature of carcinogenicity studies that used the oral route of administration. Since indigo carmine is poorly absorbed and can be metabolized somewhat by intestinal bacterial, data from oral carcinogenicity studies are not useful for predicting carcinogenicity for the intravenous route of administration. Since the drug will likely only be used once, nonclinical carcinogenicity studies are not recommended for this application.

5.5.4. Reproductive and Developmental Toxicology

No animal studies have been performed by the Applicant to evaluate reproductive and developmental toxicology of indigo carmine. The Applicant summarized findings from nonclinical scientific literature for reproductive and developmental toxicology studies that used the oral route of administration and, thus, are not useful for predicting toxicity for the intravenous roue of administration. Nonetheless, testicular toxicity was observed in a 6-week study in Swiss albino mice (Dixit and Goyal 2013). Animals administered low (17 mg/kg/day) or high dose (39 mg/kg/day) indigo carmine mixed with feed exhibited significantly decreased absolute and relative testicular weight with tubular diameter and sperm motility also significantly decreased. Indigo carmine also caused a reduction in sperm density that was significant at the high dose. Drug-related microscopic findings included thickening of the tubular basement membrane, arrest of spermatogenesis at the spermatid stage and broken sperm debris in the tubular lumen in low dose animals; dissolution of the tubular basement membrane and exfoliation of cells in the lumen leading to complete testicular blockage was observed in high dose animals. On the other hand, testicular toxicity was not reported in any other toxicity study including other mouse toxicity studies. Thus, the mouse study that resulted in treatment-related testicular toxicity appears to be an outlier finding.

Nonclinical reproductive and developmental toxicology study reports using the intravenous route of administration were not submitted and are not recommended since indigo carmine is rapidly excreted through the urine and the Applicant is recommending (b) (4)

5.5.5. Other Toxicology Studies

The Applicant submitted three special toxicology study reports (an in vitro cytotoxicity study, an in vivo albino guinea pig sensitization study, and an in vivo rabbit intracutaneous study). However, these studies were designed and performed in a manner to support safety of medical devices and are not used by FDA to evaluate the safety of drugs.

The Applicant identified seven potential impurities that could be present in the proposed drug product. The proposed limits for these impurities are shown in Table 4 below. The Applicant evaluated toxicity of impurities by The Derek Nexus System and The Leadscope Genetic Toxicity Suite in silico. This system evaluates if an impurity might significantly differ in toxicity from indigo carmine (parent structure) based on structural alerts and evaluates the relevance of any structural alerts that are present in these impurities but not in indigo carmine.

Table 4. Proposed Impurity Limits

Structure	Specification	Total exposure per injection	Maximal daily exposure
Impurity (b) (4)	NMT (b) (4) %	(b) (4)	(b) (4)
Impurity	NMT %		
Impurity	NMT %		
Impurity	NMT %		
Impurity	NMT %		
Impurity	NMT %		
Impurity	NMT %		

Source: Applicant Table from Section 4.2.3.7.6, *Impurities*
Abbreviations: (b) (4) (b) (4)

The impurity in silico study report identified the maximal daily exposure as (b) (4) mg. The study report stated "(b) (4) However, the consensus of the Review Team was that the maximum daily exposure is 40 mg. Thus, the correct "Maximum daily exposure" column in Table 4 is really the column labeled "Total exposure per injection." (b) (4) qualification threshold limits by 2-fold. The amount of these impurities present in nonclinical safety studies would normally have been considered in the nonclinical evaluation of the impurity (b) (4) proposed limits. However, nonclinical toxicity studies were not performed for this application. No relevant toxicity that was not already observed or predicted in the parent structure was identified with the Derek Nexus System.

(b) (4)

6. Clinical Pharmacology

6.1. Executive Summary

Indigotindisulfonate (indigo carmine) is a dye that is excreted through kidneys and enhances the visualization of the ureteral orifices by its deep blue color. The Applicant seeks approval of indigo carmine as a diagnostic dye for use as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures.

The recommended dose of 5 mL (40 mg/5 mL) of IC is supported by the extensive historical clinical use of indigo carmine over the last several decades as described in the scientific literature and is based primarily on the Applicant's Phase 3 study. Guidelines of the AAGL (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012) recommend the use of this dose.

Indigo carmine is excreted predominantly through kidneys into bladder. No dedicated renal impairment studies were conducted by the Applicant. Since indigo carmine exerts its pharmacological effect (i.e., dark blue coloration of urine) through renal excretion of parent compound, it is expected that patients with renal impairment (e.g., moderate, or severe renal impairment) may have higher risk of loss of efficacy especially at lower dose level due to decreases in absolute amount of indigo carmine excreted into the urine. FDA concluded that the Applicant should conduct a new clinical trial as postmarketing commitment (PMC) to evaluate the efficacy of different dosages of indigo carmine (b) (4) in patients with (b) (4) renal impairment, (b) (4).

The Office of Clinical Pharmacology has reviewed the information submitted in NDA 216264. This NDA is approvable from a clinical pharmacology perspective. The key review issues with specific recommendations/comments are summarized below:

Table 5. Key Review Issues

Review Issue	Recommendations and Comments
Principal and supportive evidence of effectiveness	The primary evidence of efficacy and safety is provided by Phase 3 Study PVP-19IC01 conducted in patients scheduled for urological or gynecological surgical procedures. The PK information is provided by a Phase 1 PK Study PVP-20IC02 conducted in healthy subjects.
General dosing instructions	The recommended dosage is 40 mg/5 mL (8 mg/mL) administered intravenously over 1 minute.
Dosing in patient subgroups (intrinsic and extrinsic factors)	The effects of hepatic impairment or renal impairment on indigo carmine PK have not been studied.
Drug-drug interactions	No dedicated drug interaction study was conducted.
Labeling	(b) (4)

Abbreviations: PI, prescribing information; PK, pharmacokinetic

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Distribution

No formal distribution studies of indigo carmine in human have been performed.

Elimination

In healthy subjects, the elimination half-life of indigo carmine following a single IV dose is approximately 12 minutes.

Metabolism

The primary metabolic pathway of indigo carmine is oxidative metabolism, which causes color fading.

Excretion

Indigo carmine is excreted by the kidney through tubular secretion. Following a single 5 mL IV dose of indigo carmine, the mean amounts of unchanged indigo carmine eliminated in urine and stool were about 16% and <2% of the indigo carmine dose administered, respectively.

Specific Populations

The effects of hepatic impairment or renal impairment on indigo carmine PK have not been studied.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The recommended dosage of indigo carmine is 40 mg/5 mL (8 mg/mL) administered intravenously over 1 minute. The recommendation is supported both by the efficacy study as well as historical clinical experience with indigo carmine reported in the scientific literature.

A scientific literature survey was performed by FDA independently and based on the Applicant's submitted literature in the NDA. The FDA survey showed that the usage of indigo carmine at 40 mg/5 mL (8 mg/mL) was reported in 15 publications from 1944 to 2016, which included one study on efficacy (Douglass and Ransom 1944), one study on PK (Lacy et al. 1955), three studies on adverse reactions (Erickson and Lauron 1960; Erickson and Widmer 1968; Scheller et al. 1986), as well as 10 case studies on adverse events (Harioka et al. 1987; Shir and Raja 1993; Naitoh and Fox 1994; Gousse et al. 2000; Graziano et al. 2005; McDonagh et al. 2007; Kim et al. 2011; Jo et al. 2013; Nandate and Voelzke 2016; Sutton and Pietrzak 2016). The FDA survey also identified the usage of indigo carmine at 20 mg/2.5 mL (8 mg/mL) or 5 mL (4 mg/mL) reported

in one manuscript and one abstract from 2010 to 2016, which included one study on efficacy and one study on adverse reactions.

It should be noted that a number of studies and review articles in the scientific literature including those referred to by the Applicant (Vakili et al. 2005; Ibeanu et al. 2009; Chi et al. 2016) are silent on the dose administered.

Moreover, the current recommended dose described in the practice guidelines of the AAGL, 2012 states that Identification of ureteral function is done by visualizing ureteral ejection of blue dye after the intravenous injection of 5 mL of indigo carmine (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012).

Therapeutic Individualization

Based on comparison of efficacy and safety data across different estimated glomerular filtration rate (eGFR, using the Modification of Diet in Renal Disease) categories in Study PVP-19IC01, no dose adjustment is recommended for patients with mild (eGFR >=60 and <90 mL/min) and moderate (eGFR >=30 and <60 mL/min) renal impairment. No patients with severe renal impairment (eGFR <30 mL/min) were included in the study.

Outstanding Issues

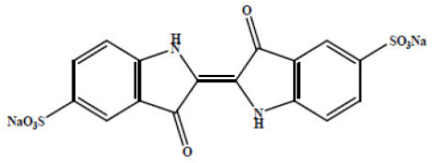
No dedicated renal impairment studies were conducted by the Applicant. Since indigo carmine exerts its pharmacological effect (i.e., dark blue coloration of urine) through renal excretion of parent compound, it is expected that patients with renal impairment (e.g., moderate, or severe renal impairment) may have higher risk of loss of efficacy especially at lower dose level due to decreases in absolute amount of indigo carmine excreted into the urine. FDA concluded that the Applicant should conduct a new clinical trial as postmarketing commitment (PMC) to evaluate the efficacy of different dosages of indigo carmine (b) (4) in patients with (b) (4)

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

In Study PVP-20IC02, the disposition of indigo carmine has been evaluated following an IV injection of 0.8% solution of indigo carmine at 2 dose levels (2.5 mL and 5 mL) in healthy volunteers. The ADME and Clinical PK Information are presented in Table 6.

Table 6. ADME and Clinical PK Information for Indigo Carmine

Parameter	Information	
Physiochemical Properties		
Chemical structure and molecular weight	Molecular Formula: $C_{16}H_8N_2Na_2O_8S_2$ Molecular Weight: 466.35 g/mole CAS Number: 860-22-0 Chemical Structure:  Source: NDA 216264, Module 3.2.S.1-2	
Pharmacology		
Mechanism of action	Indigotindisulfonate (indigo carmine) is a dye excreted through kidneys and enhances visualization of the efflux from ureteral orifices by its deep blue color.	
Imaging time window postdose	Indigo carmine causes dark blue discoloration of urine within 10 min of injection to enhance visualization of the urinary flow from each ureteral orifice.	
QT/QTc prolongation	QT/QTc prolongation was not reported in Study PVP-19IC01	
General Information		
Bioanalysis	Blood, urine, and stool PK samples were analyzed using validated LC-MS/MS assays for indigo carmine (See Section 15.3.1 for detailed information).	
Healthy volunteers vs. patients	Not applicable, no PK data collected from patients.	
Minimal effective dose or exposure	Not applicable	
Maximal tolerated dose or exposure	Not applicable	
Pharmacokinetics	2.5 mL (20 mg)	5 mL (40 mg)
C_{max} (CV%), µg/mL	2.91 (38.3)	6.33 (58.4)
$AUC_{0\text{-}INF}$ (CV%), µg·h/mL	0.564 (35.8)	1.15 (36.4)
Dose proportionality	Dose proportional PK between 20 mg and 40 mg dosages.	
Accumulation	Not applicable, indigo carmine is administered as a single dose.	
Distribution		
Mean (CV%) volume of distribution, L	10.9 (48.1)	10.7 (36.1)
Protein binding, in vitro	Approximately 90%	
Elimination		
Mean (CV%) elimination half-life, minutes	12 (39.8)	12 (34.1)
Mean (CV%) urine clearance, L/hour	7.96 (49.6)	7.08 (66.3)
Mean (CV%) total clearance, L/hour	39.0 (31.9)	40.2 (45.1)
Metabolism		
Primary metabolic pathways	Oxidative metabolism	
Excretion		
Urine (CV%)[a]	17.0% (23.6)	16.0% (44.0)
Feces	<2%	<2%

[a] Unchanged drug
Abbreviations: ADME, absorption, distribution, metabolism, excretion; $AUC_{0\text{-}INF}$, area under the plasma concentration-time curve from time of administration extrapolated to infinity; C_{max}, maximum plasma concentration; CV%, coefficient of variation; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; PK, pharmacokinetic

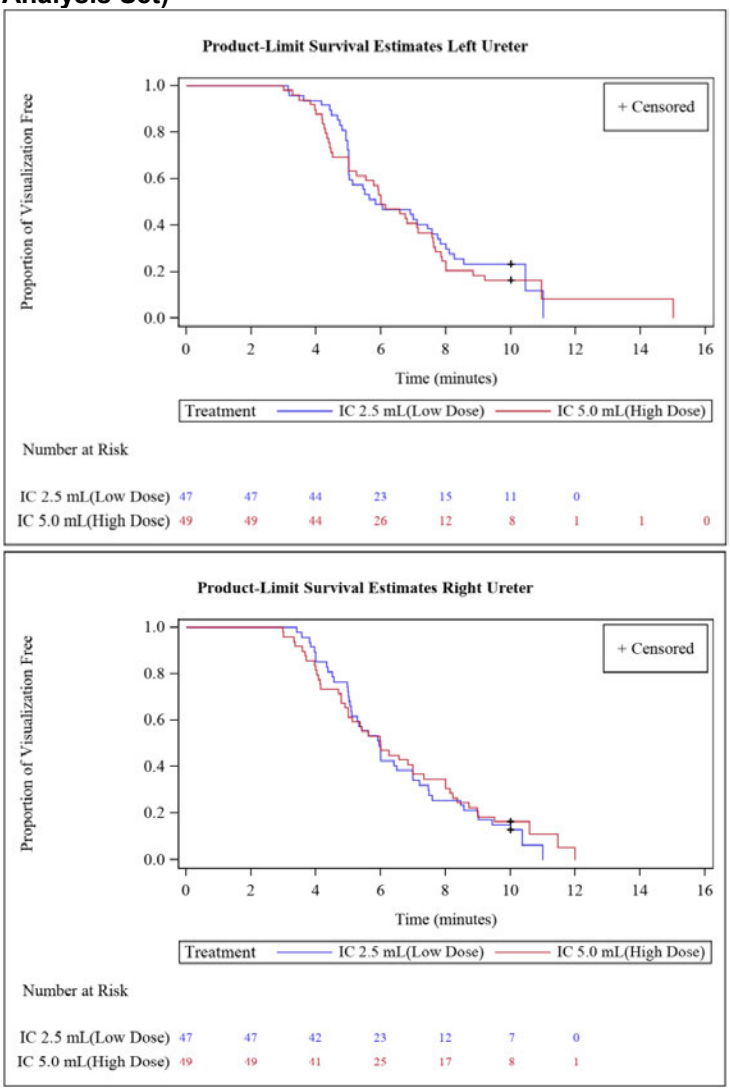
6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

Yes. In Study PVP-19IC01, a total of 121 patients were randomized into 2 dosing groups; 62 patients were randomized to the 5 mL (high dose) group and 59 patients were randomized to the 2.5 mL (low dose) group. Of the 121 randomized patients, 118 (60 high dose, 58 low dose) were administered study drug.

The primary efficacy endpoint was the urine efflux conspicuity score provided by the blinded central review process. Following administration of indigo carmine, the majority of all observations (approximately 68% in both dose groups) were scored as either a 5 or 4.5. Conversely, following administration of saline, 2 subjects (1%) had a score of 5 or 4.5, with 28% having a score of 3.5 and 25% having a score of 1, indicating that no ureter efflux was observed. Scores were generally similar for the left and right ureters. Additionally, the median time [95% CI] to visualization was similar for left and right ureters, which was 6 minutes [5.25, 7.00] in the high dose group and 5.93 minutes [5.12, 7.00] in the low dose group, and the restricted mean was 6.77 and 6.69 minutes, respectively (Figure 1).

Figure 1. Kaplan-Meier Survival Curves of Time to Visualization for Left and Right Ureters (mITT Analysis Set)



Source: Study No. PVP-19IC01 Clinical Study Report Figure 11-2.
Abbreviations: IC, indigo carmine; mITT, modified intent-to-treat

At least 1 treatment-emergent adverse event (TEAE) was reported for 30% of patients administered the indigo carmine high dose and 43% of patients administered the low dose. Four patients (3.4%) experienced SAEs, none of which were considered related to indigo carmine. No deaths or TEAEs leading to discontinuation of study drug or from the study were reported. QT/QTc prolongation was not reported in Study PVP-19IC01.

In Study PVP-20IC02, a total of 16 healthy subjects were randomized into 2 dosing groups; 8 subjects were randomized to the 5 mL (high dose) group and 8 subjects were randomized to the 2.5 mL (low dose) group. Blood, urine, and stool PK samples were collected from all subjects, and were analyzed using a validated LC-MS/MS assay. Results are presented in Table 57, Table 58, and Table 59.

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The proposed single dose of 40 mg/5 mL (8 mg/mL) administered intravenously over 1 minute is appropriate for the patient population for which the indication is being sought.

The recommended dosage of 40 mg/5 mL (8 mg/mL) administered intravenously over 1 minute is also based upon the collective clinical experience, which is also the current standard and is recommended by the AAGL.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

Renal Impairment

No dedicated renal impairment studies were conducted. In Study PVP-19IC01, the efficacy and safety results were re-analyzed following classification of renal function by eGFR. The results (Table 7) suggested that efficacy and safety of indigo carmine are generally balanced among patients with normal renal function (eGFR>=90 mL/min), mild renal impairment (eGFR >=60 and <90 mL/min) and moderate renal impairment (eGFR >=30 and <60 mL/min). It was noticed that in patients with moderate renal impairment (eGFR >=30 and <60 mL/min), the Physician Overall Satisfaction Assessment was 80% (4 out of 5 patients) in the 5 mL dosage group, and 50% (2 out of 4 patients) in the 2.5 mL dosage group. However, the data from patients with moderate renal impairment (eGFR >=30 and <60 mL/min) should be interpreted with caution due to the limited sample size and the subjective nature of the assessment. No patients with severe renal impairment (eGFR <30 mL/min) were included in this study. Since indigo carmine exerts its pharmacological effect (i.e., dark blue discoloration of urine) through renal excretion of parent compound, it is expected that patients with more severe renal impairment (e.g., moderate, or severe renal impairment) may have higher risk of loss of efficacy especially at lower dose level due to decreases in absolute amount of indigo carmine excreted into the urine. As such, the FDA concluded that the Applicant should conduct a new clinical trial as postmarketing commitment (PMC) to evaluate the efficacy of different dosages of indigo carmine (b) (4) (b) (4) in patients with renal impairment (b) (4).

Table 7. Summary of Efficacy and Safety Results by eGFR Subgroup mITT Analysis Set

	Randomized to Indigo Carmine 5 mL				Randomized to Indigo Carmine 2.5 mL			
Parameter	**eGFR ≥90 (mL/min) (N=35)**	**eGFR ≥60 and <90 (mL/min) (N=9)**	**eGFR ≥30 and <60 (mL/min) (N=5)**	**eGFR Pooled (N=49)**	**eGFR ≥90 (mL/min) (N=35)**	**eGFR ≥60 and <90 (mL/min) (N=8)**	**eGFR ≥30 and <60 (mL/min) (N=4)**	**eGFR Pooled (N=47)**
Central conspicuity scores[a]	4.4 (1.19) vs. 2.5 (1.10)	3.6 (1.17) vs. 2.3 (1.19)	2.8 (1.57) vs. 1.5 (0.80)	4.1 (1.34) vs. 2.4 (1.13)	4.5 (0.93) vs. 2.8 (1.02)	3.5 (1.60) vs. 2.4 (1.17)	3.4 (1.21) vs. 2.0 (1.04)	4.2 (1.17) vs. 2.6 (1.06)
Physician overall satisfaction assessment[b]	31 (88.6%)	9 (100.0%)	4 (80.0%)	44 (89.8%)	29 (82.9%)	7 (87.5%)	2 (50.0%)	38 (80.9%)
Time to visualization[c] (min)	6.70 (0.383)	6.13 (0.581)	7.18 (0.350)[d]	6.77 (0.309)	6.24 (0.243)	7.94 (0.711)	8.11 (1.178)	6.69 (0.249)
Responders to indigo carmine treatment[e]	Y: 91.4% N: 8.6%	Y 77.8% N: 22.2%	Y: 80% N: 20%	Y: 87.8% N: 12.2%	Y: 91.4% N: 8.6%	Y: 62.5% N: 37.5%	Y: 100% N: 0	Y: 87.2% N: 12.8%
Patients with ≥1 AE[f]	12 (28.6%)	4 (33.3%)	2(33.3%)	18(30%)	17 (39.5%)	5 (45.5%)	3 (75%)	25 (43.1%)
Serious TEAE[g]	0	0	1(16.7%)	1(1.7%)	2 (4.7%)	0	1 (25%)	3 (5.2%)

Source: Applicant's response to FDA IR conveyed on January 27, 2022.
[a] Data expressed as mean (SD) values of Central Conspicuity Scores following indigo carmine vs. saline treatment.
[b] Data expressed as number (%) patients achieved Overall Satisfaction (score 1 or 2).
[c] Data expressed as Kaplan-Meier Estimate Mean (SE) values.
[d] The mean survival time and its standard error were underestimated because the largest observation was censored and the estimation was restricted to the largest event time.
[e] Data expressed as percentage of patients with scores from either left or right ureter met the criteria of responder. Patients with missing scores from both ureters were included as nonresponders.
[f] Data expressed as number (%) of patients with ≥1 AE.
[g] Data expressed as number (%) of patients with TEAE.
Abbreviations: AE, adverse event; eGFR, estimated glomerular filtration rate; mITT, modified intent-to-treat; N, no; SD, standard deviation; SE, standard error; TEAE, treatment-emergent adverse event; Y, yes

Hepatic Impairment

No dedicated hepatic impairment studies were conducted. The primary metabolic pathway of indigo carmine is oxidative metabolism, which causes color fading. In healthy subjects, the elimination half-life of indigo carmine following a single IV dose is about 12 minutes with the estimated systemic clearance around 40 L/hour. In Study PVP-19IC01, most patients had their bilirubin (111 out of 118), aspartate transaminase (100 out of 118) and alanine transaminase (102 out of 118) value within normal range at baseline. These liver function associated blood chemistry test results also remained within the normal range at the end of study in most patients (See details in Section 8.2.4 Chemistry subsection).

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

Indigo carmine is administered intravenously, clinically relevant food-drug interaction is not expected.

No clinical studies evaluating the drug interaction potential of indigo carmine have been conducted.

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

To support this NDA 216264, the Applicant submitted the following:

- Report from the Applicant conducted Phase 3 clinical Study (PVP-19IC01)
- Report from the Applicant conducted Phase 1 clinical Study for Pharmacokinetic evaluation (PVP-20IC02)

Additional data from the scientific literature suggested that intraoperative cystoscopy improved the ureteral injury detection rate in patients undergoing minimally invasive surgery for gynecologic and pelvic surgeries.

The efficacy evaluation for the use of IC was primarily based on the results of Study PVP-19IC01, the Phase 3, randomized, prospective multi center, parallel group study. On the day of surgery, patients were randomized in a 1:1 ratio to receive a single dose of either low-dose (2.5 mL) or high-dose (5 mL) IC. The surgeon was blinded to the indigo carmine dose. Study patients served as their own control and were first injected with 5 mL of 0.9% saline IV over 1 minute. Videography of the ureteral orifices were taken from the start of the injection for about 10 minutes. This process was repeated following administration of indigo carmine. The videos were assessed by a blinded central review process (2 central reviewers and adjudicator, if needed) based on the 5-point urine efflux conspicuity scale. After the procedure, the surgeons performing the procedure reviewed the videos to provide the 5-point conspicuity score for left and right ureter after each treatment and provided their overall satisfaction with the ability to assess ureteral patency with indigo carmine compared with saline. The surgical rating of conspicuity was utilized for concordance analysis with the ratings by the central reviewers and did not have to occur on the same day as the surgery.

Table 8. Summary of Study PVP-19IC01 and Study PVP-20IC02

Trial Identity	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment	No. of Subjects Enrolled/Treated/Completed	Study Population
Controlled Studies to Support Efficacy and Safety						
PVP-19IC01 (13 February 2020 to 03 June 2021) NCT04228445 Initiated at 14 U.S. sites. Patients Randomized and Treated at 7 Study Sites.	Phase 3, multicenter, randomized, prospective, parallel-group study.	Single dose of 2.5 mL or 5 mL of 8 mg/mL indigo carmine IV at the conclusion of surgery.	Primary efficacy endpoint: the urine efflux conspicuity score provided by the blinded central review process as assessed by a 5-point ordinal scale Secondary endpoints: Proportion of surgeons who agreed that, compared to saline, indigo carmine improved visualization as an aid for the assessment of ureteral patency. Proportion of responders (≥1-point improvement in the conspicuity scores following indigo carmine versus saline injection) assessed separately for each ureter for each subject. Time to visualization (minutes) of blue color in the ureteral efflux after IC injection.	Two dose levels (2.5 mL and 5 mL)	IC 5 mL 62/60/59. IC 2.5 mL 59/58/55. 0.9% of Saline (sum of 2 IC groups) 121/118/114.	Male and female patients ≥18 and ≤85 years old scheduled for urological or gynecological surgical procedures in which the patency of the ureter was to be assessed by the surgeon at the end of the procedure.

Trial Identity	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment	No. of Subjects Enrolled/Treated/Completed	Study Population
PVP-20IC02 (14 January 2021 to 01 March 2021)	An open-label, randomized study to evaluate the PK and excretion of two different doses of 8 mg/mL solution in healthy subjects.	IC 40 mg/5 mL. 2.5 mL (low dose) 5 mL (high dose) administered as IV injection over 1 minute.	The primary objective of this study was to determine the PK profile of IC and 5-sulfo-anthranilic acid, a potential metabolite, following administration of 2 different doses of IC to healthy subjects. The secondary objectives of this study were as follows: • To determine the total excretion of IC and 5-sulfo-anthranilic acid in urine and stool • To evaluate safety.	IC 40 mg/5 mL (2.5 mL or 5 mL) IV	IC 2.5 mL: 8/ 8/ 8 IC: 2.5 mL: 8/ 8/ 8	Healthy male and female subjects ≥18 and ≤70 years old with normal renal function and no history of conditions that interfere with the absorption, distribution, metabolism, or excretion of drugs.

Source: Table 1-1, Clinical Overview 2.5.
Abbreviations: IC, indigo carmine; IV, intravenous; PK, pharmacokinetics

7.2. Review Strategy

Primary evidence of effectiveness and safety for the use of IC was provided by the Phase 3 multicenter trial with supporting evidence by the Phase 1 pharmacokinetic and safety study in healthy volunteers and by the scientific literature.

Assessment of Efficacy Consisted of:

- Review of Phase 3 clinical Study (PVP-19IC01) – confirmatory trial
- Review of Phase 1 clinical Study (PVP-20IC02) – supporting data
- Publications on the role and use of IC
- Verification of data and analyses, evaluation of the trial design and conduct, and patient populations, consistency of primary and secondary outcomes.
- OSI conducted inspections to assess the conduct of the studies. See Section 4.1 for additional details.

The complete reports of these trials submitted by the Applicant and summarized in Section 8.1 of this document were reviewed by the clinical and statistical team. Verification of efficacy analyses and additional exploratory analyses were performed by the statistical team using the data submitted by the Applicant.

Assessment of Safety:

Review of safety data from Study PVP-19IC01 and Study PVP-20IC02 submitted by the Applicant:

- DPV conducted a review of the scientific literature and FAERS data (see Section 8.2)

Additional review and analysis of the scientific literature and other premarketing safety data are provided in Section 8.2.

8. Statistical and Clinical Evaluation

8.1. Review of Individual Trials Used to Support Efficacy

8.1.1. Study PVP-19IC-01

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 0.8% Solution When Used as an Aid in the Determination of Ureteral Patency.

Study Design

This was a Phase 3, prospective, multicenter, parallel-group study to evaluate the safety, efficacy, and pharmacokinetics of indigo carmine injection 0.8% solution when used as an aid in the determination of ureteral patency.

Study Population

The following general criteria were applied.

- Protocol waivers or exemptions were not allowed.
- Individuals who were screen failures could not be rescreened; patients could not have been enrolled more than once or received study drug more than once.

Inclusion Criteria

1. Age ≥18 and ≤85 years old.
2. Signed written, institutional review board (IRB)-approved informed consent form.
3. Scheduled for urological or gynecological surgical procedures in which the patency of the ureter was to be assessed by the surgeon during the procedure.

Exclusion Criteria

1. Stage 4 or 5 chronic kidney failure, as evidenced by an eGFR ≤30 mL/min/1.73 m2 (using the Modification of Diet in Renal Disease), need for dialysis soon, or having only one kidney.
2. Known severe hypersensitivity reactions to indigo carmine or other dyes, including contrast dyes.
3. History of drug or alcohol abuse within 6 months prior to the screening visit.
4. Conditions or concomitant diseases precluding safe participation in the study (e.g., major systemic diseases), as assessed by the investigator.
5. Unable to meet specific protocol requirements (e.g., scheduled visits), uncooperative, or with a condition that could have led to noncompliance with the study procedures.
6. Participant is an investigator, subinvestigator, research assistant, pharmacist, coordinator, or relative of staff directly involved in the conduct of the study.

7. Life expectancy ≤6 months.
8. Need for concomitant treatment that could bias the primary evaluation.
9. Pregnancy or breastfeeding.

Study Drug Doses

The 5-mL 0.8 mg/mL IV dose of IC is the dose most frequently cited in the scientific literature (Pettit and Petrou 1994; Harris et al. 1997; Ribeiro et al. 1999; Jelovsek et al. 2007; Lee and Jang 2012). In addition, 5 mL is the dose recommended by the practice guidelines of the AAGL (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012), and the dose recommended in the labeling of the unapproved product marketed in the U.S. under NDC#0517-0375-05 (American Regent 2022) and the product approved and marketed in the European Union (SERB Laboratories 2015; American Regent 2017). Historically, for a similar clinical context of use, the dose volume of IC used ranged from 2.5 mL to 20 mL and the strength ranged from, 0.4% to 4%. See Table 1 in Section 1.1.

(b) (4) a study published as an abstract (Barikmo et al. 2010) that included 60 patients who were administered 50% of the traditionally accepted dose, 2.5 mL of a 8 mg/mL solution of indigo carmine. In this study, cystoscopic visualization of ureteral efflux the dye was achieved in all 60 patients. However, the Applicant notes that this study was not designed to directly compare the 5-mL dose to the 2.5-mL dose but aimed to evaluate whether 2.5 mL may also provide equivalent visualization of the urinary efflux in a timely manner.

Blinding

An unblinded administrator prepared and administered indigo carmine. Investigators, patients, and central reviewers were blinded to indigo carmine dose. The study was unblinded for the surgeon's comparison of saline versus indigo carmine. The central reviewers were also blinded to the study treatment.

Study Schema

- Screening Period (1 to 30 days prior to surgery)
- Randomization and Dosing (Day 1)
- Safety Follow-up Period (Day 30)

A listing of the study drugs administered in the study is provided in Table 9 below.

Table 9. Study Drugs Administered

Arm Name	Control (open label)	Low Dose (blinded)	High Dose (blinded)
Drug name	0.9% Saline	0.8% Indigo Carmine	0.8% Indigo Carmine
Type	Drug	Drug	Drug
Dose formulation	Pre-filled Syringe	Ampule	Ampule
Unit dose strength(s)	0.9% saline for injection	40 mg/5 mL	40 mg/5 mL
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	not applicable	Indigo Carmine 40 mg/5 mL solution for injection was supplied in 5-mL ampules	Indigo Carmine 40 mg/5 mL solution for injection was supplied in 5-mL ampules

Source: Table 9.1 Page 29 of 112 Clinical study report.
Abbreviations: IMP, investigational medicinal product; IV, intravenous; NIMP, noninvestigational medicinal product

Efficacy Endpoints

Primary Efficacy Endpoint

- The urine efflux conspicuity score for indigo carmine injection compared to the score for saline injection provided by the blinded central review process as assessed by the 5-point ordinal scale.

Secondary Efficacy Endpoints

- Proportion of surgeons who agreed that, compared to saline, indigo carmine improved visualization as an aid for the assessment of ureteral patency, based on responses on the 5-point Physician Satisfaction Agreement Scale (PSAS).
- Proportion of responders; a subject was a responder when there was ≥1-point improvement in the conspicuity scores for indigo carmine compared to saline(indigo carmine – saline ≥1) and the conspicuity score of the indigo carmine injection was 3, 4, or 5. The responder criteria were assessed separately for each ureter for each subject based on the blinded central reviewer's conspicuity score.
- Time to visualization (TTV; minutes) of blue color in the ureteral efflux following indigo carmine treatment.

Other Efficacy Endpoints

- Conspicuity score provided by the operating surgeon.
- Concordance of conspicuity scores between the surgeons' assessments and the blinded central reviewer assessments.
- Comparison of conspicuity score between the indigo carmine high dose and low dose assessed by surgeons blinded to the dose.

Assessment of Endpoints

Conspicuity

The following were recorded:

- Type of surgical procedure/visualization (cystoscopic, robotic, open)
- Time each study drug injection started and ended
- Time of visualization of blue urine following indigo carmine administration
- The amount and type of fluid for procedures requiring fluid instillation into the bladder
- Type, amount, and timing of hydration and use of any diuretics during the procedure.

Physician Overall Rating of Visualization

After the completion of the procedure, the surgeon rated the experience of using indigo carmine for each subject using a 5-point PSAS, that assessed the level of agreement with the following statement:

"Compared to the use of saline treatment, my ability to assess ureteral patency was improved after the addition of indigo carmine."

1 = Strongly Agree; 2 = Agree; 3 = Neither Agree nor Disagree; 4 = Disagree; and 5 = Strongly Disagree

The operating surgeons were considered satisfied with the indigo carmine injection if their rating was either 1 (strongly agree) or 2 (agree).

Videography and Central Review Process

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

Recording began immediately prior to the 0.9% saline injection and continued uninterrupted for 10 minutes. A second video commenced with the injection of indigo carmine and continued uninterrupted for another 10 minutes. If both ureteral orifices could not be recorded simultaneously in the same field of view, the camera alternated between the ureters approximately every 15 to 30 seconds for the entire 10 minutes of each recording.

During videography, the surgeon assessed the patency of the ureters by identifying the efflux of urine from the ureteral orifices; each video was to be recorded for a full 10 minutes, regardless of if and when the ureters were deemed patent. Once identified, the surgeon noted the time of identification of efflux of the first blue urine following indigo carmine injection.

The videos were submitted electronically to a central imaging group, for anonymization and to confirm adequate image quality and adherence to the acquisition parameters. De-identified videos were provided to 2 blinded central reviewers for conspicuity assessment using the same

conspicuity scale used by the operating surgeon. Each ureter efflux was scored for conspicuity, independent of the other ureter; hence, each subject had 4 assessments for ureter efflux conspicuity (2 following saline administration and 2 following indigo carmine administration).

A consistency check between the 2 central reviewers was performed; the 2 reviewers were consistent if their scores for a given ureter were within ±1 point; in this case, the average score of the 2 reviewers was the final score for efficacy analysis. Otherwise, a third reviewer served as an adjudicator. The adjudicator reviewed the questioned video, and the score from the adjudicator was the final score for the efficacy analysis.

The surgeon also reviewed the videos and scored the ureteral efflux using the same conspicuity scale. The surgeon's score was based on a review of the videos and did not have to occur on the day of surgery; the conspicuity score was not to be completed during the procedure. For consistency and ease of comparison between the central reviewer and surgeon conspicuity scores, ureter efflux and conspicuity scores were referred to as left and right based on the perceived orientation on the videos irrespective of the actual orientation of the subject.

Statistical Analysis Plan

All qualified subjects were randomized to receive either low-dose indigo carmine (2.5 mL) or high-dose indigo carmine (5 mL) after receiving a dose of normal saline resulting in 2 randomization groups (IC high dose and IC low dose) and 3 treatment groups (IC high dose, IC low dose, and saline). Data presentation for the conspicuity score analyses was based on the 3 treatment groups and data presentation for the physician overall satisfaction and TTV was based on the 2 randomization groups.

All efficacy analyses were based on the modified intent-to-treat (mITT) analysis set, defined as all subjects who had a surgical procedure to assess ureteral patency, who received both study drugs (saline and indigo carmine), and in whom a video approximately 10 minutes in length was available after each treatment.

Study Hypothesis

There were 2 null hypotheses for the primary efficacy endpoint i.e., no difference in conspicuity score between indigo carmine high dose and saline and no difference in score between indigo carmine low dose and saline. Multiplicity was controlled by the Hochberg method.

The treatment effect of odds ratio of indigo carmine versus saline for the conspicuity score was evaluated via a generalized estimating equation (GEE) for repeated measures. For additional details of Statistical analyses refer to Section 8.3.

Protocol Amendments

Table 10 below summarizes chronologically the protocol amendments related to the protocol submitted for this NDA from 7/1/2019 through 6/26/2021. Study PVP-19IC01 was conducted from 2/13/2020 when the first patient signed the consent through 06/03/2021 the date of last

patient's last visit. Study PVP-20IC02 was conducted from 01/14/2021, when the first subject signed the informed consent through 03/01/2021, the date of the last subject contact.

Table 10. Summary of Protocol Amendments Related to the NDA

Date	Comments
07/01/2019	Original protocol
10/18/2019	• Added a reference cited in the document to the bibliography. • Clarified the timing for prohibited medications, food, supplements. • Clarified packaging for indigo carmine
12/10/2019	• Revised the sample size to include plans to randomize 96 subjects in a 1:1 ratio (48 per indigo carmine dose), including approximately 16 subjects (8 per indigo carmine dose) for PK assessment. • Added the following: documentation of UOVS score following the 0.9% saline injection; urine collection for PK analysis at specified time points; and pregnancy follow-up for pregnancies that may have occurred during the study in female partners of male subjects. • Added a reference to support dose justification.
06/23/2020	• Clarified and updated the videography process: 2 separate 10-minute recordings were to be made and saved as separate video files for the saline and indigo carmine administrations.
09/02/2020	• Revised wording of the primary objective to include the 5-point conspicuity score, with definitions for each score, to provide an objective tool applicable to the visualization of the urine efflux when determining ureteral patency. • Modified the secondary objectives based on the revised conspicuity scale and revised procedures.

Source: Clinical Study Report Section 9.8.1.
Abbreviations: NDA, new drug application; PK, pharmacokinetics; SAP, statistical analysis plan; UOVS, ureteral orifice visualization scale

8.1.1.1. Study Results

Compliance With Good Clinical Practice

The study was performed in compliance with GCP and with oversight from the local IRB, guided by ethical considerations and provisions of Title 21 CFR Part 56 and 21 CFR Part 50 Financial Disclosure.

Financial Disclosures

There was no disclosable information for all the study principal investigators and subinvestigators.

Data Quality and Integrity

The data quality and integrity were considered adequate

Patient Disposition

A total of 121 patients were randomized at 7 study sites; 62 patients were randomized to the 5 mL (high dose) group and 59 patients were randomized to the 2.5 mL (low dose) group. Of the 121 randomized patients, 118 (60 high dose, 58 low dose) were administered study drug. Of the 118 treated patients, 114 patients completed the study; 4 patients prematurely discontinued: 2 were lost to follow-up and 1 patient each withdrew due to physician decision and patient decision. Patient disposition is summarized in Table 11 below.

Table 11. Disposition of Patients by Randomization Group

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose)	2.5 mL (low dose)	Overall
	Number (%) of Subjects		
Randomized (ITT analysis set)	62	59	121
Treated with saline	60 (96.8)	58 (98.3)	118 (97.5)
Treated with indigo carmine	60 (96.8)	58 (98.3)	118 (97.5)
Not treated with saline and indigo carmine	2 (3.2)	1 (1.7)	3 (2.5)
Reason not treated			
Physician decision	1 (1.6)	1 (1.7)	2 (1.7)
Other[a]	1 (1.6)	0	1 (0.8)
Completed the study	59 (95.2)	55 (93.2)	114 (94.2)
Prematurely discontinued the study	3 (4.8)	4 (6.8)	7 (5.8)
Reason for study discontinuation			
Lost to follow-up	1 (1.6)	1 (1.7)	2 (1.7)
Physician decision	1 (1.6)	2 (3.4)	3 (2.5)
Subject decision	0	1 (1.7)	1 (0.8)
Other[a]	1 (1.6)	0	1 (0.8)

Source: Table 14.1.1.1
[a] Patient completed screening and randomization, but plan of care changed, and the planned procedure was not performed, so study drug was not administered (see Listing 15.2.1.1).
Abbreviations: ITT, intent-to-treat

The numbers of randomized patients by study site are shown in Table 12 below. Sites 106, 202 and 101 enrolled the highest number of patients in that order respectively.

Table 12. Number of Randomized Patients by Site for Each Analysis Set

	ITT Set			Safety Set			mITT Set			Efficacy PP Set		
Site ID (Investigator Name)	IC 5.0 mL	IC 2.5 mL	Total	IC 5.0 mL	IC 2.5 mL	Total	IC 5.0 mL	IC 2.5 mL	Total	IC 5.0 mL	IC 2.5 mL	Total
106 (Steven Robison)	18	20	38	17	20	37	14	18	32	14	18	32
202 (Lucas Wiegand)	11	10	21	10	10	20	10	10	20	10	10	20
101 (D. Eric Bolster)	10	10	20	10	10	20	8	7	15	7	7	14
116 (David Talley)	9	8	17	9	7	16	9	7	16	9	7	16
109 (Kalpesh Patel)	5	5	10	5	5	10	3	3	6	2	3	5
102 (Rebecca McCrery)	5	4	9	5	4	9	1	0	1	1	0	1
113 (Richard David)	4	2	6	4	2	6	4	2	6	3	1	4

Source: Table 14.1.1.2
Abbreviations: IC, indigo carmine; ITT, intent-to-treat; mITT, modified intent-to-treat; PP, per protocol

Protocol Deviations

Protocol deviations were reported for 64% of the patients. Most deviations, such as missing posttreatment or laboratory assessments or visits outside the window, were not considered to be important deviations. Six patients (5%) had important deviations. Protocol deviations deemed important are listed in Table 13 below with details .

Table 13. Important Protocol Deviations (Safety Analysis Set)

	Indigo Carmine		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
	Number (%) of Subjects		
Subjects with ≥1 important deviation	4 (6.7)	2 (3.4)	6 (5.1)
Inclusion/exclusion criteria not met	1 (1.7)	1 (1.7)	2 (1.7)
Did not complete eligible criteria confirmation	1 (1.7)	0	1 (0.8)
Treatment initiation without randomization code	1 (1.7)	0	1 (0.8)
Video length was shorter than requirement	1 (1.7)	0	1 (0.8)
Serious adverse event follow-up	0	1 (1.7)	1 (0.8)

Source: Table 14.1.4.
Abbreviations: N, number of subjects in group

Details of important deviations are provided below.

- Patient (b) (6) (high dose) was status post left nephrectomy and met exclusion criterion #1 but was randomized and dispensed study drug before the site identified the deviation.
- Patient (b) (6) (high dose), a 36-year-old female, did not complete a pregnancy test prior to surgery; the pregnancy test order was cancelled by the care team without notifying the study team.
- Randomization of patient (b) (6) (high dose) was delayed due to a system malfunction; the patient had been anesthetized and, after 15 minutes of waiting, the investigator unblinded the dose.
- After saline was injected into patient (b) (6) (high dose), a video malfunction occurred, resulting in no recording initially. The video was restarted but the length of video following the saline injection was 3 minutes, 54 seconds rather than the full 10 minutes.
- Patient (b) (6) (low dose) was 88 years old and did not meet age inclusion criteria.
- SAE follow-up report for patient (b) (6) (low dose) was not submitted within 1 day of receipt of hospital records.

A review of these deviations indicates that in two subjects, (b) (6), the lack of advanced warning to the study team was deemed significant from a safety perspective; however, no adverse events were reported in these patients. The deviations did not affect the study's data quality.

Demographic Characteristics

Demographic characteristics were balanced between the 2 randomization groups. Overall, the majority of the patients were white (89%), non-Hispanic (83%), female (74%), and younger than 65 years of age (70%). Median age was 48 years and age ranged from 20 to 88 years. Median body mass index (BMI) at baseline was 29 kg/m^2 and ranged from 17.2 to 56.7 kg/m^2. See Table 14 and Table 15 below.

Table 14. Demographic Characteristics (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
Age (years)			
Mean (standard deviation)	51.3 (16.85)	51.4 (16.86)	51.3 (16.78)
Median	47.5	48.5	48.0
Minimum, maximum	20, 82	24, 88[a]	20, 88[a]
	Number (%) of Subjects		
Age group			
<65 years	42 (70.0)	41 (70.7)	83 (70.3)
≥65 years	11 (18.3)	12 (20.7)	23 (19.5)
≥75 years	7 (11.7)	5 (8.6)	12 (10.2)
Sex			
Male	18 (30.0)	13 (22.4)	31 (26.3)
Female	42 (70.0)	45 (77.6)	87 (73.7)
Race			
White	56 (93.3)	49 (84.5)	105 (89.0)
Black or African American	4 (6.7)	4 (6.9)	8 (6.8)
Asian	0	2 (3.4)	2 (1.7)
Other	0	1 (1.7)	1 (0.8)
Unknown	0	2 (3.4)	2 (1.7)
Ethnicity			
Hispanic	10 (16.7)	8 (13.8)	18 (15.3)
Non-Hispanic	50 (83.3)	48 (82.8)	98 (83.1)
Unknown	0	2 (3.4)	2 (1.7)

Source: Table 14.1.2.1.
[a] Violation of inclusion criterion#1. (Subjects ≥18 and ≤ 85)
Abbreviations: N, number of subjects in group

Table 15. Additional Baseline Characteristics (SAS)

Characteristics Statistics	IC 5 mL (N=60)	IC 2.5 mL (N=58)	Overall (N=118)
Baseline height (cm)			
n	60	58	118
Mean	166.6	167.9	167.23
SD	10.05	8.2	9.2
Min	144.8	149.9	144.8
Median	165.1	169.5	167.6
Max	187.9	187.9	187.9
Baseline weight (kg)			
n	60	58	118
Mean	83.3	88.7	85.9
SD	20.0	26.5	23.5
Min	49.9	53.5	49.9
Median	80.6	81.6	81.2
Max	154.2	165.5	156.5
Baseline BMI (kg/m^2)			
n	60	58	118
Mean	29.94	31.3	30.6
SD	6.5	8.6	7.6
Min	17.2	20.8	17.2
Median	28.9	28.9	28.9
Max	56.7	55.7	56.7

Source: Table 14.1.2.1 and Listing 16.2.4.1
Abbreviations: BMI, body mass index; IC, indigo carmine; kg, N/n, number of subjects in group; SAS, safety analysis set; SD, standard deviation

Medical and Surgical History

All patients reported at least 1 medical or surgical condition, with 80% reporting prior surgical procedures. The 3 most common surgical procedures were female sterilization (21%), hysterectomy (17%), and cholecystectomy (14%). Medical history conditions reported by >15% of subjects overall included hypertension (37%), anxiety (33%), gastroesophageal reflux disease (30%), depression (28%), menorrhagia (22%), stress urinary incontinence (20%), hypothyroidism (19%), migraine (18%), and seasonal allergy (17%).

Surgery Characteristics

The characteristics of the surgery performed were generally similar between the 2 randomization groups. Overall, the majority of patients had surgery performed cystoscopically (58%) by either a gynecologist (48%) or urologist (44%). Few patients required diuretics (6%), although 56% of patients required fluid to be instilled into the bladder. During surgery, 26 subjects (22%) had a concomitant procedure performed; the most common concomitant procedures were additional cystoscopy (14%), salpingectomy (9%), and adhesiolysis (6%). See Table 16 below.

Table 16. Surgery Characteristics (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
Surgery duration (hours)			
Mean (standard deviation)	0.98 (0.497)	1.25 (1.237)	1.11 (0.943)
Median	0.90	1.00	1.00
Minimum, maximum	0.1, 2.4	0.1, 8.3	0.1, 8.3
Amount (cc) of IV fluid administered during surgery	(N=55)	(N=54)	(N=109)
Mean (standard deviation)	1023.6 (550.66)	1082.0 (651.59)	1052.6 (600.69)
Median	1000.0	1000.0	1000.0
Minimum, maximum	100, 2900	0, 3000	0, 3000
	Number (%) of Subjects		
Method of surgery			
Cystoscopic	37 (61.7)	31 (53.4)	68 (57.6)
Robotic	15 (25.0)	18 (31.0)	33 (28.0)
Open	0	1 (1.7)	1 (0.8)
Laparoscopic	0	1 (1.7)	1 (0.8)
Other	8 (13.3)	7 (12.1)	15 (12.7)
Surgeon's specialty			
Gynecologist	27 (45.0)	30 (51.7)	57 (48.3)
Urogynecologist	4 (6.7)	4 (6.9)	8 (6.8)
Urologist	29 (48.3)	23 (39.7)	52 (44.1)
Other	0	1 (1.7)	1 (0.8)
Were diuretics given? Yes	3 (5.0)	4 (6.9)	7 (5.9)
Was fluid required to be instilled into the bladder? Yes	36 (60.0)	30 (51.7)	66 (55.9)

Source Table: 14.1.5.2
Abbreviations: cc, cubic centimeters; IV, intravenous

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The study drug was administered during the surgical procedure to the patients directly by a designated unblinded administrator. The date and time of each dose (a dose of saline followed by a dose of indigo carmine) administered at the study site were recorded. The IC randomized dose assignment was provided by the randomization system to the designated unblinded site staff.

Prior and Concomitant Therapy - Prohibited Concomitant Medications

Prior therapy included medications a subject received within 30 days of study enrollment. Any medication the patient was receiving at the time of enrollment or received during the study was recorded.

Medications, foods, or supplements that could discolor the urine were prohibited. The length of time required from last dose to study randomization was based on the known duration of the effect on urine coloration. Examples of prohibited medications are presented in Table 17 below:

Table 17. Examples of Prohibited Medications

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	phenindione		cascara
metoclopramide	nefopam		levodopa
triamterene	clofazimine		phenytoin

Source: Table 9-2 of Clinical Study Report.

Efficacy Results

Data Sets Analyzed

The data sets used for efficacy analyses are provided in Table 18 below.

Table 18. Analysis Sets

	Indigo Carmine		
	5.0 mL (high dose) (N=62)	2.5 mL (low dose) (N=59)	Overall (N=121)
	Number (%) of Subjects		
Safety analysis set[a]	60 (96.8)	58 (98.2)	118 (97.5)
Efficacy analysis set (mITT)[b]	49 (79.0)	47 (79.7)	96 (79.3)
Efficacy PP analysis set[c]	46 (74.2)	46 (78.0)	92 (76.0)

Source: Table 14.1.1.1
Abbreviations: mITT, modified intent-to-treat; PP, per protocol.

All efficacy analyses were based on the mITT analysis set.

Primary Efficacy Endpoint

The conspicuity scores for the visualization of urinary efflux after the high and low dose indigo carmine injections were greater than the scores after saline injection ($p<0.0001$). There was no difference in conspicuity scores between the high dose and low dose IC injections. Overall, for the left and right ureters there were a total of 96 observations following indigo carmine high dose injection, 92 observations following indigo carmine low dose injection, and 180 observations following saline injection.

Generally similar differences in conspicuity score favoring indigo carmine were reported in various subgroup analyses (e.g., surgery method, surgeons' specialty, patients' age, sex, and race). Table 19 below shows the distribution of conspicuity scores for IC doses, saline, and left and right ureters separately and combined.

Table 19. Conspicuity Scores Based on the Blinded Central Reviewers' Assessment

	Indigo Carmine 0.8% Solution								
	5.0 mL (high dose) (N=49)			2.5 mL (low dose) (N=47)			Saline (N=96)		
Score[a]	Left	Right	Pooled	Left	Right	Pooled	Left	Right	Pooled
n	49	47	96	46	46	92	91	89	180
	Number (%) of Observations								
1	6 (12.2)	4 (8.5)	10 (10.4)	3 (6.5)	4 (8.7)	7 (7.6)	24 (26.4)	21 (23.6)	45 (25.0)
1.5	3 (6.1)	0	3 (3.1)	0	0	0	9 (9.9)	4 (4.5)	13 (7.2)
2	0	0	0	0	1 (2.2)	1 (1.1)	12 (13.2)	7 (7.9)	19 (10.6)
2.5	1 (2.0)	0	1 (1.0)	1 (2.2)	0	1 (1.1)	3 (3.3)	10 (11.2)	13 (7.2)
3	4 (8.2)	3 (6.4)	7 (7.3)	2 (4.3)	2 (4.3)	4 (4.3)	13 (14.3)	10 (11.2)	23 (12.8)
3.5	2 (4.1)	3 (6.4)	5 (5.2)	6 (13.0)	6 (13.0)	12 (13.0)	24 (26.4)	27 (30.3)	51 (28.3)
4	2 (4.1)	3 (6.4)	5 (5.2)	2 (4.3)	2 (4.3)	4 (4.3)	6 (6.6)	8 (9.0)	14 (7.8)
4.5	8 (16.3)	11 (23.4)	19 (19.8)	7 (15.2)	5 (10.9)	12 (13.0)	0	1 (1.1)	1 (0.6)
5	23 (46.9)	23 (48.9)	46 (47.9)	25 (54.3)	26 (56.5)	51 (55.4)	0	1 (1.1)	1 (0.6)
4.5 or 5	31 (63.3)	34 (72.3)	65 (67.7)	32 (69.6)	31 (67.4)	63 (68.5)	0	2 (2.2)	2 (1.1)
Mean (SD)	3.9 (1.47)	4.3 (1.17)	4.1 (1.34)	4.3 (1.11)	4.2 (1.24)	4.2 (1.17)	2.4 (1.08)	2.6 (1.12)	2.5 (1.10)
Median	4.5	4.5	4.5	5.0	5.0	5.0	2.5	3.0	2.8

Source: Table 14.2.1.1
Ref 'a'- Conspicuity score: 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).
Abbreviations: N, number of subjects in each group; n, number of observations with nonmissing score; SD, standard deviation

It should be noted that because the average conspicuity score of 2 central reviewers was the final score for efficacy analysis when the 2 reviewers were consistent (i.e., their scores for a given ureter/video were within ±1 point), it was possible that conspicuity scores by the central reviewers could also be rated as fractions (see column 1 of Table 19).

Secondary Efficacy Endpoints

Conspicuity Score by Operating Surgeon

Based on the surgeons' assessment, the majority of observations following indigo carmine injection were scored as a 5 (60% in both high and low doses). However, following saline injection only 6 observations (3%) were scored as 5 and 26% were scored as a 1, indicating that no urine efflux was observed. Scores were generally similar for the left and right ureters. See Table 20 below.

Table 20. Conspicuity Score Based on the Surgeons' Assessment

	Indigo Carmine 0.8% Solution						Saline (N=93)		
	5.0 mL (high dose) (N=47)			2.5 mL (low dose) (N=46)					
Score[a]	Left	Right	Pooled	Left	Right	Pooled	Left	Right	Pooled
n	47	47	94	46	46	92	93	93	186
	Number (%) of Observations								
1	7 (14.9)	4 (8.5)	11 (11.7)	4 (8.7)	3 (6.5)	7 (7.6)	28 (30.1)	21 (22.6)	49 (26.3)
2	1 (2.1)	0	1 (1.1)	3 (6.5)	2 (4.3)	5 (5.4)	25 (26.9)	26 (28.0)	51 (27.4)
3	2 (4.3)	2 (4.3)	4 (4.3)	6 (13.0)	3 (6.5)	9 (9.8)	29 (31.2)	34 (36.6)	63 (33.9)
4	10 (21.3)	12 (25.5)	22 (23.4)	6 (13.0)	10 (21.7)	16 (17.4)	9 (9.7)	8 (8.6)	17 (9.1)
5	27 (57.4)	29 (61.7)	56 (59.6)	27 (58.7)	28 (60.9)	55 (59.8)	2 (2.2)	4 (4.3)	6 (3.2)
Mean (SD)	4.0 (1.44)	4.3 (1.16)	4.2 (1.31)	4.1 (1.34)	4.3 (1.18)	4.2 (1.26)	2.3 (1.06)	2.4 (1.07)	2.4 (1.07)
Median	5.0	5.0	5.0	5.0	5.0	5.0	2.0	2.0	2.0

Source: Table 14.2.5.1
Ref 'a'- Conspicuity score: 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).
Abbreviations: N, number of subjects in each group; n, number of observations with nonmissing score; SD, standard deviation

Concordance between the central reviewers' and surgeons' conspicuity scores for the left and right ureters was 91% and 96%, for the high dose and 88% and 86% for the low dose respectively. There was no difference in dichotomized concordance between the central readers' and surgeons' assessments of conspicuity score (nominal p=0.2920). See Table 21 below

Table 21. Concordance Between Surgeons and Central Reviewers' Assessment of Conspicuity Score

		Indigo Carmine 0.8% Solution		
Analysis	Summary[a]	5.0 mL (high dose) (N=45)	2.5 mL (low dose) (N=43)	Saline (N=88)
Left Ureter	Total Number of Pairs	45	43	88
	Number (%) Agreement	41 (91.1)	38 (88.4)	67 (76.1)
	Number (%) Disagreement	4 (8.9)	5 (11.6)	21 (23.9)
Right Ureter	Total Number of Pairs	45	43	88
	Number (%) Agreement	43 (95.6)	37 (86.0)	63 (71.6)
	Number (%) Disagreement	2 (4.4)	6 (14.0)	25 (28.4)

Source Table 14.2.5.3
Reference 'a'- If the difference between raters in conspicuity score was within ±1 (i.e., the difference ranged from -1 to +1, inclusive), the scores were considered to agree.
Abbreviations: N, number of subjects in each group

Physician's Overall Assessment of Conspicuity

The physician's overall assessment of conspicuity of indigo carmine is a subjective endpoint. The proportion of favorable responses (PSAS score of 1 or 2) was 90% for the high dose and 81% for the low dose. The proportion of physicians satisfied with the high dose and the low dose was numerically similar. See Table 22 below.

Table 22. Physician's Overall Assessment of Conspicuity

Physician's Response to Statement: "Compared to the saline treatment, my ability to assess ureter patency was improved after the addition of indigo carmine."	Indigo Carmine		
	5.0 mL (high dose) (N=49)	2.5 mL (low dose) (N=47)	Overall (N=96)
	Number (%) of Subjects		
1 Strongly agree	36 (73.5)	34 (72.3)	70 (72.9)
2 Agree	8 (16.3)	4 (8.5)	12 (12.5)
3 Neither agree nor disagree	3 (6.1)	3 (6.4)	6 (6.3)
4 Disagree	0	1 (2.1)	1 (1.0)
5 Strongly disagree	2 (4.1)	5 (10.6)	7 (7.3)
Overall satisfaction (score of 1 or 2)	44 (89.8)	38 (80.9)	82 (85.4)
95% CI using Wilson confidence limits	[78.2, 95.6]	[67.5, 89.6]	[77.0, 91.1]
Binomial superiority test: H0=0.5 vs H0 >0.5	<0.0001	<0.0001	<0.0001
CMH test: row mean score difference (Indigo carmine: 5.0 mL vs 2.5 mL)	0.2807		

Source Table 14.2.2
Abbreviations: CI, confidence interval; CMH, Cochran-Mantel-Haenszel; N, number of subjects in each group

Time to Visualization of Ureteral Efflux of Dye

Time To Visualization was achieved when the surgeon unequivocally visualized the ureter efflux within 10 minutes after the injection start. Each patient contributed 2 independent TTV observations, 1 for the left ureter and 1 for the right ureter. TTV (in minutes) of blue color in the ureter efflux was evaluated using the Kaplan-Meier survival analysis approach. The median times to visualization of the two IC doses were similar (6 min). Refer to Clinical Pharmacology for details (see Section 6).

Comparison Between IC High Dose and Low Dose for Efficacy Outcomes

Exploratory analyses showed no difference between the 2 doses of indigo carmine in all efficacy variables.

Covariate Analyses of Primary Efficacy Endpoint

The potential impact of covariates related to demographic and surgery characteristics on the primary efficacy endpoint was evaluated. The covariates included surgery method, surgeons' specialty type, age group, sex, and race group. Each variable was assessed individually by adding the variable to the primary efficacy models.

The overall (left and right ureter) group means and medians of the conspicuity scores for each covariate subgroup are provided in Table 23. Within each subgroup, scores for both indigo carmine doses were higher than the scores for saline. Within a given treatment (IC high dose, IC low dose, or saline), the magnitude of the difference in median score and mean score between 2 subgroups of a covariate was ≤1 point, with 1 exception in the subgroup by sex. The difference in median conspicuity score between females and males was less than 1 point for IC

high dose and was 1.5 points for the low dose (median scores were 5.0 for females and 3.5 for males).

Statistically significant treatment-by-surgical specialty interaction (nominal p=0.0064) and treatment-by-sex interaction (nominal p=0.0181) were also observed for the low dose group. The nature of the interaction was investigated by further inspection of the differences between the subgroups within each treatment. The interaction was due to the size of the difference and not the direction of the difference. That is, the magnitude of the difference between the subgroups defined by surgical specialty and by gender was associated with treatment, but the score was always higher following indigo carmine injection than saline injection in each subgroup.

The effect of treatment, that is, the difference in conspicuity score between the indigo carmine and saline groups, was statistically significant in all covariate analyses, indicating that, although the score in 1 subgroup could be higher than the score in another subgroup, the indigo carmine treatment effect on improving the visualization of urine efflux over saline was not changed after the adjustment of the impact of covariates. See Table 23 below.

Table 23. Mean and Median Conspicuity Scores Overall (Left or Right Ureter) by Subgroup (mITT Analysis Set)

	Conspicuity Overall (Left or Right Ureter) by Treatment[a] and Subgroup Within a Covariate														
	Method[b]			Specialty[c]			Age[d]			Sex[e]			Race[f]		
	5.0 mL	2.5 mL	Saline	5.0 mL	2.5 mL	Saline	5.0 mL	2.5 mL	Saline	5.0 mL	2.5 mL	Saline	5.0 mL	2.5 mL	Saline
Group 1															
Mean (SD)	3.8 (1.45)	4.0 (1.19)	2.3 (1.06)	4.4 (1.18)	4.6 (1.02)	2.8 (1.12)	4.2 (1.32)	4.3 (1.26)	2.6 (1.10)	4.2 (1.26)	4.4 (1.13)	2.5 (1.12)	4.9 (0.18)	4.2 (1.25)	2.8 (0.76)
Median	4.5	4.5	2.0	5.0	5.0	3.0	5.0	5.0	3.0	5.0	5.0	3.0	5.0	5.0	3.0
Group 2															
Mean (SD)	4.5 (0.99)	4.5 (1.10)	2.8 (1.10)	3.8 (1.40)	3.8 (1.21)	2.2 (1.00)	3.7 (1.31)	4.1 (0.96)	2.1 (1.01)	3.8 (1.49)	3.7 (1.13)	2.3 (1.04)	4.0 (1.37)	4.3 (1.16)	2.4 (1.14)
Median	5.0	5.0	3.0	4.5	4.3	2.0	4.5	4.5	2.0	4.5	3.5	2.5	4.5	5.0	2.5

Source: Table 14.2.6.1.1, Table 14.2.6.2.1, Table 14.2.6.3.1, Table 14.2.6.4.1, and Table 14.2.6.5.1
[a] Treatment Group: indigo carmine 5 mL (high dose); indigo carmine 2.5 mL (low dose); saline (pooled across indigo carmine high and low doses)
[b] Surgery Method: Group 1 = Cystoscopic (N=56); Group 2 = Not Cystoscopic (N=40)
[c] Surgeon Specialty: Group 1 = Gynecologist (N=48); Group 2 = Urologist (N=48)
[d] Age: Group 1 = Age <65 years (N=67); Group 2 = Age ≥65 years (N=29)
[e] Sex: Group 1 = Female (N=67); Group 2 = Male (N=29)
[f] Race: Group 1 = Non-white (N=13); Group 2 = White (N=83)
Abbreviations: mITT, modified intent-to-treat; SD, standard deviation

Proportion of Responders by Central Reviewers' Assessment

The proportion of responders, assessed separately for each ureter for each patient based on the blinded central reviewer's conspicuity score, was a secondary efficacy endpoint. A patient was a responder if there was ≥1-point improvement in the conspicuity scores favoring indigo carmine (indigo carmine – saline ≥1) and the conspicuity score for indigo carmine was ≥3 (color contrast or significant jet flow). For each ureter and each indigo carmine dose, approximately three-quarters of the subjects were rated as responders (Table 24 below). Overall, 92% of subjects were rated as a responder for either the left or right ureter. A sensitivity analysis in which missing data were counted as nonresponse resulted in 88% of subjects being rated as a

responder for either the left or right ureter, with 76% of left ureters and 71% of right ureters rated as a responder.

Table 24. Proportion of Responders to Indigo Carmine Treatment Based on Central Reviewers' Assessment (mITT Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=49)	2.5 mL (low dose) (N=47)	Overall (N=96)
Responder	Number (%) of Subjects		
Subjects by left ureter			
Yes	36 (75.0)	37 (86.0)	73 (80.2)
No	12 (25.0)	6 (14.0)	18 (19.8)
Missing score	1	4	5
Subjects by right ureter			
Yes	36 (78.3)	32 (74.4)	68 (76.4)
No	10 (21.7)	11 (25.6)	21 (23.6)
Missing score	3	4	7
Overall subjects (either left or right)[a]			
Yes	43 (89.6)	41 (95.3)	84 (92.3)
95% CI	[80.9, 98.2]	[89.1, 100]	[86.8, 97.8]
Difference (high vs low)	-5.8		
95% CI	[-16.5, 4.9]		
Sensitivity analysis: Overall subjects (either left or right)[b]			
Yes	43 (87.8)	41 (87.2)	84 (87.5)
95% CI	[78.6, 96.9]	[77.7, 96.8]	[80.9, 94.1]
Difference (high vs low)	0.5		
95% CI	[-12.7, 13.8]		

Source: Table 14.2.3.1 and Table 14.2.3.2
[a] Primary analysis in which missing data were excluded.
[b] Sensitivity analysis in which missing data were imputed as not responder.
Abbreviations: CI, confidence interval; mITT, modified intent-to-treat

Durability of Response and Persistence of Effect

Following IV administration, indigo carmine is excreted by the kidneys, allowing the urine to be sufficiently colored blue within approximately 10 minutes. It appears that these characteristics, short duration of action and clearance appear to make it a suitable agent for timely assessment of ureteral patency during surgery. For additional details, see Clinical Pharmacology Section 6.

Additional Analyses Conducted on the Individual Trial

Please refer to Statistical Section 8.3.

8.1.2. Assessment of Efficacy Across Trials

This section is not applicable because a single adequate and well-controlled Study (PVP-19IC-01) assessed the efficacy of IC.

8.1.3. Review of the Scientific Literature

The Applicant conducted a systematic review of the scientific literature using the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) and meta-analysis guidelines (Liberati et al. 2009). The search focused on controlled studies of cystoscopy with or without the use of indigo carmine to compare detection rates for injury to the ureter and bladder using the following databases and search engines: Pubmed/PubMed Central, OVID, Cochrane Central Trials register, Clintrials.gov, and World Health Organization-International Clinical Trial Registry Platform (WHO ICTRP). A total of 474 unique article citations were obtained by the Applicant. Additional references were discovered during review of these initial articles. Neither the initial searches nor the follow-on searches identified an adequate and well-controlled clinical study that directly compared the diagnostic performance of cystoscopy with or without the use of indigo carmine.

The scientific literature search identified 30 studies between 1990 and 2020, covering a total of 9,850 patients, who routinely underwent cystoscopy with indigo carmine to detect iatrogenic lower urinary tract injury during abdominal and pelvic surgery. Most of the studies were retrospective series. The prevalence of injury was 2.3%; the studies were generally not adequate for a reliable assessment of injury detection rates with IC. See the Meta-Analysis Publications Lists (FDA 2022).

A total of 11 published studies provided some supportive evidence of the value of cystoscopy with or without a dye for detection of ureteral and bladder injuries. However, the studies were generally not designed to evaluate the contribution of IC to the detection of the injury.

Among these studies, three systematic reviews examined the value of intraoperative cystoscopy during urogenital surgeries (Gilmour et al. 1999; Gilmour et al. 2006; Teeluckdharry et al. 2015). These reviews suggest that rates of intraoperative detection of ureteral injury are higher in patients who undergo cystoscopy. Moreover, two prospective studies (Vakili et al. 2005; Ibeanu et al. 2009; Chi et al. 2016) showed a high rate of ureteral injury detection with cystoscopy using IC.

A retrospective study (Chi et al. 2016) compared pre- and post-universal cystoscopy rates of urologic injury detection with inconclusive results. Studies comparing current urologic injury detection rates to historical precystoscopy rates are not interpretable because of multiple confounding factors. For example, a longitudinal observational study (Makinen et al. 2013) reported a decrease over a decade in overall surgical complications (including ureteral injury) post hysterectomy. The decrease is attributable to multiple factors including changes in medical and surgical practice and patient populations.

The Applicant also identified information useful for selection of IC doses (Barikmo et al. 2010), route, and timing of administration and visualization for the development of the clinical study protocol.

8.1.4. Study PVP-20IC02

Study PVP-20IC02 was a Phase 1, single-dose, open-label, randomized study to evaluate the PK, excretion, and safety of 2 different doses (2.5 mL and 5 mL) of indigo carmine 0.8% solution for injection administered in 16 healthy subjects. The study consisted of a screening period, check-in, treatment period, and a follow-up visit at the end of the study.

Overall Study Design

On Day 1, subjects were randomly assigned in a 1:1 ratio and received a single IV dose of either an IC high dose (5,mL) or IC low dose (2.5 mL). Subjects were confined to the clinical unit from Day -1 until discharge on Day 2. The follow-up visit occurred on Day 8. Male and female subjects aged 18 to 70 years, with a BMI of 18 to 35 kg/m^2, were enrolled.

Safety was assessed by evaluation of AEs, clinical laboratory testing (hematology, serum chemistry, and urinalysis), vital signs, and 12-lead electrocardiogram (ECG). All AEs observed after study drug dosing were reported regardless of the relationship to study drug or clinical significance.

8.1.4.1. Study Results

Subject Disposition

Subject disposition is summarized in Table 25 below. All study subjects completed the study.

Table 25. Summary of Subject Disposition (Safety Population)

Table 10–1 Summary of Subject Disposition (Safety Population)

	IC 2.5 mL (N=8) n (%)	IC 5.0 mL (N=8) n (%)	Total (N=16) n (%)
Total Number of Subjects			
Completed	8 (100.0)	8 (100.0)	16 (100.0)
Discontinued	0	0	0
Analysis Populations			
Safety Population[a]	8 (100.0)	8 (100.0)	16 (100.0)
PK Population[b]	8 (100.0)	8 (100.0)	16 (100.0)

Source: End of text table 14.1.1.
[a] The safety population included all subjects who received at least 1 dose of study drug.
[b] The PK population included all subjects who received at least 1 dose of IC and had sufficient concentration data to support accurate estimation of at least 1 PK parameter.
Percentages were based on the number of subjects in the safety population.
Abbreviations: IC, indigo carmine; PK, pharmacokinetic.

Demographic and Other Baseline Characteristics

The majority of the subjects were male (62%), Black or African American (62%), and not Hispanic or Latino (88%). Subjects had a mean age of 45 years (range 20 to 66 years), mean body weight of 80.6 kg, and mean BMI of 27.03 kg/m^2. See Table 26 below.

Table 26. Demographic and Baseline Characteristics (Safety Population)

	IC 2.5 mL (N=8)	IC 5.0 mL (N=8)	Total (N=16)
Age (years)			
n	8	8	16
Mean (SD)	43.0 (15.78)	46.1 (15.84)	44.6 (15.36)
Median	44.5	49.0	47.0
Min, Max	20, 62	23, 66	20, 66
Sex, n (%)			
Male	5 (62.5)	5 (62.5)	10 (62.5)
Female	3 (37.5)	3 (37.5)	6 (37.5)
Race, n (%)			
Black or African American	6 (75.0)	4 (50.0)	10 (62.5)
White	2 (25.0)	4 (50.0)	6 (37.5)
Ethnicity, n (%)			
Not Hispanic or Latino	6 (75.0)	8 (100.0)	14 (87.5)
Hispanic or Latino	2 (25.0)	0	2 (12.5)
Height (cm)			
n	8	8	16
Mean (SD)	175.55 (8.819)	168.66 (3.985)	172.11 (7.507)
Median	172.55	169.50	169.80
Min, Max	166.0, 187.0	162.0, 174.0	162.0, 187.0
Weight (kg)			
n	8	8	16
Mean (SD)	84.05 (20.567)	77.06 (11.237)	80.56 (16.412)
Median	82.85	79.20	79.20
Min, Max	58.7, 108.1	55.4, 89.5	55.4, 108.1
Body Mass Index (kg/m^2)			
n	8	8	16
Mean (SD)	27.06 (5.158)	27.00 (3.143)	27.03 (4.126)
Median	26.80	27.75	27.75
Min, Max	20.7, 33.6	21.1, 30.6	20.7, 33.6

Source: End-of-Text Table 14.1.2.
Percentages were based on the number of subjects in the safety population.
Abbreviations: IC, indigo carmine; max, maximum; min, minimum; SD, standard deviation

Serial blood (plasma), urine, and stool PK samples were collected for analysis of IC and 5-sulfo-anthranilic acid at multiple time points. Plasma, urine, and stool PK parameters for IC and 5-

sulfo-anthranilic acid were calculated as endpoints. Details are provided in Clinical Pharmacology Section 6.

Data Quality Assurance for PVP-19IC01 and PVP-20IC02:

All aspects of the study were monitored for compliance with applicable government regulatory requirements, current Good Clinical Practice, the protocol, and standard operating procedures. Electronic CRFs and electronic data capture compliant with 21 CFR Part 11 were utilized.

8.2. Review of Safety

8.2.1. Safety Review Approach

Safety evaluation for this NDA included the following.

- Review of safety data collected in the Phase 3 Study PIV-19IC01 and the supporting Phase 1 Study PVP-20IC-02 along with data and information obtained from the scientific literature on the clinical use of indigo carmine over several decades.
- Supplemental information obtained by request including the potential for drug induced changes to cardiovascular function and interference with oximetry measurements.
- Systematic review by the Division of Pharmacovigilance (DPV)/Office of Surveillance and Epidemiology (OSE).

  In this Review of Safety, the safety data in healthy volunteers (Study PVP-20IC-02) will be presented first (see Section 8.2.3), followed by safety data in the indicated patient population (Study PIV-19IC01) (See Section 8.2.4) and by safety reports in the scientific literature (see Section 8.2.5). For the systematic safety review by DPV see Section 8.2.10.

Categorization of Adverse Events

Adverse Effects were classified using standard terminology from the verbatim description according to Medical Dictionary for Regulatory Activities (MedDRA) Coding Dictionary (Version 22.1. TEAEs were reported as mild, moderate, severe, life-threatening, or fatal and classified as short-term (within 1 week of surgery), midterm (within the first 6 weeks) and long-term. Adverse events reported from Day 1 beginning at the saline injection through the final follow-up were considered TEAEs. Adverse events reported after saline injection and prior to IC injection were summarized with saline treatment, while AEs after indigo carmine injection, including events reported during the follow-up period, were summarized with IC treatment.

Participating investigators assessed the severity of each AE and their relationship to study drugs using standard categories and definitions. Serious adverse events of special interest were also summarized. Adverse events of special interest were summarized because of their association with indigo carmine in clinical use. These events included transient elevation of blood pressure and reflex bradycardia, tachycardia, hypotension, hypertension, rash or erythema, and

respiratory symptoms, such as dyspnea or bronchospasm. All AEs were reviewed before database lock to identify AEs of special interest.

The adverse events could be divided into drug-related events and procedure-related events and were reported at the patient level. The adverse events were tabulated by MedDRA system organ class and preferred term (PT), by relationship to investigational product and by intensity. Subgroup analyses of adverse events were performed by gender, age group, race, ethnicity, BMI, and study sites.

8.2.2. Adequacy of Applicant's Clinical Safety Assessments

Data Integrity and Quality for Studies PVP-19IC01 and PVP-20IC02

The primary safety data in this Summary of Clinical Safety are derived from the Phase 3 Study in the indicated patient population. The quality of the study data is acceptable. Data from drug exposure in healthy volunteers in the phase 1 study and reports from FAERS and the scientific literature contributed to the assessment of safety. Collectively these data provide an overview of laboratory data and adverse events for IC and allow exploration of findings across groups based on age, race, ethnicity, BMI, disease stage, and surgery types. Descriptive statistics are used to describe demographic and baseline characteristics.

Adequacy of the Safety Database

We deem the safety data submitted by the Applicant together with a safety review conducted by the DPV/OSE adequate to evaluate the safety of the proposed use of IC. Since the Applicant's clinical trials (PVP-19IC01 and PVP-20IC02) did not include pediatric patients, the Applicant will be required to conduct a clinical trial in the pediatric patient population (2 years to 18 years of age) per the Agreed initial Pediatric Study Plans (iPSP) as part of a Pediatric postmarketing requirement (PMR).

Overall Exposure

In Study PVP-19IC01 the 118 IC treated subjects (60 high dose, 58 low dose) comprised the safety analysis set. Of the 118 treated patients, 114 patients completed the study. Patient disposition for Study PVP-19IC01 is summarized in Table 11. A total of 16 subjects were enrolled in Study PVP-20IC-02, and all subjects completed the study. All 16 subjects were included in the safety population.

8.2.3. Safety Results Study PVP-20IC02

This Phase 1, single-dose, open-label, randomized study was designed to evaluate the PK, excretion, and safety of 2 different doses (2.5 mL and 5 mL) of IC 0.8% solution for injection when administered to healthy subjects. Subjects fasted overnight before study drug administration and maintained fast for 4 hours after dosing with study drug. Subjects were confined to the clinical unit from Day -1 until discharge on Day 2. The follow-up visit occurred on Day 8.

Sixteen healthy male and female subjects (mean age: 44.6 years; range: 20 to 66 years) were enrolled in the study: 8 subjects each received a single IV dose of indigo carmine 5 mL and indigo carmine 2.5 mL. All subjects completed the study.

No deaths or SAEs were reported during the study, and no subject had a TEAE that led to early discontinuation from the study. All TEAEs resolved by end of study (EOS). The mean vital sign measurements and mean changes from baseline were generally similar at all time points assessed. No treatment-related trends were observed in vital sign measurements, and no individual vital sign measurement was reported as a TEAE by the Investigator. No abnormal ECG finding was considered clinically significant or reported as a TEAE by the Investigator.

Summary of Adverse Events

- Overall, 3 of the 16 subjects (19%) reported a total of 3 TEAEs: 2 subjects in the IC 2.5 mL and 1 subject in the IC 5 mL group.
- The TEAEs included presyncope considered related (two subjects) and headache considered unrelated (one subject).
- Except for one moderate TEAE of presyncope, all TEAEs were mild in severity.

An overall summary of adverse events for subjects is provided in Table 27.

Table 27. Overall Summary of Adverse Events (Safety Population) PVP-20IC02

	IC 2.5 mL (N=8) n (%) [E]	IC 5.0 mL (N=8) n (%) [E]	Total (N=16) n (%) [E]
Any TEAE	2 (25.0) [2]	1 (12.5) [1]	3 (18.8) [3]
Any Treatment-Related TEAE	2 (25.0) [2]	0	2 (12.5) [2]
Any Moderate TEAE	1 (12.5) [1]	0	1 (6.3) [1]
Any Treatment-Related Moderate TEAE	1 (12.5) [1]	0	1 (6.3) [1]
Any Severe TEAE	0	0	0
Any Treatment-Related Severe TEAE	0	0	0
Any SAE	0	0	0
Any Treatment-Related SAE	0	0	0
Any TEAE Leading to Early Discontinuation	0	0	0
Any Death	0	0	0

Source: End-of-Text Table 14.3.1.1.
A TEAE was defined as any event not present before exposure to study drug or any event already present that worsened in intensity or frequency after exposure.
A treatment related TEAE was defined as TEAE that was related to study treatment.
At each level of subject summarization, a subject was counted once if the subject reported one or more events.
n represents the number of subjects at each level of summarization. Percentages were based on the number of subjects in the safety population within each treatment and overall. [E] represents the number of events at each level of summarization.
Abbreviations: IC, indigo carmine; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

Clinical Laboratory Evaluations

Overall, mean hematology, serum chemistry, and urinalysis (specific gravity, urobilinogen, and pH) values were within the reference ranges at the time points assessed and mean values

observed on Day 8/EOS were similar to those at baseline. None of the abnormal hematology, serum chemistry, or urinalysis values were considered clinically significant or reported as a TEAE by the investigator.

The laboratory parameters for which 2 or more subjects in at least 1 group shifted from normal at baseline to low or high values at Day 8/EOS based on reference ranges were in serum chemistry measurements as follows.

- Creatinine (µmol/L): In IC 5 mL treatment group, 2 subjects (25.0%) shifted from normal at baseline to low on Day 8/EOS.
- Glucose (mmol/L): In IC 5 mL treatment group, 2 subjects (25.0%) shifted from normal at baseline to high at Day 8/EOS and 1 subject (12.5%) shifted from normal at baseline to low on Day 8/EOS.

No shifts from normal at baseline to low, high, or abnormal values based on reference ranges were reported in 2 or more subjects in each group for any hematology or urinalysis parameters. The safety profile appeared similar for both the high dose and low dose treatment groups.

8.2.4. Safety Results Study PVP-19-IC01

All patients underwent general anesthesia for the urogynecological surgeries. Safety monitoring included the tests listed in Table 28 below that were performed at the times specified in the Table were collected immediately prior to each injection, 5 minutes after each injection, and approximately every 15 minutes thereafter through 1 hour following the surgical procedure. Mean changes from baseline (immediately prior to saline injection) were small and no clinically concerning trends were observed. The investigators recorded (heart rate, temperature, blood pressure (BP), respiration rate) immediately prior to and following IC injection at 15 min, 30 min, 45 min, 1 hr. and 2 hr. Laboratory evaluation included complete blood count, clinical chemistry panel and a pregnancy test. SeeTable 28 below.

Table 28. Tabular Schedule for Assessments for Study PVP-19IC01

	Screening period Day -30 to -1[a]	Randomization/ Treatment Day 1	Follow-up Visit Day 7 to Day 32 or Early Termination[b]	Telephone follow-up if Follow-up Visit occurred prior to Day 28
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Medical and surgical history, demography, medication review	X			
Physical examination	X		X	
Height	X			
Weight	X			
Vital signs	X	X[c]	X	
12-lead electrocardiogram	X		X	
Randomization		X		
Concomitant medications	X	X	X	X
Adverse event/serious adverse event		X	X	X
Blood safety laboratory assessments[d]	X		X[e]	
Surgery		X		
0.9% saline injection		X		
0.8% indigo carmine injection		X		
Video filming of ureteral jet flow with saline and with indigo carmine		X		
Surgeon satisfaction with indigo carmine treatment		X		
Surgeon conspicuity score of urine jet flow[f]		X		
Urine pregnancy test[g]		X		

Source: Table 9.3 CSR PVP-19IC01
[a] Bloodwork/electrocardiogram completed as part of the preoperative work-up (within 30 days of surgery) was acceptable for study use and did not have to be repeated for study participation.
[b] Subjects who had an onsite visit between Day 7 and Day 27 were to have safety follow-up completed, with an additional telephone call between Day 28 and Day 32.
[c] Continuous monitoring, including heart rate and rhythm, was required during the procedure and in the immediate postoperative period. were collected at the following 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.
[d] Blood safety laboratory assessments included hematology (white blood cell count, red blood cell count, hemoglobin, hematocrit, platelet count, differential blood count [neutrophils, lymphocytes, monocytes, eosinophils, basophils]) and serum chemistry (total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine, blood urea nitrogen, sodium, chloride, potassium, bicarbonate, phosphorus, calcium, glucose, albumin).
[e] Must have been performed at the same laboratory as the initial bloodwork.
[f] The surgeon was to review the videos postsurgery to perform the conspicuity scoring of the urine efflux; scoring was not to be done during surgery.
[g] Urine pregnancy tests were done on the day of surgery on all women of childbearing potential

Past Medical History and Types of Surgeries Performed for Study

All subjects reported at least 1 medical or surgical history condition, with 80.5% reporting prior surgical procedures. The 3 most common surgical procedures were female sterilization (21.2%), hysterectomy (16.9%), and cholecystectomy (13.6%). Medical history conditions reported by >15% of subjects overall included hypertension (37.3%), anxiety (33.1%), gastroesophageal reflux disease (29.7%), depression (28.0%), menorrhagia (22.0%), stress urinary incontinence (19.5%), hypothyroidism (18.6%), migraine (17.8%), and seasonal allergy (16.9%).

The characteristics of the surgery performed were generally similar between the 2 randomization groups. See Table 29 below. Overall, the majority of subjects had surgery performed cystoscopically (58%) by either a gynecologist (48%) or urologist (44%). Few subjects

required diuretics (6%), although 56% of subjects required fluid to be instilled into the bladder. See Table 29 below.

Table 29. Surgery Characteristics (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
Surgery duration (hours)			
Mean (standard deviation)	0.98 (0.497)	1.25 (1.237)	1.11 (0.943)
Median	0.90	1.00	1.00
Minimum, maximum	0.1, 2.4	0.1, 8.3	0.1, 8.3
Amount (cc) of IV fluid administered during surgery	(N=55)	(N=54)	(N=109)
Mean (standard deviation)	1023.6 (550.66)	1082.0 (651.59)	1052.6 (600.69)
Median	1000.0	1000.0	1000.0
Minimum, maximum	100, 2900	0, 3000	0, 3000
	Number (%) of Subjects		
Method of surgery			
Cystoscopic	37 (61.7)	31 (53.4)	68 (57.6)
Robotic	15 (25.0)	18 (31.0)	33 (28.0)
Open	0	1 (1.7)	1 (0.8)
Laparoscopic	0	1 (1.7)	1 (0.8)
Other	8 (13.3)	7 (12.1)	15 (12.7)
Surgeon's specialty			
Gynecologist	27 (45.0)	30 (51.7)	57 (48.3)
Urogynecologist	4 (6.7)	4 (6.9)	8 (6.8)
Urologist	29 (48.3)	23 (39.7)	52 (44.1)
Other	0	1 (1.7)	1 (0.8)
Were diuretics given? Yes	3 (5.0)	4 (6.9)	7 (5.9)
Was fluid required to be instilled into the bladder? Yes	36 (60.0)	30 (51.7)	66 (55.9)

Abbreviations: cc, cubic centimeters; IV, intravenous

Deaths

No deaths were reported in the clinical studies conducted by the Applicant. However, one death caused by myocardial infarction deemed related to IC was identified in a review of FAERS safety reports (5792376, 2005). This event in a 65-year-old male who underwent a urological procedure was associated with rapid decrease in blood pressure, sinus arrhythmia and ventricular tachycardia and cardiac arrest, with a plausible time relationship to IC administration.

Serious Adverse Events

Four subjects, 1 in the high dose group and 3 in the low dose group, experienced SAEs, all of which were considered unrelated to the study drug. Subjects with SAEs are summarized in Table 30 below.

Table 30. Subjects With Serious Adverse Events (Safety Analysis Set) PVP-19IC01

Subject ID (dose)	Sex/Age/ Race	Surgery Performed	MedDRA preferred term (verbatim term)	Study Day start/ duration	Intensity	Relationship to study drug	AE outcome
(b) (6) (2.5 mL)	F/40/black or AA	Hysterectomy	Pneumonia aspiration (Aspiration pneumonia)	2/ 4 days	Moderate	Unrelated	Resolved
(b) (6) (2.5 mL)	F/74/white	TRL sacral colpopexy, lysis of adhesions, cystoscopy, colporrhaphy, anterior and posterior repair, bladder sling	Pulmonary embolism (Bilateral PE) Deep vein thrombosis (Left iliac DVT)	2 / unknown 2 / unknown	Severe Severe	Unrelated Unrelated	Resolving Resolving
(b) (6) (2.5 mL)	F/69/white	Transvaginal tape	Atrioventricular block complete (3rd Degree heart block required pacemaker/hospitalization	27 / 3 days	Severe	Unrelated	Resolved
(b) (6) (5 mL)	M/66/white	Cystoscopy, right retrograde pyelogram, right ureteroscopy, right ureteral stent exchange, fulguration of bladder neck	Pyelonephritis (Pyelonephritis)[a] Nausea (Nausea [exacerbation])[a] Vomiting (Vomiting [exacerbation])[a]	12 / unknown 32 / unknown 32 / unknown	Severe Severe Severe	Unrelated Unrelated Unrelated	Resolving Unknown Unknown

Source: Table 2-3 Summary of Clinical Safety.
Abbreviations: AA, African American; AE, adverse event; DVT, deep vein thrombosis; F, female; ID, identification; M, male; MedDRA, Medical Dictionary for Regulatory Activities; PE, pulmonary embolism; TRL, total robotic laparoscopy

Treatment-Emergent Adverse Events and Adverse Reactions

Overall, the most common TEAEs by PT were constipation (5.1%) and dysuria (4.2%). By PT, most TEAEs were reported for a single patient.

TEAEs reported for ≥2 patients overall are displayed by system organ class and PT in Table 31 below. No clinically concerning differences in types or incidences of TEAEs were noted between the 2 indigo carmine dose groups.

Most patients had TEAEs that were mild or moderate in intensity. Five patients (4.2%), 2 in the high dose group and 3 in the low dose group, experienced severe TEAEs. In the high dose group, one patient had severe colitis and one patient had severe oliguria, pyelonephritis, nausea, and vomiting. In the low dose group, one patient experienced severe post procedural urine leak, pulmonary embolism, and deep vein thrombosis; one patient had severe atrioventricular block complete; and one patient had severe ureteral injury and urinary retention.

All TEAEs were assessed by the Applicant as unrelated to study drug. The most common TEAEs were those typically associated with the surgical procedures performed and the medications used operatively and postoperatively. See Table 31 below.

Table 31. Treatment-Emergent Adverse Events Reported for ≥2 Patients Overall (Safety Analysis Set)

System Organ Class MedDRA Preferred Term	Indigo Carmine 0.8% Solution 5.0 mL (high dose) (N=60)	 2.5 mL (low dose) (N=58)	 Overall (N=118)
	Number (%) of Subjects		
Subject with ≥1 TEAE	18 (30.0)	25 (43.1)	43 (36.4)
Gastrointestinal disorders	6 (10.0)	5 (8.6)	11 (9.3)
Constipation	3 (5.0)	3 (5.2)	6 (5.1)
Abdominal pain	2 (3.3)	1 (1.7)	3 (2.5)
Nausea	2 (3.3)	1 (1.7)	3 (2.5)
Vomiting	2 (3.3)	0	2 (1.7)
Dyspepsia	0	2 (3.4)	2 (1.7)
Renal and urinary disorders	3 (5.0)	7 (12.1)	10 (8.5)
Dysuria	1 (1.7)	4 (6.9)	5 (4.2)
Investigations	3 (5.0)	3 (5.2)	6 (5.1)
Alanine aminotransferase increased	2 (3.3)	0	2 (1.7)
General disorders and administration site conditions	3 (5.0)	1 (1.7)	4 (3.4)
Pyrexia	2 (3.3)	1 (1.7)	3 (2.5)
Respiratory, thoracic and mediastinal disorders	0	4 (6.9)	4 (3.4)
Pulmonary embolism	0	2 (3.4)	2 (1.7)

Source: Table 2.2. Module 2.7.4 Summary of Clinical Safety.
Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event

A total of two patients in the low dose group had TEAEs associated with cardiac disorders (bradycardia and atrioventricular block complete in one patient; tachycardia in one patient); all cardiac events were considered unrelated to indigo carmine. One patient in each dose group had a TEAE associated with immune system disorders (hypersensitivity to Dilaudid in the high dose group; urticaria [hives] on Day 9 in low dose group). No anaphylactoid symptoms or signs considered related to indigo carmine were reported.

No patients had TEAEs that led to discontinuation of study drug or from the study.

Nine patients, 4 in the high dose group and 5 in the low dose group, had TEAEs designated as special interest because of their association with the pharmacologic actions of IC including vasopressor effects or because of reports of serious reaction to IC from long-term clinical use. By PT, no TEAE of special interest was reported for more than a single patient. Most of the TEAEs of special interest were considered unrelated to indigo carmine (See Table 32 below).

Table 32. Treatment-Emergent Adverse Events of Special Interest (Safety Analysis Set)

System Organ Class MedDRA Preferred Term	Indigo Carmine 0.8% Solution 5.0 mL (high dose) (N=60)	 2.5 mL (low dose) (N=58)	 Overall (N=118)
Subject with ≥1 TEAE of special interest	4 (6.7)	5 (8.6)	9 (7.6)
Immune system disorders	1 (1.7)	1 (1.7)	2 (1.7)
Drug hypersensitivity	1 (1.7)	0	1 (0.8)
Urticaria	0	1 (1.7)	1 (0.8)
Vascular disorders	1 (1.7)	1 (1.7)	2 (1.7)
Hypertensive crisis	1 (1.7)	0	1 (0.8)
Hypertension	0	1 (1.7)	1 (0.8)
Cardiac disorders	0	2 (3.4)	2 (1.7)
Atrioventricular block complete	0	1 (1.7)	1 (0.8)
Bradycardia	0	1 (1.7)	1 (0.8)
Tachycardia	0	1 (1.7)	1 (0.8)
Injury, poisoning and procedural complications	0	2 (3.4)	2 (1.7)
Post procedural urine leak	0	1 (1.7)	1 (0.8)
Ureteric injury	0	1 (1.7)	1 (0.8)
Investigations	1 (1.7)	0	1 (0.8)
Blood creatinine increased	1 (1.7)	0	1 (0.8)
Renal and urinary disorders	1 (1.7)	0	1 (0.8)
Bladder spasm	1 (1.7)	0	1 (0.8)
Respiratory, thoracic and mediastinal disorders	0	1 (1.7)	1 (0.8)
Dyspnoea	0	1 (1.7)	1 (0.8)

Source: Table 12-4 CSR, PVP-19IC01
Abbreviation: MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event

Patients with TEAEs of special interest are summarized in Table 33 below. Of the nine patients with TEAEs of special interest, six were >65 years of age (age range: 69 to 82 years), five of whom had medical histories or ongoing conditions related to the event.

Table 33. Details of Patients With TEAEs of Special Interest

Sub ID	Sex/Age/Race	Surgery	MedDRA Term	Study Day Start/Dur.	Intensity	Rel./	AE Outcome
(b) (6) (2.5 mL)	F/56/W	Robotic H&BSO	Urticaria	9/4 days	Mod.	UR	Resolved
(b) (6) (2.5 mL)	F/74/W	TRL sacral colpopexy	Post procedure urine leak	2/1 day	Sev	UR	Resolved
(b) (6) (2.5 mL)	M/77/W	Cystoscopy	Hypertension	5/UK	Mod	UR	Resolving
(b) (6) (2.5 mL)	F/69/W	Transvaginal tape	Dyspnea Bradycardia AV 3rd deg	10/UK 22/UK 27/3 days	Mod Mod Sev	UR	NR NR Resolved
(b) (6) (2.5 mL)	F/34/B or AA	Lap appendectomy & TAH-BSO	Ureteral injury Tachycardia	1/1 day 2/2/ days	Sev Mod	UR	Resolved
(b) (6) (5 mL)	F/36/W	Hysterectomy	Drug hypersensitivity -Dilaudid	1/1 day	Mild	UR	Resolved
(b) (6) (5 mL)	F/75/W	Cystoscopy	Bladder spasm	2/UK	Mod	UR	NR
(b) (6)	M/72/W	Cystoscopy	Blood creatinine increased	25/UK	Mod	UR	NR
(b) (6)	M/82/W	TU resection of bladder tumor	Hypertensive crisis	1/1 day	Mod	UR	Resolved

Source: PVP-19IC01 CSR Table 12-5; Listing 16.2.5.2 and Listing 16.2.7.1
a Subject had a medical history of urge incontinence and ongoing cystocele (Listing 16.2.4.2).
b Subject had a medical history of myocardial infarction and coronary angioplasty and ongoing hypertension (Listing 16.2.4.2).
c Subject had a medical history of hydronephrosis (Listing 16.2.4.2).
d Subject had a medical history of hematuria and urinary bladder suspension and ongoing urinary tract infection, stress urinary incontinence, pollakiuria, nephrolithiasis, and ureterolithiasis (Listing 16.2.4.2).
e Subject had a medical history of ongoing hematuria (Listing 16.2.4.2).
f Subject had a medical history of ongoing hypertension, stroke, and cardiac failure (Listing 16.2.4.2).
Abbreviations: AA, African American; AE, adverse event; AV, atrioventricular; B, black; F, female; ID, identification; M, male; Mod, moderate; MedDRA, Medical Dictionary for Regulatory Activities; NR, not reported; Sev, severe; TAH-BSO, total abdominal hysterectomy with bilateral salpingo-oophorectomy; TEAE,4 treatment-emergent adverse event; TRL, total robotic laparoscopy; TU, trans urethral; UR, unrelated; W, white

Clinical Laboratory Findings

Hematology

Most patients had hematology test results that were within normal range at baseline and remained within normal range at the end of study. Overall, the most common (≥5%) shifts from normal at baseline to high or low at end of study were for high platelets (11%), low hematocrit (7%), and high eosinophils (6%) (Table 34 below).

Table 34. Number (%) of Patients With Hematology Test Results Within Normal Range at Baseline and Low or High Results at End of Study (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
	n/N (%) of Subjects		
White blood cell count (10^9/L)			
High	1/53 (1.9)	1/51 (2.0)	2/104 (1.9)
Red blood cell count (10^{12}/L)			
Low	0/51	3/45 (6.7)	3/96 (3.1)
High	1/51 (2.0)	0/45	1/96 (1.0)
Hemoglobin (g/dL)			
Low	1/47 (2.1)	1/41 (2.4)	2/88 (2.3)
High	1/47 (2.1)	1/41 (2.4)	2/88 (2.3)
Hematocrit (%)			
Low	2/46 (4.3)	4/38 (10.5)	6/84 (7.1)
High	1/46 (2.2)	1/38 (2.6)	2/84 (2.4)
Platelets (10^9/L)			
Low	1/53 (1.9)	0/47	1/100 (1.0)
High	6/53 (11.3)	5/47 (10.6)	11/100 (11.0)
Neutrophils (%)			
Low	1/49 (2.0)	0/46	1/95 (1.1)
High	1/49 (2.0)	0/46	1/95 (1.1)
Lymphocytes (%)			
Low	4/47 (8.5)	0/45	4/92 (4.3)
High	1/47 (2.1)	1/45 (2.2)	2/92 (2.2)
Eosinophils (%)			
High	3/50 (6.0)	3/47 (6.4)	6/97 (6.2)
Basophils (%)			
High	2/49 (4.1)	0/48	2/97 (2.1)

Source: Table 14.3.3.1.1 in CSR

Abnormal clinically significant hematology test results were observed postbaseline for four patients, all of whom received the low dose of indigo carmine; at Follow-up, low values were

reported for hematocrit in one subject, hemoglobin and hematocrit in one subject, and platelets in two subjects.

- Patient (b) (6) who had an ongoing history of anemia, had low screening hematocrit (33.2%; reference range: 38 to 44%) and hemoglobin (9.7 g/dL; reference range 11.3 to 15.3 g/dL); at Follow-up on Day 26, hematocrit was 32.3% and hemoglobin was 9.2 g/dL. A TEAE of anemia was reported on Day 27 that was considered mild in intensity and unrelated to study drug; the event was noted as not resolved.
- Patient (b) (6) had a screening hematocrit of 39.8% (reference range: 38 to 44%) that had decreased to 36.9% at Follow-up on Day 15.
- Patient (b) (6), with an ongoing history of hematuria, had a low screening platelet count of 143×109/L (reference range: 150 to 450×109/L) that had decreased to 123×109/L at Follow-up on Day 22. A TEAE of thrombocytopenia was reported on Day 22 that was considered mild in intensity and unrelated to study drug; the event was noted as not resolved.
- Patient (b) (6), with an ongoing history of immune thrombocytopenic purpura, had a low platelet count of 61×109/L (reference range: 150 to 450×109/L) at Follow-up on Day 11; screening platelet count was not provided.

Chemistry

Most patients had chemistry test results that were within normal range at baseline and remained within normal range at the end of study. Normal glucose values at baseline were reported for 66% of patients. Overall, the most common (≥5%) shifts from normal at baseline to high or low at end of study were for high glucose (31%), high phosphorus (10%), low sodium (8%), low bicarbonate (7%), and high blood urea nitrogen (6%) (Table 35 below).

Table 35. Number (%) of Patients with Chemistry Test Results Within Normal Range at Baseline and Low or High Results at End of Study (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
	n/N (%) of Subjects		
Calcium (mg/dL)			
Low	3/50 (6.0)	0/53	3/103 (2.9)
High	0/50	2/53 (3.8)	2/103 (1.9)
Glucose (mg/dL)			
Low	0/42	1/36 (2.8)	1/78 (1.3)
High	13/42 (31.0)	11/36 (30.6)	24/78 (30.8)
Albumin (g/dL)			
Low	2/54 (3.7)	2/53 (3.8)	4/107 (3.7)

Source: Table 14.3.3.1.2 CSR PVP-19IC01.
Abbreviations: dL, deciliter; N, number of subjects

Table 36. Number (%) of Patients with Chemistry Test Results Within Normal Range at Baseline and Low or High Results at End of Study (Safety Analysis Set)

	Indigo Carmine 0.8% Solution		
	5.0 mL (high dose) (N=60)	2.5 mL (low dose) (N=58)	Overall (N=118)
	n/N (%) of Subjects		
Total bilirubin (mg/dL)			
Low	1/56 (1.8)	2/55 (3.6)	3/111 (2.7)
High	4/56 (7.1)	0/55	4/111 (3.6)
Aspartate transaminase (U/L)			
Low	4/52 (7.7)	2/48 (4.2)	6/100 (6.0)
High	1/52 (1.9)	0/48	1/100 (1.0)
Alanine transaminase (U/L)			
Low	0/51	2/51 (3.9)	2/102 (2.0)
High	2/51 (3.9)	3/51 (5.9)	5/102 (4.9)
Alkaline phosphatase (U/L)			
Low	0/54	1/54 (1.9)	1/108 (0.9)
High	0/54	1/54 (1.9)	1/108 (0.9)
Creatinine (mg/dL)			
Low	2/47 (4.3)	2/45 (4.4)	4/92 (4.3)
High	1/47 (2.1)	2/45 (4.4)	3/92 (3.3)
Blood urea nitrogen (mg/dL)			
Low	2/48 (4.2)	0/50	2/98 (2.0)
High	1/48 (2.1)	5/50 (10.0)	6/98 (6.1)
Sodium (mmol/L)			
Low	4/53 (7.5)	4/48 (8.3)	8/101 (7.9)
Potassium (mmol/L)			
Low	0/54	1/52 (1.9)	1/106 (0.9)
Chloride (mmol/L)			
Low	2/50 (4.0)	1/52 (1.9)	3/102 (2.9)
High	2/50 (4.0)	3/52 (5.8)	5/102 (4.9)
Bicarbonate (mmol/L)			
Low	3/50 (6.0)	4/50 (8.0)	7/100 (7.0)
High	0/50	1/50 (2.0)	1/100 (1.0)
Phosphorus (mg/dL)			
Low	1/45 (2.2)	1/48 (2.1)	2/93 (2.2)
High	2/45 (4.4)	7/48 (14.6)	9/93 (9.7)

Source: Table 14.3.3.1.2 CSR PVP-19IC01.
Abbreviations: dL, deciliter; L, liter; mg, milligram; mmol, millimole; N, number of subjects

Abnormal clinically significant chemistry test results were observed postbaseline in six patients who received the high dose of indigo carmine and one patient who received the low dose of indigo carmine. Five of the seven patients had chemistry test results outside the reference

range at screening. The following is a compilation of summaries for all instances and all referenced listings are from the clinical study report (CSR) for PVP-19IC01:

- Patient (b) (6) (low dose) at screening had normal values for alkaline phosphatase (80 U/L, reference range: 38 to 126 U/L) and sodium (140 mmol/L, reference range: 137 to 145 mmol/L). At Follow-up on Day 15, alkaline phosphatase was high (307 U/L), and sodium was low (133 mmol/L). A TEAE of blood sodium decreased was reported on Day 15 that was mild in intensity and unrelated to study drug; the event was noted as resolving.
- Patient (b) (6) (high dose) had a high alanine aminotransferase value at screening (49 U/L; reference range: 0 to 44 U/L) that increased to 80 U/L at follow-up on Day 28. A TEAE of alanine aminotransferase increased was reported on Day 28 that was considered mild in intensity and unrelated to study drug; outcome of the event was unknown.
- Patient (b) (6) (high dose) had a high glucose value at screening (183 mg/dL; reference range: 65 to 99 mg/dL) that increased to 257 mg/dL at Follow-up on Day 30.
- Patient (b) (6) (high dose) had a high creatinine value at screening (1.85 mg/dL; reference range: 0.76 to 1.27 mg/dL) that increased to 2.04 mg/dL at Follow-up on Day 25. A TEAE of blood creatinine increased was reported on Day 25 that was considered moderate in intensity and unrelated to study drug; the event was noted as not resolved.
- Patient (b) (6) (high dose) had a low potassium value at screening (2.9 mmol/L; reference range 3.5 to 5.3 mmol/L) that decreased to 2.8 mmol/L at Follow-up on Day 27.
- Patient (b) (6) (high dose) had a high screening alanine aminotransferase (101 U/L; reference range: 9 to 52 U/L), not considered clinically significant, that was 88 U/L at Follow-up on Day 13 and considered clinically significant. A TEAE of alanine aminotransferase increased was reported on Day 13 that was mild in intensity and unrelated to study drug; the event was noted as not resolved.
- Patient (b) (6) (high dose) at screening, had normal values for chloride (101 mmol/L; reference range: 96 to 106 mmol/L) and sodium (137 mmol/L; reference range: 134 to 144 mmol/L); at Follow-up on Day 22, low values were reported for chloride (92 mmol/L) and sodium (129 mmol/L). TEAEs of hypochloremia and hyponatremia were reported on Day 22, both of which were considered moderate in intensity and unrelated to study drug; both events were noted as not resolved.

Blood Pressure and Heart Rate Findings

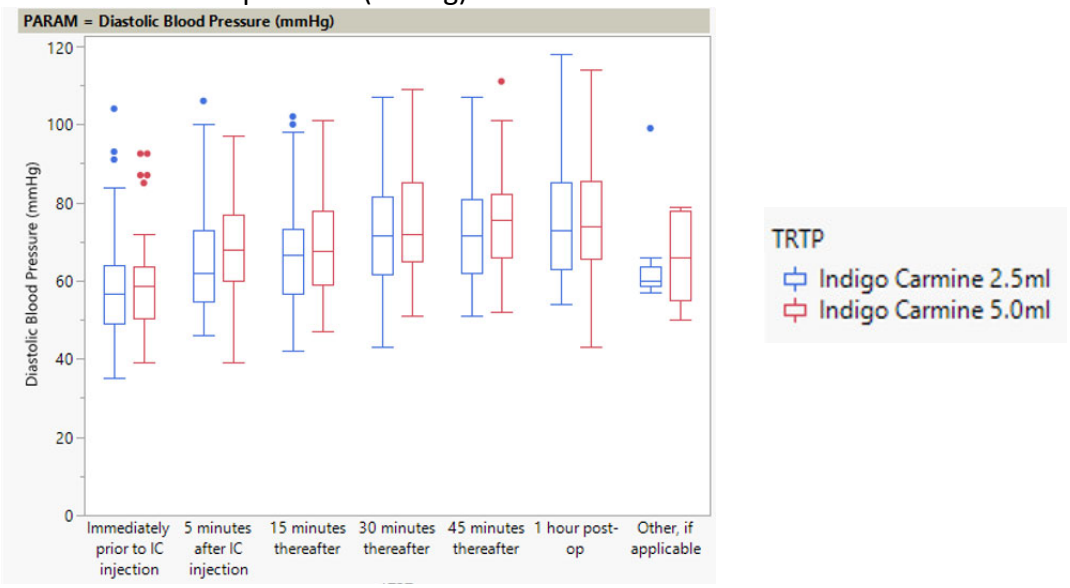
On a review of the safety data related to the administration of indigo carmine that included FAERS reports, VigiBase and the published scientific literature (Naitoh and Fox 1994; Kim et al. 2011; Jeon et al. 2012; Lee and Jang 2012), FDA identified additional reports of hypotension, hypertension, bradycardia, second degree atrioventricular (AV) block and tachycardia.

FDA summarized the blood pressure measurements in patients following administration of indigo carmine 2.5 mL or 5 mL dosages. Both diastolic blood pressure (Figure 2A) and systolic

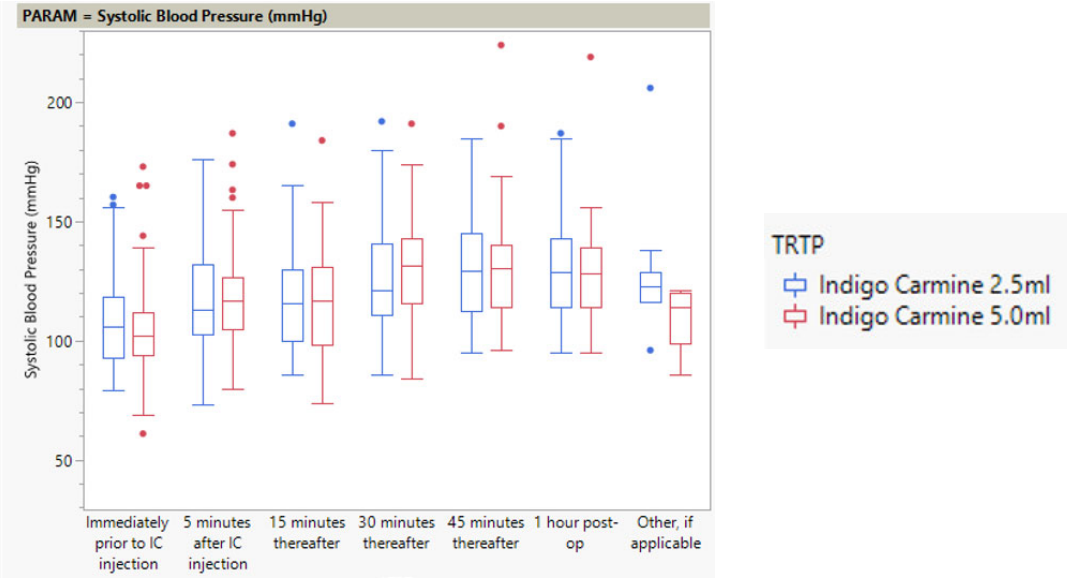
blood pressure (Figure 2B) following both dosages appeared to be similar from baseline to 1-hour postinjection in the overall patient population. Also, it was noted that the variability of both diastolic blood pressure and systolic blood pressure increased following the administration of indigo carmine 2.5 mL or 5 mL dosages.

Figure 2. FDA Analyses of Diastolic Blood Pressure (A) and Systolic Blood Pressure (B) in Patients After Receiving Indigo Carmine 2.5 mL or 5 mL Dosages

A. Diastolic blood pressure (mmHg)



B. Systolic blood pressure (mmHg)

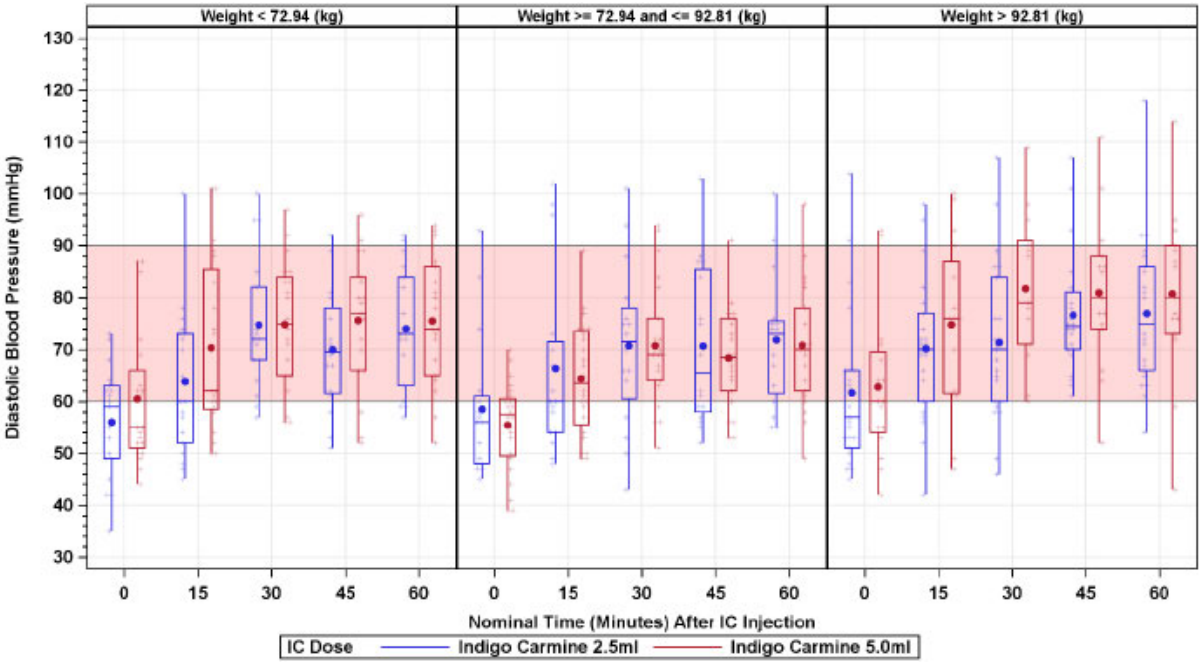


Abbreviations: IC, indigo carmine; mmHg, millimeter of mercury

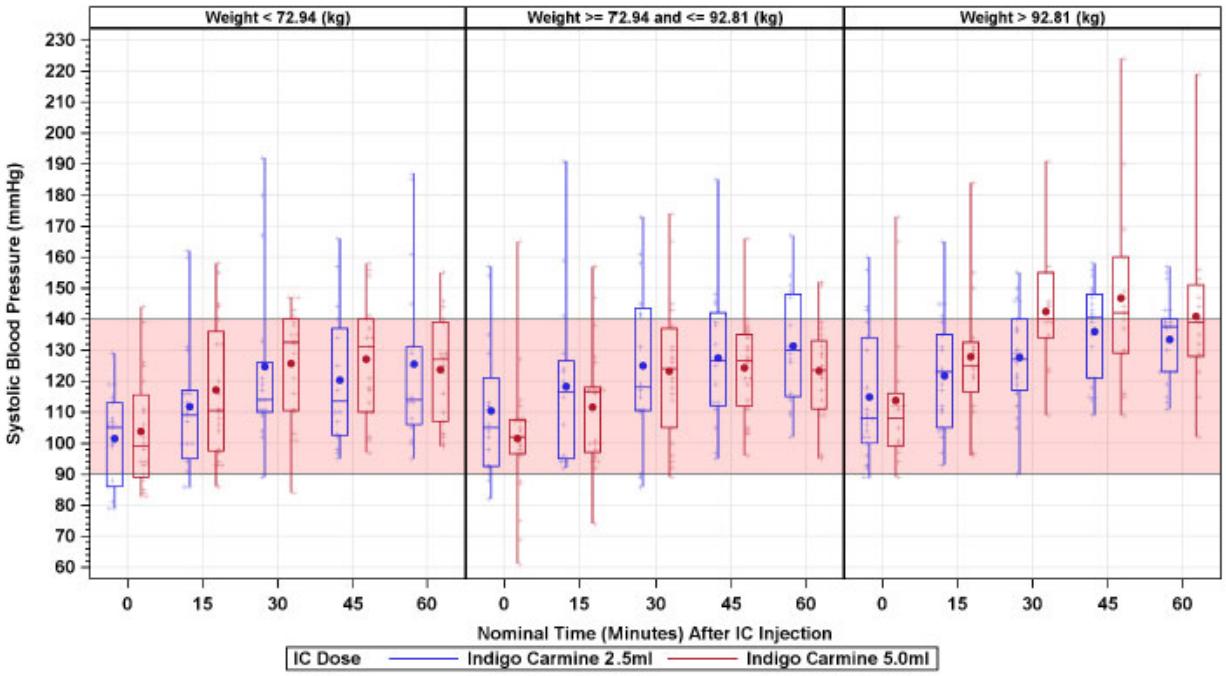
The Applicant conducted additional analyses to evaluate blood pressure and heart rate in patients with different body weight categories (i.e., <72.94 kg, 72.94 - <=92.81 kg, >92.81 kg) following the administration of indigo carmine 2.5 mL or 5 mL dosages. The results of these analysis suggest that both indigo carmine 2.5 mL and 5 mL dosages have the potential to raise diastolic blood pressure (Figure 3A) and systolic blood pressure (Figure 3B). Heart rates did not appear to be affected (Figure 3C). However, group means in a relatively small dataset might be difficult to interpret and the results might be confounded by multiple factors including patients' comorbidities, obesity, and variability of drug exposure.

Figure 3. Diastolic Blood Pressure (A), Systolic Blood Pressure (B) and Heart Rate (C) Observed Value and Group Mean (95% CI) Before (Time 0) and After Indigo Carmine Injection (Safety Analysis Set)

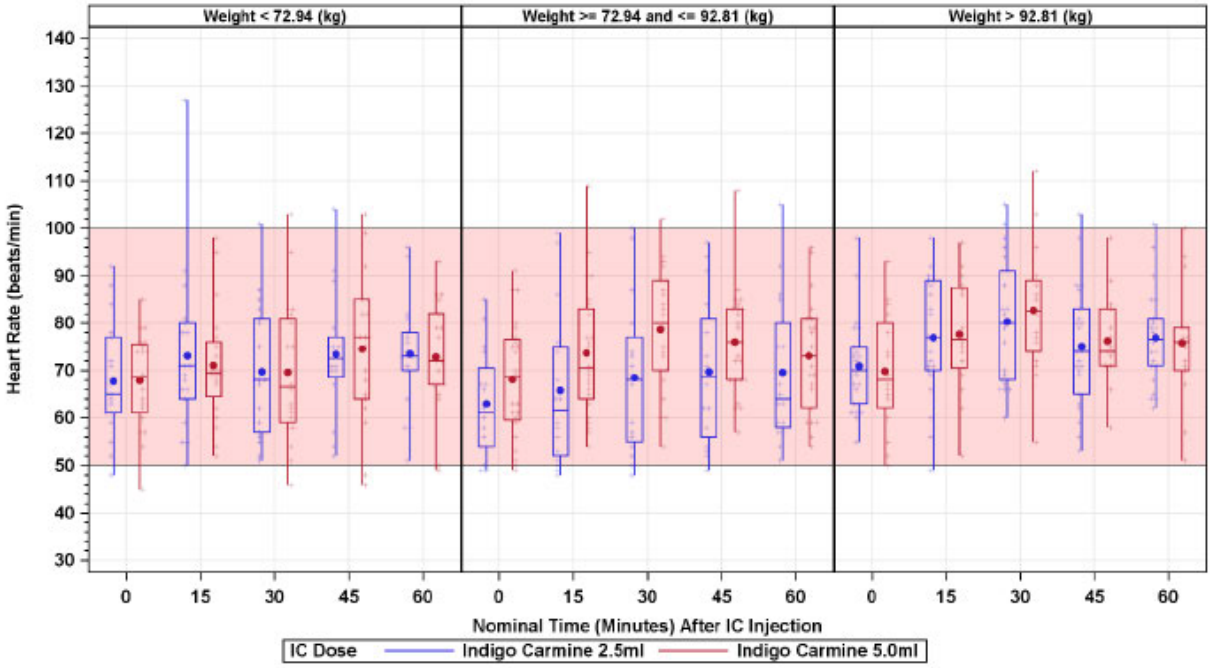
A. Diastolic blood pressure (mmHg)



B. Systolic blood pressure (mmHg)



C. Heart Rates (beats/min)



Source: Applicant's IR response submitted on June 7th, 2022.
The dot indicates the mean; the box shows Q1 (bottom) and Q3 (top) with median line in the middle; the vertical lines are the extreme values. The pink shades represent the normal ranges of vital signs for the adult population.
Abbreviations: IC, indigo carmine; mmHg, millimeter of mercury

Examination of individual patent responses identified three cases of interest. One patient experienced elevation in blood pressure intraoperatively and much later developed atrioventricular block and two patients experienced a hypertension event (PTs: hypertension and hypertensive crisis).

- Patient (b) (6), a 69-year-old female, had an extensive medical and surgical history and was morbidly obese, weighing 121.7 kg (BMI of 40.8). On Day 1 during surgery, the diastolic blood pressure increased from 57 mm Hg to 76 mm Hg and the systolic blood pressure increased from 94 mm Hg to 150 mm Hg, both peaked 45 and 30 minutes, respectively, after the administration of indigo carmine 2.5 mL. The patient developed dyspnea on Day 10, bradycardia on Day 22, and a 3rd degree heart block that required a pacemaker on Day 27.

- Patient (b) (6), a 77-year-old male, had a history of uncontrolled hypertension. On Day 1 during surgery, the diastolic blood pressure increased from 81 mm Hg to 100 mm Hg and the systolic blood pressure increased from 138 mm Hg to 191 mm Hg, both peaked 5 and 15 minutes, respectively, after the administration of 2.5 mL indigo carmine. The patient also developed "worsening uncontrolled hypertension" on Day 5.

- Patient (b) (6), an 82-year-old male had a history of hypertension, stroke, and cardiac failure. On Day 1 during surgery, at 45 minutes after the administration of 5 mL indigo carmine, the systolic blood pressure increased from 117 mm Hg to 224 mm Hg. the

diastolic blood pressure increased from 66 mm Hg to 114 mm Hg and peaked at 1 hour postop. The event resolved within 1 day.

Based on the above assessments, (b) (4)

ECG Assessments

Two patients in the low dose group had ECG-associated TEAEs. Patient (b) (6) had moderate bradycardia on Day 22 that was noted as not resolved (no vital sign or ECG data were available on Day 22) and complete atrioventricular block on Day 27 that resolved in 3 days; both events were considered unrelated to study drug. Patient (b) (6), who had a medical history of an abnormal electrocardiogram and a screening heart rate on ECG of 109 beats/min had moderate tachycardia on Day 2 that was considered unrelated to study drug and that resolved the same day.

8.2.5. Safety Reports in the Scientific Literature

Cardiovascular Effects of IC

Cardiovascular Effects of IC appear to be the most common adverse reactions reported in the scientific literature. Erickson et al found that blood pressure increased after IV administration of indigo carmine in all the 28 patients they studied (Erickson and Lauron 1960). They suggested that IC acts as a peripheral vasoconstrictor. Several other studies in healthy subjects reported the occurrence of elevation in blood pressure and conduction abnormalities soon after the injection of IC (Erickson and Widmer 1968; Kennedy et al. 1968; Jeffords et al. 1977; Harioka et al. 1987). Hobai reported that atrioventricular block developed in two patients immediately after the injection of IC (Hobai 2008). Takeyama who noted second-degree heart block (Wenckebach-type) after IC administration (Takeyama et al. 2014) suggested a cholinergic (vagal) mediated effect coupled with anesthetic drugs as a causative mechanism for the induction of the heart block.

The similar molecular structures of indigo carmine and serotonin suggest a common molecular basis for these hemodynamic reactions. Hypertension associated with indigo carmine has been attributed to vasoconstriction caused by a direct α-adrenergic activation or by the release of catecholamines leading to an increase in the total peripheral resistance and increases in diastolic and systolic blood pressures (i.e., in the same manner as serotonin) (Jo et al. 2013).The study by Chang et al evaluated the potential mechanism by which indigo carmine produces hypertension, using isolated rat thoracic aortic rings tested in vitro and precontracted with phenylephrine (Chang et al. 1996). Indigo carmine significantly inhibited receptor- and nonreceptor-mediated endothelium-dependent vasorelaxation. Indigo carmine also inhibited endothelium-independent vasorelaxation induced by sodium nitroprusside, although to a lesser extent than vasodilation from acetylcholine and histamine. IC had no effect on the vasodilation

induced by isoproterenol, indicating that indigo carmine selectively inhibits nitric oxide-mediated responses. The main action of indigo carmine appears to be at the level of nitric oxide generation and/or release from the endothelial cells. In addition, indigo carmine appears to inhibit vascular smooth muscle guanylyl cyclase. Thus, indigo carmine may elevate blood pressure by interfering with these nitric oxide-mediated vasodilatory mechanisms.

Effect of IC on Oximetry

In common with other intravenous dyes such as methylene blue, indocyanine green, and fluorescein, indigo carmine can cause erroneously low pulse oximeter saturation, SPO_2, readings (Ralston et al. 1991a) with pulse due to un usual light absorption characteristics (Scheller et al. 1986; Barker and Tremper 1987). These false readings are transitory and easily recognized if the user is aware of the potential for error (Ralston et al. 1991b). The effect of the dye is usually minimal within a minute of administration due to the dilution of the dye and its rapid distribution and clearance (Eide et al. 1987; Unger and Scheller 1987). Recovery to baseline readings occurred within 3 minutes in all subjects with decreases. It is important that anesthesiologists be aware of the potential for falsely low SPO_2 readings temporarily induced by IC (Scheller et al. 1986).

Other Adverse Reactions to IC

Other idiosyncratic and rare adverse effects of indigo carmine resembling allergic or anaphylactoid reactions have been described, involving 1 or more of the following: hypotension, shortness of breath, bronchospasm, or dermatological reactions (urticaria, erythema). Sudden life threatening cardiovascular reactions to IC leading to cardiac arrest and suggestive of an anaphylactoid reaction have been described in the scientific literature (Shir and Raja 1993; Nguyen et al. 1998; Gousse et al. 2000; Graziano et al. 2005).

8.2.6. Analysis of Submission-Specific Safety Issues

GI Reactions

Gastrointestinal (GI) reactions and renal and urinary disorders were the most common AEs with incidence of 9.3% and 8.5% respectively. These types of TEAEs are typically associated with the surgical procedures performed during the study and the medications used operatively and postoperatively. None of the TEAEs reported were considered by the Applicant or Investigator to be related to IC.

8.2.7. Safety Analyses by Demographic Subgroups

Similarly, to the demographic characteristics of the overall study population, the majority of patients in each dose group who had TEAEs were white, female, and <65 years of age. No notable differences were observed between patients who had and those that did not have TEAEs. Hence, no subgroup analyses were performed for TEAEs. Subjects with and without TEAEs are presented by demographic subgroup in Table 37 below.

Table 37. Patients With and Without TEAEs by Demographic Characteristics (SAS)

	Indigo Carmine 0.8% Solution			
	5 mL (high dose)		2.5 mL (low dose)	
	Had TEAE (N=18)	No TEAE (N=42)	Had TEAE (N=25)	No TEAE (N=33)
Age (years)				
Mean (standard deviation)	51.7 (18.64)	51.1 (16.26)	53.3 (16.50)	49.9 (17.24)
Median	47.0	49.0	52.0	46.0
Minimum, maximum	25, 82	20, 78	24, 77	26, 88
	Number (%) of Subjects			
Age group				
<65 years	12 (66.7)	30 (71.4)	16 (64.0)	25 (75.8)
≥65 years	6 (33.3)	12 (28.6)	9 (36.0)	8 (24.2)
Sex				
Female	12 (66.7)	30 (71.4)	17 (68.0)	28 (84.8)
Male	6 (33.3)	12 (28.6)	8 (32.0)	5 (15.2)
Race				
White	16 (88.9)	40 (95.2)	19 (76.0)	30 (90.9)
Black or African American	2 (11.1)	2 (4.8)	3 (12.0)	1 (3.0)
Asian	0	0	1 (4.0)	1 (3.0)
Other	0	0	1 (4.0)	0
Unknown	0	0	1 (4.0)	1 (3.0)

Source: Table 6-1 Summary of Clinical Safety.
Abbreviations: SAS, safety analysis set; TEAE, treatment-emergent adverse event.

Use in Geriatric Patients

Of the total number of patients in the clinical study, 23 (20%) were 65 to 74 years of age, and 12 (10%) were 75 and older. No overall differences in safety or effectiveness were observed between these patients and younger patients.

8.2.8. Specific Safety Studies/Clinical Trials

None were provided and none are needed.

8.2.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

There are no human data available to evaluate for risk for drug-associated carcinogenicity or tumor development and none are needed.

Human Reproduction and Pregnancy

There are no human data available to evaluate for a drug-associated risk of major birth defects, miscarriage, or other adverse maternal or fetal outcomes.

Pediatrics and Assessment of Effects on Growth

Pediatric patients were not studied in the clinical trials. A Postmarketing Requirement (Refer to approved PMR) study is planned in patients from 2 to 18 years of age.

No studies on effects on growth are planned and none are needed.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No adverse reactions have been reported in the scientific literature in patients receiving up to 80 mg of IC intravenously. Drug abuse potential, withdrawal and rebound issues are not applicable to the use of IC.

8.2.10. Safety in the Postmarketing Setting

Safety Concerns Identified Through Postmarket Experience of Unapproved IC Drug Product

While the limited safety data from PVP-19IC01 and PVP20IC-02 show that both 2.5 mL and 5 mL IV dose of IC have an acceptable safety profile, the broad clinical experience has identified the occurrence of serious adverse reactions, albeit rare, that warrant specific Warnings and Precautions in the PI as risk mitigation steps, (b) (4)

IC has been marketed in the U.S. for several decades for the intraoperative use in patients undergoing urogynecological surgeries. The Applicant submitted data from this clinical use obtained from EudraVigilance database from January 01, 1980, to October 31, 2020. In all, they identified a total of 57 AEs during that period including 12 cases of Immune system disorders, 12 cases of skin and subcutaneous disorders, 4 cases of GI disorders, 2 cases of cardiac disorders (2nd degree AV block and tachycardia). The Applicant also submitted details of safety in the scientific literature.

A review by FDA's DPV identified 57 unique AE reports with IC drug causality categorized as "probable" in 45 cases and "possible" in 12 cases. Causality was judged based on temporal relationship between IC administration and the adverse event, in particular rapid onset after an otherwise stable surgical procedure and prompt reversal with treatment. In 33 of the 49 cases where time-to-onset was reported, the adverse events occurred within 5 minutes of administration of IC. Among the FAERS cases (n=25), 24 were associated with serious outcomes including death (n=1), life threatening reactions (n=6), hospitalization (n=5), and "other" serious (n=13). The most frequently reported PTs included hypotension (n=22), hypertension (n=8), bradycardia (n=7), oxygen saturation decreased (n=7) and anaphylactic reaction (n=5).

In this case series, DPV identified reports of rapid changes in blood pressure, both in patients experiencing hypotension and patients experiencing hypertension. Cardiac rhythm disturbances included atrioventricular block second degree, ventricular tachycardia, ventricular extrasystoles with bradyarrhythmia, and sinus tachycardia. There were four cases of cardiac arrest with three documenting asystole and one fatal myocardial infarction. The majority of patients with

hypotension required treatment (intravenous fluids, vasopressors, cardiopulmonary resuscitation) and some patients with hypertension required treatment (atropine, hydralazine, nitroglycerine). Injection site discoloration was also reported.

The cases of anaphylaxis manifested with the classic presentations involving cutaneous (urticaria, erythema, rash), respiratory (bronchospasm, wheezing), cardiovascular (hypotension, bradycardia), and gastrointestinal system (nausea) events. Many of the patients required treatment with epinephrine. Ten of the 11 cases reported one or more treatments including epinephrine, injectable corticosteroids, and cardiopulmonary resuscitation.

Based on the above findings, the PI contains a Warning regarding hypersensitivity reactions (including anaphylactic reactions) and a Warning with a complete listing of cardiovascular reactions.

The complete DPV report can be found in the pharmacovigilance review (Cotter et al. 2022).

8.2.11. Integrated Assessment of Safety

The integrated analyses of safety data were conducted using the integrated SAS, defined as all patients exposed to indigo carmine injection. All qualified subjects were randomized to receive either low dose indigo carmine (2.5 mL or high dose indigo carmine (5 mL) after receiving a dose of normal saline. The safety analysis set, which included all randomized subjects who received any study drug (saline or indigo carmine), was used for safety and tolerability assessments.

Aspects of the safety of indigo carmine injection in the datasets were assessed by extent of exposure, the occurrence of TEAEs, serious TEAEs, clinical laboratory results, vital sign measurements, and ECG results. The following observations were made on the safety of indigo carmine injection in these studies.

No deaths and no TEAEs leading to discontinuation of IC were reported. Four patients (3.4%) experienced SAEs, none of which are considered related to indigo carmine. Five patients experienced severe TEAEs. In the IC high dose group, one patient had severe colitis and one patient had severe oliguria, pyelonephritis, nausea, and vomiting. In the IC low dose group, one patient experienced severe post procedural urine leak, pulmonary embolism, and deep vein thrombosis; one patient had severe atrioventricular block complete; and one patient had severe ureteral injury and urinary retention. These events were considered unrelated to indigo carmine.

Most patients had TEAEs that were mild or moderate in intensity. At least 1 TEAE was reported for 30% of patients administered the indigo carmine high dose and 43% of patients administered the low dose. Overall, the most common TEAEs were constipation (5.1%) and dysuria (4.2%).

Generally, no clinically important changes in clinical laboratory testing were observed and no study-drug-related ECG changes were observed.

8.3. Statistical Issues

8.3.1. Study PVP-19IC01

A total of 121 patients scheduled for urological or gynecological surgical procedures were enrolled from 7 sites in the U.S. and randomized in a 1:1 ratio to receive a single dose of either 5 mL (high dose) or2.5 mL (low dose) of indigo carmine: 62 were randomized to the high dose arm and 59 to the low dose arm. Of the 121 subjects, 118 were administered indigo carmine injection. Of the 118 treated subjects, 114 completed the study; 4 prematurely discontinued from the study (2 were lost to follow-up, 1 withdrew due to physician decision and 1 due to subject decision). Each randomized patient serves as his/her own control by receiving 5 mL 0.9% saline injection prior to receiving the randomized indigo carmine dose to evaluate urine efflux from right and left ureters.

For the first enrolled 18 of the 114 patients (9 randomized to the high dose arm and 9 to the low dose arm), the primary efficacy endpoint of a conspicuity score for the ureteral efflux was measured by the 3-point ureteral orifice visualization scale (UOVS) as per the initial protocol:

- 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.

During routine monitoring for the first 18 subjects, it was noted that the UOVS score by a surgeon provided no differentiations between indigo carmine and saline injections possibly due to a ceiling effect as shown in Table 38.

Table 38. Average UOVS Score* in the Training Set

	Indigo Carmine		Saline	
Treatment Arm	**Left**	**Right**	**Left**	**Right**
Indigo carmine 2.5 mL arm (n=9)	3.00	3.00	3.00	2.89
Indigo carmine 5 mL arm (n=9)	3.00	3.00	3.00	3.00

Source: FDA primary statistical reviewer
* Ureteral orifice visualization scale (UOVS) with 3-point scale

This observation prompted the Applicant to request guidance from the Agency via a type-C meeting on 4/16/2020. During the meeting, the Applicant was advised to develop a new objective scale that would be able to detect the ureteral efflux visualization difference between indigo carmine and saline injections. At the time when this advice was received, ureteral efflux visualization videos were available for the first 18 subjects. Using these 18 videos, the Applicant developed and tested a 5-point conspicuity scale by asking 4 independent urologists not aware of treatment associated with each video to score the videos using the 5-point conspicuity scale:

- 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 test results of the 5-point conspicuity scale by the 4 independent urologists are shown in Table 39. The Applicant implemented this 5-point conspicuity score as the primary efficacy endpoint in the protocol amendment dated 9/2/2020.

Table 39. Average of 5-Point Conspicuity Scores From 4 Independent Urologists in the Training Set

	Indigo Carmine		Saline	
Treatment Arm	**Left**	**Right**	**Left**	**Right**
Indigo carmine 2.5 mL arm (n=9)	3.00	3.39	1.75	1.75
Indigo carmine 5 mL arm (n=9)	2.83	3.39	2.05	1.86

Source: FDA primary statistical reviewer

According to the statistical analysis plan dated 6/26/2021, the 18 patients who were randomized and treated prior to the development of the 5-point conspicuity scale were excluded from the efficacy population. These patients were excluded from the efficacy analysis because the initial protocol only required investigators to record a single video that covered the images of ureteral efflux after indigo carmine and saline injections. Although these vides could be split into 2 separate segments based on the times of saline and indigo carmine injections to facilitate testing of the new conspicuity score, the 2 video segments were not of uniform duration. This lack of consistency might potentially unblind treatment to the scoring reviewers biasing the study efficacy results. The videos for these 18 patients were not subject to the formal central review process used to evaluate the primary efficacy for the study.

Study Results

For the remaining 96 of the 114 patients, video of urine efflux from both right and left ureters was recorded for 10 minutes post saline injection and another 10-minute video post randomized dose of indigo carmine. The 96 patients (47 in the indigo carmine 2.5 mL arm and 49 in the indigo carmine 5 mL arm) constitute the mITT population consisting of all patients who received both study drugs (indigo carmine and saline) and had a surgical procedure to assess ureteral patency with a video approximately 10 minutes in length available after each treatment. Videos from the 96 patients were sent to a central imaging group that pooled and blinded the videos. Videos were assessed by independent central reviewers for assessment of conspicuity of ureteral urine flow using the 5-point conspicuity scale. Average conspicuity scores for the mITT population are shown in Table 40.

Table 40. Average Conspicuity Scores for the mITT Population

	Indigo Carmine		Saline	
Treatment Arm	**Left**	**Right**	**Left**	**Right**
Indigo carmine 2.5 mL arm	4.3	4.2	2.53	2.74
Indigo carmine 5 mL arm	3.9	4.3	2.23	2.48

Source: FDA primary statistical reviewer
Abbreviations: modified intent-to-treat

The frequency of missing conspicuity scores in the mITT population was nonsignificant as shown in Table 41.

Table 41. Frequency of Available Conspicuity Scores

	Indigo Carmine			Saline		
Treatment Arm	**Left**	**Right**	**%**	**Left**	**Right**	**%**
Indigo carmine 2.5 mL arm (n=47)	46	46	92/94=0.98	43	43	86/94=0.92
Indigo carmine 5 mL arm (n=49)	49	47	96/98=0.98	48	46	94/98=0.96

Source: FDA primary statistical reviewer

Two null hypotheses regarding the primary efficacy endpoint of the conspicuity score were tested on the mITT population:

1. There is no difference in the 5-point conspicuity score between indigo carmine low dose (2.5 mL) and saline injections,
2. There is no difference in the 5-point conspicuity score between indigo carmine high dose (5 mL) and saline injections.

Applicant's Efficacy Analysis

The treatment effect of odds ratio of indigo carmine versus saline for the conspicuity score was evaluated via a generalized estimating equation (GEE) for repeated measures to control for the possible intrasubject correlation by fitting a proportional odds model (ordered logit model) including treatment (indigo carmine or saline), ureter (left or right) and treatment by ureter interaction as covariates. Multiplicity was controlled by the Hochberg method. SAS codes used to fit the GEE model are shown below (usubjid stands for subject id, parcat2 for left or right side of ureter, trtp for indigo carmine or saline treatment and aval for conspicuity score):

```
proc genmod data =;
    class usubjid parcat2 (ref = 'right ureter') trtp (ref = 'saline');
    model aval = trtp parcat2 trtp*parcat2 / link = clogit dist = mult type3;
    repeated subject = usubjid / type = ind;
    estimate 'OR: indigo carmine vs saline' trtp 1 -1/ exp;
run;
```

The GEE model was fitted separately for each comparison: indigo carmine high dose versus saline and indigo carmine low dose versus saline. Efficacy analysis results of conspicuity scores of ureteral efflux for indigo carmine versus saline injections are summarized in Table 42.

Table 42. Efficacy Analysis Results of Conspicuity Scores of Indigo Carmine Versus Saline

Parameter	Indigo Carmine 2.5 mL Arm N=47	Indigo Carmine 5 mL Arm N=49
Odds ratio: indigo carmine vs. saline	16.77	14.03
(95% confidence interval)	(8.62, 32.62)	(7.41, 26.58)
p-value	<0.001	<0.001

Source: Table 2-5, Summary of Clinical Efficacy

Statistical Reviewer's Sensitivity Analysis 1

Missing conspicuity scores in the mITT population dataset were imputed using a worst-case scenario: the lowest value of 1 for missing scores of indigo carmine injection and the highest value of 5 for missing scores for saline injection. Sensitivity analysis 1 results of conspicuity scores of ureteral efflux of indigo carmine versus saline injections by the GEE model used in the primary efficacy analysis are summarized in Table 43.

Table 43. Sensitivity Analysis 1 Results of Conspicuity Scores of Indigo Carmine Versus Saline[a]

Parameter	Indigo Carmine 2.5 mL Arm N=47	Indigo Carmine 5 mL Arm N=49
Odd ratio: indigo carmine vs. saline	8.63	9.45
(95% confidence interval)	(3.80, 18.58)	(5.11, 17.51)
p-value	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] Missing conspicuity score is imputed with the lowest score of 1 for missing scores of indigo carmine injection and with the highest score of 5 for missing scores of saline injection.

Statistical Reviewer's Sensitivity Analysis 2

The conspicuity score is treated as a continuous variable rather than an ordinal one as in the GEE model used for the primary efficacy analysis. The treatment effect of mean difference in the conspicuity score between indigo carmine and saline injections was evaluated using a mixed-effect model including treatment (indigo carmine or saline), ureter (left or right) and treatment by ureter interaction as fixed covariates and subject as a random covariate. The mixed-effect model is fitted on the mITT population. Since observations of conspicuity score were repeated per ureter and treatment within a subject, ureter by treatment grouping is to be imposed upon the errors. Unstructured covariance structure of the errors is specified. Note that unlike the GEE model that assesses the overall treatment effect while accounting for the correlation of measurements within individual subjects, the mixed-effect model accounts for subject-specific contribution in assessing the treatment effect.

SAS codes used to fit a mixed-effect model are shown below (usubjid stands for subject id, parcat2 for left or right side of ureter, trtp for indigo carmine or saline treatment and aval for conspicuity score):

```
proc mixed data =;
    class usubjid parcat2 (ref = 'right ureter') trtp (ref = 'saline');
```

```
    model aval = trtp parcat2 trtp*parcat2 / ddfm = kr;

    repeated parcat2*trtp / subject = usubjid type = un;

    lsmeans trtp / cl pdiff;

run;
```

The sensitivity analysis 2 results of conspicuity scores of ureteral efflux of indigo carmine versus saline injections are summarized in Table 44.

Table 44. Sensitivity Analysis 2 Results of Conspicuity Scores of Indigo Carmine Versus Saline[a]

Parameter	Indigo Carmine 2.5 mL Arm N=47	Indigo Carmine 5 mL Arm N=49
Mean difference: indigo carmine vs. saline	1.63	1.72
(95% confidence interval)	(1.30, 1.95)	(1.41, 2.04)
p-value	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] Conspicuity score was analyzed as a continuous endpoint

Statistical Reviewer's Sensitivity Analysis 3

As another approach to evaluate the treatment effect of mean difference in the conspicuity score between indigo carmine and saline injections, a paired t-test was applied to conspicuity scores of indigo carmine and saline injections for left and right ureters separately in low and high dose arms in the mITT population. The sensitivity analysis 3 results of conspicuity scores of ureteral efflux of indigo carmine versus saline injections are summarized in Table 45.

Table 45. Sensitivity Analysis 3 Results of Conspicuity Scores of Indigo Carmine Versus Saline

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	Left	Right	Left	Right
Mean difference: indigo carmine vs. saline	1.76	1.55	1.66	1.76
(95% confidence interval)	(1.37, 2.14)	(1.15, 1.94)	(1.28, 2.03)	(1.39, 2.13)
p-value[a]	<0.001	<0.001	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] Paired t-test

Statistical Reviewer's Sensitivity Analysis 4

The conspicuity of ureteral efflux was assessed by 2 central, independent, reviewers who provided main scores and by an adjudicator who intervened and provided a final score in cases where the scores of the 2 reviewers deviated by more than 1 point. Scores from the 2 reviewers and videos were presented to the adjudicator. All 3 reviewers including the adjudicator were blinded to any subject clinical data.

Table 46 and Table 47 summarize mean conspicuity score difference in indigo carmine versus saline for each of the 2 central reviewers. A paired t-test was applied to conspicuity scores of

indigo carmine and saline injections for left and right ureters separately in low and high dose arms from the 2 reviewers in the mITT population.

Table 46. Mean Conspicuity Score Difference of Indigo Carmine Versus Saline for Reviewer 1

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	**Left**	**Right**	**Left**	**Right**
Mean difference: indigo carmine vs. saline	1.28	1.26	1.21	1.50
(95% confidence interval)	(0.77, 1.79)	(0.78, 1.74)	(0.80, 1.62)	(1.07, 1.93)
p-value[a]	<0.001	<0.001	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] paired t-test

Table 47. Mean Conspicuity Score Difference of Indigo Carmine Versus Saline for Reviewer 2

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	**Left**	**Right**	**Left**	**Right**
Mean difference: indigo carmine vs. saline	1.79	1.69	1.85	2.00
(95% confidence interval)	(1.41, 2.16)	(1.33, 2.05)	(1.50, 2.20)	(1.67, 2.33)
p-value[a]	<0.001	<0.001	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] paired t-test

Ninety-six patients of the mITT population resulted in 192 individual videos (saline and indigo carmine injections) requiring 2 conspicuity scores for each video (left ureter and right ureter) providing 384 total individual conspicuity scores. Of those 384 scores 52 (13.5%) required adjudication.

Table 48 summarizes the breakdown of adjudication cases according to how the initial conspicuity scores from the 2 reviewers differ per treatment (saline and indigo carmine) and arm (2.5 and 5 mL). Major observations from this summary are:

1. Adjudication cases occur at a similar rate in 2.5 mL and 5 mL arms, but extreme cases where the scores from the 2 reviewers differed by 3 or 4 points all occur in 5 mL arm.
2. A majority of adjudication cases occur in saline injection.
3. The adjudicator tends to provide a lower score for saline injection and a higher score for indigo carmine injection if conspicuity scores from the 2 reviewers differ.

Table 48. Breakdown of Adjudication Cases[a]

	Saline Injection						Indigo Carmine Injection						
Treatment Arm	**Scores Differ by 2 Points**	**Scores Differ by 3 Points**	**Scores Differ by 4 Points**	**1 From a Reviewer and NA From the Other**	**NAs From Both Reviewers**	**Total**	**Scores Differ by 2 Points**	**Scores Differ by 3 Points**	**Scores Differ by 4 Points**	**1 From a Reviewer and NA From the Other**	**NAs From Both Reviewers**	**Total**	**Total by Arm**
2.5 mL arm (adj's verdict)	14 (10: adj picks lower score)	0	0	2 (2: adj picks 1)	0	16	8 (2: adj picks lower score)	0	0	0	0	8	24
5 mL arm (adj's verdict)	14 (9: adj picks lower score)	3 (3: adj picks lower score)	0	3 (3: adj picks 1)	1 (1: adj picks NA)	21	2 (1: adj picks lower score)	0	2 (1: adj picks lower score)	2 (2: adj picks 1)	1 (1: adj picks NA)	7	28
Total by treatment						37						15	52

Source: FDA primary statistical reviewer
[a] Adjudication cases are counted by ureter,
Abbreviations: adj, adjudicator; NA, not available

To evaluate the impact of the variability of conspicuity scores from 2 reviewers on the robustness of the efficacy of indigo carmine in visualizing ureteral efflux, the conspicuity scores of the 2 central reviewers were replaced with a maximum conspicuity score from 2 reviewers for saline injection and with a minimum conspicuity score from 2 reviewers for indigo carmine injection. Then, a paired t-test was applied to conspicuity scores of indigo carmine and saline injections for left and right ureters separately in low and high dose arms on the mITT population. The sensitivity analysis 4 results of conspicuity scores of ureteral efflux of indigo carmine versus saline injections are summarized in Table 49.

Table 49. Sensitivity Analysis 4 Results of Conspicuity Scores of Indigo Carmine Versus Saline[a]

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	**Left**	**Right**	**Left**	**Right**
Mean difference: indigo carmine vs. saline	0.91	0.93	0.85	1.24
(95% confidence interval)	(0.43, 1.38)	(0.46, 1.40)	(0.41, 1.30)	(0.83, 1.66)
p-value[b]	<0.001	<0.001	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] Conspicuity scores of the central review panel were replaced with a maximum conspicuity score from 2 reviewers for saline injection and with a minimum conspicuity score from 2 reviewers for indigo carmine injection
[b] Paired t-test

Statistical Reviewer's Sensitivity Analysis 5

Missing conspicuity scores in the mITT population dataset were imputed using a worst-case scenario: the lowest value of 1 for missing scores of indigo carmine injection and the highest value of 5 for missing scores of saline injection. Table 50 summarizes the sensitivity analysis 5 results of conspicuity scores of ureteral efflux of indigo carmine versus saline injections by a paired t-test was applied to conspicuity scores of indigo carmine and saline injections for left and right ureters separately in low and high dose arms.

Table 50. Sensitivity Analysis 5 Results of Conspicuity Scores of Indigo Carmine Versus Saline[a]

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	**Left**	**Right**	**Left**	**Right**
Mean difference: indigo carmine vs. saline	1.47	1.20	1.61	1.49
(95% confidence interval)	(1.00, 1.93)	(0.70, 1.71)	(1.23, 1.99)	(1.01, 1.97)
p-value[b]	<0.001	<0.001	<0.001	<0.001

Source: FDA primary statistical reviewer
[a] Missing conspicuity score is imputed with the lowest score of 1 for missing scores of indigo carmine injection and with the highest score of 5 for missing scores of saline injection
[b] Paired t-test

Statistical Reviewer's Exploratory Analysis

In this study, the discrepancy was observed about the 3-point scale and 5-point scale conspicuity score with 18 patient-images. The secondary statistical reviewer reduced the 5-point scale to the 3-point UOVS scoring according to the following scheme:

- 1 = No jet observed, or Weak jet, little color contrast, (combine scores 1 and 2 in the 5-point scale)

- 2 = Color contrast or significant jet flow, (score 3 in the 5-point scale)
- 3 = Strong jet flow with good color contrast, or Strong jet flow with striking contrast in color. (combine scores 4 and 5 under 5-point scale)

The paired t-test analysis of this derived conspicuity score based on 96 patient images in mITT population with worst case scenario imputation was performed. The algorithm of the worst-case imputation is as follows: impute with the lowest score of 1 for missing scores of indigo carmine injection and impute with the highest value of 3 for missing scores of saline injection. The results are shown in Table 51. It is interesting to note that when the 5-point scale conspicuity score is collapsed into the 3-point scale with 18 patient- images in the training set, the derived conspicuity score was able to show the indigo carmine effect for both 2.5 mL dose and 5 mL dose.

Table 51. Mean Difference of Conspicuity Scores Using a 3-Point Scale Derived From the 5-Point Scale[a]

	Indigo Carmine 2.5 mL Arm N=47		Indigo Carmine 5 mL Arm N=49	
Parameter	**Left**	**Right**	**Left**	**Right**
Mean difference: indigo carmine vs. saline	0.65	0.37	0.64	0.58
(95% confidence interval)	(0.37, 0.93)	(0.07, 0.67)	(0.37, 0.91)	(0.27, 0.89)
p-value[b]	<0.001	<0.02	<0.001	<0.001

Source: FDA secondary statistical reviewer
[a] Impute a score of 1 if derived conspicuity score is missing during indigo carmine and a score of 3 if derived conspicuity score is missing during saline
[b] Paired t-test

8.3.2. Reviewer Agreement

To investigate the consistency in evaluating the conspicuity of ureteral efflux for indigo carmine injection versus saline injection among reviewers, the paired score difference (conspicuity score for indigo carmine injection minus conspicuity score for saline injection on the same subject) for a given dose (2.5 mL or 5 mL) and ureter (left L or right R) is considered. Note that urologists U gave conspicuity scores in the training set (first 18 subjects) and reviewer 1 R1, reviewer 2 R2 and surgeon S gave conspicuity scores in the validation set (96 subjects in the mITT population). As the training set was used to develop a new scoring system (after observing a ceiling effect in the original UOVS score) and see if there would be separation between indigo carmine and saline injections in the 5-point conspicuity score, reading training was only available for the reviewers in the validation dataset, not for the urologists in the training dataset.

Table 52 describes the distribution of the paired score difference by data set, dose, ureter, and reviewer, ranging from -4 to 4 (minus difference occurs when a reviewer gave a higher score for saline injection) with bold % representing score difference with the highest frequency. Table 53 includes major distributional statistics of the paired score difference by ureter, dose and reviewer in the validation set.

Table 52. Distribution of the Paired Conspicuity Score Difference (IC - Saline)

	Training Set				Validation Set											
	2.5 mL		5 mL		2.5 mL			5 mL			2.5 mL			5 mL		
Percentage	L	R	L	R	L	L	L	L	L	L	R	R	R	R	R	R
Score	U	U	U	U	R1	R2	S	R1	R2	S	R1	R2	S	R1	R2	S
% sc-4	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0
% sc-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0.	0	0
% sc-2	0	0	0	0	2	2	0	0	0	0	4	0	0	2	0	2
% sc-1	0	0	0	0	6	0	2	8	0	2	7	2	0	2	0	0
% sc 0	11	11	22	11	9	11	19	25	14	23	16	12	9	15	9	14
% sc 1	**56**	11	**44**	33	**41**	19	15	**35**	21	4	**35**	29	19	**46**	23	8
% sc 2	11	**78**	22	**44**	16	**40**	**39**	8	**38**	**38**	16	**36**	**39**	6	**36**	**52**
% sc 3	22	0	11	11	9	21	13	14	14	23	9	14	24	15	22	17
% sc 4	0	0	0	0	11	4	10	8	10	8	12	7	7	13	9	6

Source: FDA primary statistical reviewer
Bolded % represents scores with the highest frequencye
Abbreviations: IC, indigo carmine; L, left; R, right; R1, reviewer 1; R2, reviewer 2; S, surgeon; U, urologist

Table 53. Summary Statistics of the Paired Conspicuity Score Difference (IC - Saline) in the Validation Set

Ureter Arm Statistics	Reviewer 1	Reviewer 2	Surgeon
Left			
2.5 mL			
N	43	42	46
Mean (SD)	1.28 (1.65)	1.79 (1.20)	1.74 (1.29)
95% CI	0.77, 1.79	1.41, 2.16	1.36, 2.12
Median	1	2	1
Range	-4, 4	-2, 4	-1, 4
5 mL			
N	48	47	47
Mean (SD)	1.21 (1.41)	1.85 (1.18)	1.83 (1.32)
95% CI	0.80, 1.62	1.50, 2.20	1.44, 2.22
Median	1	2	2
Range	-1, 4	0, 4	-1, 4
Mean (median) difference between 2.5 mL and 5 mL (95% CI)	0.07 (0) (-0.57, 0.71)	0/06 (0) (-0.44, 0.56)	0.09 (1) (-0.45, -0.63)
Right			
2.5 mL			
N	43	42	46
Mean (SD)	1.26 (1.56)	1.69 (1.16)	1.87 (1.36)
95% CI	0.78, 1.74	1.33, 2.05	1.47, 2,27
Median	1	2	2
Range	-2, 4	-1, 4	-4, 4
5 mL			
N	46	44	48
Mean (SD)	1.50 (1.44)	2.00 (1.10)	1.83 (1.19)
95% CI	1.07, 1.93	1.67, 2.33	1.49, 2.18
Median	1	2	2
Range	-2, 4	0, 4	-2, 4
Mean (median) difference between 2.5 mL and 5 mL (95% CI)	.24 (0) (-0.39, 0.87)	0.31 (0) (-0.17, 0.79)	0.04 (0) (-0.48, 0.56)

Abbreviations: CI, confidence interval; IC, indigo carmine; SD, standard deviation

From Table 52 and Table 53, distributions of the paired score difference are skewed and peaked at score difference of 1 for left ureter and 2 for right ureter in the training set, while the peaked paired score difference for reviewer 1 is 1 and is 2 for reviewer 2 in the validation set. There is no statistical evidence that paired score difference between 2.5 mL and 5 mL is different for either reviewer in the validation set, the median is the same.

Table 54 summarizes the agreement rate of the paired score difference between reviewers 1 and 2 by ureter and dose.

Table 54. Agreement Rate of the Paired Score Difference (IC - Saline) Between Reviewers 1 and 2

Ureter Arm	% Agree	% ±1 Score Diff	% ±2 Score Diff	% ±3 Score Diff	% ±4 Score Diff
Left					
2.5 mL	33	40	21	2	2
5 mL	38	40	17	4	0
Right					
2.5 mL	33	52	12	2	0
5 mL	39	43	18	0	0

Source: FDA primary statistical reviewer
Abbreviations: IC, indigo carmine

Percentage of more than 1 point difference in the paired score difference between reviewers 1 and 2 are 25%, 21%, 14% and 18%. None of the cases meet the criteria of reasonable consistency of no more than 5 to 10% of score difference being greater than 1 point. Therefore, disagreement between the 2 reviewers cannot be ignored.

These analyses attempted to assess the consistency in scoring the conspicuity between the reviewers by ureter and by dose. Statistical test accounting for ureter and dose suggested there is significant reviewer effect (nominal p-value<0.001), namely, scoring by reviewer 1 and reviewer 2 are different. Assuming reviewer agreement issue can be ignored, statistical significance of the difference in conspicuity score between indigo carmine and saline injections remains based on the worst case analysis, shown in the Reviewer's sensitivity analysis 4.

As a way of presenting the visualization efficacy results of indigo carmine in the presence of a reviewer agreement issue, a responder analysis was conducted (reviewer disagreement in conspicuity score is less visible with count of units whose scores exceed a threshold compared to with actual numerical scores from reviewers). A responder is defined as a ureter whose difference in conspicuity score between indigo carmine and saline injections is greater than or equal to 1 while conspicuity from indigo carmine injection is greater than or equal to 3, with 3 being color contrast or significant jet flow. The results of the responder analysis by ureter, dose and reviewer are given in Table 55.

Table 55. Proportion of Responders (IC - Saline Score ≥1 and IC Score ≥3).

Ureter Dose	Reviewer 1	Reviewer 2	Surgeon
Left			
2.5 mL	33 / 43=0.77	36 / 42=0.86	35 / 46=0.76
5 mL	31 / 48=0.65	38 / 47=0.81	35 / 47=0.74
Right			
2.5 mL	31 / 43=0.72	36 / 42=0.86	40 / 46=0.87
5 mL	37 / 46=0.80	40 / 44=0.91	40 / 48=0.83

Source: FDA primary statistical reviewer
Abbreviations: IC, indigo carmine

8.3.3. Statistical Comments on Scientific Literature Review

The following two publications offer supportive evidence of efficacy of indigo carmine for the endpoint of the urinary tract injuries, not visualization of conspicuity of ureteral efflux. These two publications do not mention the dose of indigo carmine.

Ibeanu, 2009[1]

This study assessed urinary tract injury during hysterectomy based on universal cystoscopy. The objective was to estimate the incidence and location of injury to the urinary tract during hysterectomy for benign gynecological disease.

This prospective clinical study was conducted in an academic environment at three sites in the U.S. Diagnostic cystourethroscopy using indigo carmine was performed on all patients after hysterectomy for benign disease. A total of 839 patients were enrolled. The rate of bladder injury was 2.9% (24 of 839 cases), and rate of ureteral injury was 1.8% (15 of 839 cases). There were three cases of simultaneous bladder and ureteral injuries, resulting in a cumulative injury rate of 4.3% (36 of 839 cases). There was one false negative (normal dye efflux on cystoscopy, but injury subsequently detected). The most common site of injury to the ureter was at the junction of the ureter and the uterine artery in 80% (12 of 15 cases) of ureteral injuries. Transection and kinking injuries were the most frequent type of injury. There were 21 cases of subnormal dye efflux from the ureteral orifices, with no subsequent injury detected on further evaluation (false positive). The authors concluded that ureteral injury occurred most commonly at the level of the uterine artery, transection and kinking injuries were most frequent, and diminished dye efflux from ureteral orifices was not associated with injury.

Secondary statistical reviewer comments: *This publication is about multicenter demonstration of efficacy of indigo carmine for detection of ureteral injury. It is about estimation of the incidence and location of injury to the urinary tract during hysterectomy for benign gynecological disease. IC dose is not mentioned in this paper.*

[1] (Ibeanu et al. 2009)

Table 56. Ureteral and Bladder Injury Incidence

Parameter	Ureteral and Bladder Injury Present - Surgeon's Assessment	Ureteral and Bladder Injury Absent - Surgeon's Assessment	PPV/NPV Percentage
IC Dye +	36	21	PPV =63%
IC Dye -	1	781	NPV =99.9%
PPA/NPA Percentage	PPA =97%	NPA =97%	

Abbreviations: IC, indigo carmine; PPA, positive percent agreement; PPV, positive predictive value; NPA, negative percent agreement; NPV, negative predictive value

Vakili, 2005[2]

This was a prospective study to evaluate the incidence of urinary tract injury during hysterectomy for benign disease. Patients were enrolled prospectively from three sites in the U.S. All patients undergoing abdominal, vaginal, or laparoscopic hysterectomy for benign disease underwent diagnostic cystourethroscopy using indigo carmine.

Four hundred and seventy-one patients participated. Ninety-six percent (24/25) of urinary tract injuries were detected intraoperatively. There was one urinary tract injury (bladder) that was not seen during surgery but presented after 7 days. There were 8 cases of ureteral injury and 17 cases of bladder injury. The authors concluded that the incidence of urinary tract injury during hysterectomy was 4.8%, that surgery for prolapse or incontinence increases the risk, and that routine use of cystoscopy during hysterectomy should be considered.

Secondary statistical reviewer comments: This publication is based on multicenter (3 centers) prospective experience for estimation purpose. During hysterectomy, detection rate for urinary tract injury using indigo carmine was 4.8% using surgeon's assessment as SOR. Out of 23 injuries, 7 were detected before cystoscopy and 16 additional injuries were detected by cystoscopy (with indigo carmine). The authors state that the reported incidence of urinary tract injury during abdominal hysterectomy is 2.2%. The incidence of 4.8% in this study is higher than the reported incidence. IC dose is not mentioned in this paper.

8.4. Conclusions and Recommendations

Intra-operative identification of ureteral injury with cystoscopy in patients undergoing urogynecologic surgeries is critical for early intervention to reduce serious morbidity. The review team unanimously recommends approval of this New Drug Application for the use of IC, a diagnostic dye, as a visualization aid in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures. The finding of efficacy and safety relies primarily on one Phase 3 trial (PVP-19IC01) with supporting evidence from a Phase 1 trial (PVP-20IC02). In addition, the scientific literature, including two prospective study reports, provides support for the clinical utility and the safety of IC in the same clinical context of use, namely cystoscopic visualization of ureteral efflux to evaluate the integrity of the ureters (Vakili et al. 2005; Ibeanu et al. 2009).

[2] (Vakili et al. 2005)

[redacted] effective in assessing the urine efflux from the ureters, [redacted] the 5 mL dose of IC is based on the support of this dose by the scientific literature. It is noted that this dose is recommended by a practice guideline and by existing labeling.

In summary, the 5 mL (0.8 mg/mL) IC dose is:

- The most commonly used dose of IC used in the same clinical context as that of the indicated population in studies reported in the scientific literature (Pettit and Petrou 1994; Harris et al. 1997; Ribeiro et al. 1999; Jelovsek et al. 2007; Lee and Jang 2012)
- The recommended dose described in the practice guidelines of the American Association of Gynecological Laparoscopists (AAGL Advancing Minimally Invasive Gynecology Worldwide 2012)
- The dose listed in the non-U.S. approved product labeling (SERB Laboratories 2015; American Regent 2017)
- The dose listed in the PI of the unapproved indigo carmine product marketed by American Regent in the U.S. (NDC#0517-0375-05 (American Regent 2022))

[redacted]

A review of the safety experience with IC use identified serious and life threatening, albeit rare, adverse reactions. The safety review conducted by DPV included the analysis of FAERS, the scientific literature, VigiBase, and the Applicant's clinical safety data. The serious adverse reactions include cardiac arrest, acute elevation or decrease in blood pressure, cardiac conduction abnormalities including heart block, as well as hypersensitivity reactions. Accordingly, information on safety risks and mitigation steps have been included in the Warnings and Precautions and Adverse Reactions sections of the PI.

In addition, the FDA reached an agreement with the Applicant for a PMC to provide [redacted] and dosage of IC through a postmarketing clinical trial in which [redacted] doses will be tested. [redacted] The FDA also reached an agreement with the Applicant for a separate PMC study [redacted] in patients with renal insufficiency.

9. Advisory Committee Meeting and Other External Consultations

The Application did not raise safety or efficacy issues requiring discussion at an Advisory Committee. No external consultation was needed.

10. Pediatrics

The two Applicant-conducted studies, PVP-19IC01 and PVP-20IC02, did not include pediatric patients. Hence, FDA's PeRC required that a clinical trial be conducted under a Pediatric Research Equity Act (PREA) PMR to establish the efficacy and safety of IC in the pediatric population. The Applicant has an Agreed iPSP to conduct a clinical trial (b) (4)

(b) (4)

The following observations provide the rationale for the PMC study. The recommended dose of IC for adults will be 5 mL of an 8 mg/mL solution administered intravenously. The dose is supported by the single efficacy trial and by published studies from long-term use of marketed, unapproved IC. (b) (4)

administered for the indicated use of IC and might lead to important, albeit rare adverse reactions. The PMC study of the two IC doses (b) (4) (b) (4)

11. Prescription Drug Labeling

The Applicant submitted a draft PI in module 1 at the time of initial NDA submission. The labeling was reviewed independently and jointly by the respective teams – CMC, Pharmacology/Toxicology, Clinical Pharmacology, Division of Medication Error Prevention and Analysis (DMEPA), OSE, Division of Pediatric and Maternal Health (DPMH), Biometrics and Clinical in multiple meetings with several information requests (IR) to the Applicant as needed for clarification and or additional information. As a result, the following changes were made and reflect the final version of the PI.

Prescribing Information

The following points and changes have been addressed in the PI.

- Nonproprietary Name of the Drug Product
- (b) (4) the USP monograph for the drug product is "indigotindisulfonate sodium injection." The proposed labeling used (b) (4) (b) (4) "indigotindisulfonate sodium injection", "indigotindisulfonate sodium" or "indigotindisulfonate" as appropriate to be consistent with the established name of the USP monograph.
- Indications and Usage
- (b) (4)
- Dosage and Administration
  - (b) (4) 5 mL in the labeling. The approved dose for visualization in the cystoscopic assessment of the integrity of the ureters in adults following urological and gynecological open, robotic, or endoscopic surgical procedures is 5 mL.
  - (b) (4) When the 2.5 mL dose is mentioned, the following statement "The 2.5 mL dose is not approved" is added as the labeling should not imply endorsement of an unapproved dose that is not included in the Dosage and Administration section per 21 CFR 201.57(c)(3)(ii).
  - Instructions to monitor blood pressure and cardiac rhythm during and following the injection are included for safety.

  - The drug is dark blue in color and is packaged in an amber glass ampoule. As a result, it is difficult to inspect the vial for particulate matter and discoloration. An instruction is added to withdraw the drug using a 5 micron or smaller filter to ensure that the withdrawn solution contains no particulates.
  - Due to the lack of compatibility data for drugs that can be coadministered, the instruction of not administering with infusion assemblies used with other drugs is added.

- Warnings and Precautions
  - This section is revised to include warnings for serious adverse reactions reported from the use of indigotindisulfonate sodium injection products. The warnings include cardiovascular reactions and hypersensitivity reactions. The drug is contraindicated in patients with a known hypersensitivity to indigotindisulfonate. Recommendations for close monitoring for blood pressure, cardiac rhythm, and anaphylactic reactions are added.
  - A warning for spurious changes to sPO2 measurements is added.
- Adverse Reactions
- The adverse reactions (≥1%) observed in the clinical trials are listed in order of decreasing frequency.
- The adverse reactions from the FAERS report are listed by body system, then by severity of the reaction.
- The revised section is consistent with the FDA labeling regulation for the Adverse Reactions section.
- Pregnancy and Lactation
  - Limitations of available evidence are reflected in the risk summary of pregnancy and lactation.
  - (b) (4)
- Renal Impairment
  - Indigotindisulfonate sodium injection is not recommended for patients with eGFR <30 mg/mL due to the lack of clinical data to support the safe and effective use of the drug.

For additional information related to the points described above, please refer to the approved labeling.

Other Prescription Drug Labeling

Refer to the DMEPA review in DARRTS for the container label and carton labeling.

12. Risk Evaluation and Mitigation Strategies (REMS)

No risk evaluation and mitigation strategies are required beyond the information and risk mitigation steps included in the Warning and Precautions section of the labeling.

13. Postmarketing Requirements and Commitments

The Applicant commits to conduct a PMC study of [redacted] (b) (4) doses of IC [redacted] (b) (4)

The Applicant also commits to conduct a [redacted] (b) (4) PMC study [redacted] (b) (4) in patients with renal insufficiency.

Further, the Applicant has an Agreed PREA iPSP under a PREA PMR to study the efficacy and safety of IC in the pediatric population [redacted] (b) (4)

14. Division Director (Clinical, Designated Signatory Authority) Comments

I concur with the unanimous recommendation by all the review disciplines for the approval of this application for indigo carmine injection (trade name Bludigo), a diagnostic renally excreted dye in long-term clinical use. The dye stains the urine blue and is proposed as an aid for the cystoscopic visualization (b) (4)

The evidence of the efficacy of the recommended dose of the dye (5 mL of 8mg/ml injection IV) is provided by one adequate and well controlled trial that demonstrated greater conspicuity of urinary efflux from the ureters after dye injection compared to saline injection and by support from the scientific literature. The visualization efficacy of IC was supported by several sensitivity analyses and was represented in the labeling by a responder analysis based on patients who met specific criteria for the level of increased conspicuity following IC injection. The contribution of IC to the procedure for the intraoperative recognition of ureteral injuries is meaningful because it allows for prompt repair of the injury. This benefit outweighs the risk of serious, rare hypersensitivity and cardiovascular reactions reported in the scientific literature and FAERS.

Unresolved review questions include the need to: confirm the efficacy of (b) (4) dose of indigo carmine (b) (4) IC for patients with renal insufficiency; and efficacy of IC in pediatric patients. These questions will be addressed by two trials under two PMCs in adults and a PREA PMR study. The pediatric study will be delayed (b) (4) .

15. Appendices

15.1. References

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15.2. Financial Disclosure

Covered Clinical Study (Name and/or Number): PVP-19IC01

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 15		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): None		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 0 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator 0 Sponsor of covered study: Provepharm Inc		
Is an attachment provided with details of the disclosable financial	Yes ☐	No ☐ (Request details from Applicant)

interests/arrangements:		
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

15.3. OCP Appendices (Technical documents supporting OCP recommendations)

15.3.1. Summary of Bioanalytical Method Performance

Table 57. Summary Method Performance of a Bioanalytical Method to Measure Indigo Carmine in Human Plasma

Parameter	Details		
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS		
Method description	LC-MS/MS Assay for the Determination of Indigo Carmine and 5 Sulfo Anthranilic Acid in Human Plasma		
Materials used for calibration curve & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 0.100, 0.250, 1.50, 3.50, 5.00, 7.00, 9.00, 10.0 µg/mL		
Validated assay range	0.100 to 10.0 µg/mL		
Material used for QCs & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 0.100, 0.300, 4.00, 8.00 µg/mL		
Regression model & weighting	Quadratic $1/x^2$		
Validation parameters	Method validation summary		Acceptability
Standard calibration curve performance during accuracy & precision	Number of standard calibrators from LLOQ to ULOQ	8	Acceptable
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-2.0 to 1.0%	Acceptable
	Cumulative precision (%CV) from LLOQ to ULOQ	≤8.6%	Acceptable
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs QCs: LLOQ, Low, Medium, High	-5.2 to 8.9%	Acceptable
	Interbatch %CV QCs: LLOQ, Low, Medium, High	≤12%	Acceptable
Selectivity & matrix effect	Six lots of human plasma were tested. For indigo carmine, the range of observed matrix effect was 1.1 to 1.4. No issues were observed.		Acceptable
Interference & specificity	Six lots of human plasma were tested. For indigo carmine, the observed bias was -9.3%. No issues were observed.		Acceptable

Parameter	Details	
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS	
Hemolysis effect	One lot of hemolyzed plasma containing approximately 2% lysed red blood cells was tested. For indigo carmine, the range of observed bias was -2.4% to 3.0%. The results indicate that hemolysis does not affect the ability to accurately measure analyte concentrations in plasma.	Acceptable
Lipemic effect	One lot of hyperlipidemic plasma (>150 mg/dL) was tested. For indigo carmine, the range of observed bias was 6.0% to 6.3%. The results indicate that hyperlipidemia does not affect the ability to accurately measure analyte concentrations in plasma.	Acceptable
Dilution linearity	A 40.0-µg/mL QC sample was diluted ten-fold (10X) with human plasma to a concentration of 4.00 µg/mL. For indigo carmine, the percent difference from the nominal concentration was 0.8% and the precision (CV) was 2.9%.	Acceptable
Bench-top/process stability	To determine quantitative reproducibility of extracted samples following storage at 2-8°C for an extended time period, six replicates of low and high QC samples were extracted and analyzed as one analytical run. The extracts were stored for 51 hours, then reanalyzed against a fresh standard curve in another analytical run. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). The results met acceptance criteria and established that processed extracts are stable at 2-8°C for 51 hours prior to analysis.	Acceptable
Freeze-Thaw stability	Six replicates of low and high QC samples were subjected to a series of freeze-thaw cycles and subsequently analyzed. Each sample was initially frozen for at least 24 hours. Each cycle consisted of unassisted thawing on wet ice (2-8°C) and storage at -80°C for at least 12 hours. The mean concentrations after the final freeze-thaw cycle were compared to the nominal concentration to obtain the percent difference. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). Results met acceptance criteria and established that the analytes are stable in matrix after at least four freeze-thaw cycles.	Acceptable
Long-term storage	Stability was determined by comparing six replicates of the stability samples against freshly prepared calibrators and freshly prepared QC samples in the same run. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%).	Acceptable

Parameter	Details	
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Plasma by LC-MS/MS	
	The results met acceptance criteria and established that the analytes are stable when stored in matrix at -80°C for at least 546 days. The results met acceptance criteria and established that the analytes are stable when stored in matrix at -20°C for at least 11 days.	
Carry over	For both analytes in all three accuracy and precision runs, all blanks in the run were found to be free of analyte or internal standard responses.	Acceptable
Method performance in Study PVP-20IC02 An Open-Label, Randomized Study to Evaluate the Pharmacokinetics and Excretion of Two Different Doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium 0.8% Solution in Healthy Subjects		
Assay passing rate	100%	
Standard curve performance	Cumulative bias range: -1.3 to 0.8% Cumulative precision: ≤6.07% CV	Acceptable
QC performance	Cumulative bias range: -2.0 to 7.5% Cumulative precision: ≤10.31% CV TE: ≤ x% (LBA only) - NOT APPLICABLE	Acceptable
Method reproducibility	Incurred sample reanalysis was performed in 11% of study samples and 75% of samples met the prespecified criteria for indigo carmine.	Acceptable
Study sample analysis/ stability	The maximum plasma sample storage period of 39 days was calculated from the first sample collection date (January 22, 2021) to the last extraction date (March 2, 2021). Long-term matrix stability has been established at -80°C for 546 days for both indigo carmine and 5-sulfo-anthranilic acid.	

Abbreviations: CV, coefficient of variation; LBA, liqand binding assay; LC/MS-MS, liquid chromatography/ tandem mass spectrometry; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification

Table 58. Summary Method Performance of a Bioanalytical Method to Measure Indigo Carmine in Human Urine

Parameter	Details		
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Urine by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Urine by LC-MS/MS		
Method description	LC-MS/MS Assay for the Determination of Indigo Carmine and 5 Sulfo Anthranilic Acid in Human Urine		
Materials used for calibration curve & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 0.250, 0.500, 1.50, 3.50, 5.00, 7.00, 9.00, 10.0 µg/mL		
Validated assay range	0.250 to 10.0 µg/mL		
Material used for QCs & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 0.250, 0.750, 4.00, 8.00 µg/mL		
Regression model & weighting	Quadratic $1/x^2$		
Validation parameters	Method validation summary		Acceptability
Standard calibration curve performance during accuracy & precision	Number of standard calibrators from LLOQ to ULOQ	8	Acceptable
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.3 to 2.3%	Acceptable
	Cumulative precision (%CV) from LLOQ to ULOQ	9.4%	Acceptable
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs QCs: LLOQ, Low, Medium, High	-9.6 to 15.2%	Acceptable
	Interbatch %CV QCs: LLOQ, Low, Medium, High	≤3.2%	Acceptable
Selectivity & matrix effect	Six lots of human urine were tested. For indigo carmine, the range of observed matrix effect was 0.85 to 1.0. No issues were observed.		Acceptable
Interference & specificity	Ten lots of human urine were tested. For indigo carmine, the observed bias was 6.8%. No issues were observed.		Acceptable
Hemolysis effect	NOT APPLICABLE		
Lipemic effect	NOT APPLICABLE		
Dilution linearity	A 40.0-µg/mL QC sample was diluted ten-fold with human urine to a concentration of 4.00 µg/mL. For indigo carmine, the percent difference from the nominal concentration was -3.0% and the precision (CV) was 4.2%.		Acceptable
Bench-top/process stability	After initial injection and acceptance, an entire run was stored in the autosampler at 2-8°C for 34 hours, then the entire run was reinjected and quantitated. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). The results met acceptance criteria and established processed extract integrity of indigo carmine at 2-8°C for 34 hours prior to analysis.		Acceptable

Parameter	Details	
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Urine by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Urine by LC-MS/MS	
Freeze-thaw stability	Six replicates of low and high QC samples were subjected to a series of freeze-thaw cycles and subsequently analyzed. Each sample was initially frozen for at least 24 hours. Each cycle consisted of unassisted thawing on wet ice (2-8°C) and storage at -80°C for at least 12 hours. The mean concentrations after the final freeze-thaw cycle were compared to the nominal concentration to obtain the percent difference. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). Results met acceptance criteria and established that the analytes are stable in matrix after at least three freeze-thaw cycles.	Acceptable
Long-term storage	Stability was determined by comparing six replicates of the stability samples against freshly prepared calibrators and freshly prepared QC samples in the same run. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). The results met acceptance criteria and established that the analytes are stable when stored in matrix at -80°C for at least 167 days. The results met acceptance criteria and established that the analytes are stable when stored in matrix at -20°C for at least 14 days.	Acceptable
Carry over	For both analytes in all three accuracy and precision runs, all blanks in the run were found to be free of analyte or internal standard responses.	Acceptable
Method performance in Study PVP-20IC02An Open-Label, Randomized Study to Evaluate the Pharmacokinetics and Excretion of Two Different Doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium 0.8% Solution in Healthy Subjects		
Assay passing rate	100%	
Standard curve performance	Cumulative bias range: -1.6 to 2.6% Cumulative precision: ≤6.05% CV	Acceptable
QC performance	Cumulative bias range: -4.9 to 3.3% Cumulative precision: ≤8.33% CV TE: ≤ x% (LBA only) - NOT APPLICABLE	Acceptable
Method reproducibility	Incurred sample reanalysis was performed in 16% of study samples and 89% of samples met the prespecified criteria for indigo carmine.	Acceptable
Study sample analysis/ stability	The maximum urine sample storage period of 39 days was calculated from the first sample collection date (January 22, 2021) to the last extraction date (March 2, 2021). Long-term matrix stability has been established at -80°C for 167 days for both indigo carmine and 5-sulfo-anthranilic acid.	

Abbreviations: CV, coefficient of variation; LBA, liqand binding assay; LC/MS-MS, liquid chromatography/ tandem mass spectrometry; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification

Table 59. Summary Method Performance of a Bioanalytical Method to Measure Indigo Carmine in Human Feces

Parameter	Details		
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Feces by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Feces by LC-MS/MS		
Method description	LC-MS/MS Assay for the Determination of Indigo Carmine and 5 Sulfo Anthranilic Acid in Human Feces		
Materials used for calibration curve & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 1.25, 2.50, 7.50, 17.5, 25.0, 35.0, 45.0, 50.0 µg/mL		
Validated assay range	1.25 to 50.0 µg/mL		
Material used for QCs & concentration	Indigo Carmine (Indigotindisulfonate Sodium) 1.25, 3.75, 20.0, 40.0 µg/mL		
Regression model & weighting	Quadratic $1/x^2$		
Validation parameters	Method validation summary		Acceptability
Standard calibration curve performance during accuracy & precision	Number of standard calibrators from LLOQ to ULOQ	8	Acceptable
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.1 to 2.9%	Acceptable
	Cumulative precision (%CV) from LLOQ to ULOQ	12%	Acceptable
QCs performance during accuracy & precision	Cumulative accuracy (%bias) in 5 QCs QCs: LLOQ, Low, Medium, High	-12.0 to 7.3%	Acceptable
	Interbatch %CV QCs: LLOQ, Low, Medium, High	≤14%	Acceptable
Selectivity & matrix effect	Six lots of human feces were tested. For indigo carmine, the range of observed matrix effect was 0.88 to 0.69. The experiment did not meet acceptance criteria but was accepted as is with a deviation.		Acceptable
Interference & specificity	Eight lots of human feces were tested. For indigo carmine, the observed bias was 18.4%. No issues were observed. The experiment did not meet acceptance criteria but was accepted as is with a deviation.		Acceptable
Hemolysis effect	NOT APPLICABLE		
Lipemic effect	NOT APPLICABLE		
Dilution linearity	A 200-µg/mL QC sample was diluted ten-fold with human feces to a concentration of 20.0 µg/mL. For indigo carmine, the percent difference from the nominal concentration was 10.5% and the precision (CV) was 5.0%.		Acceptable
Bench-top/process stability	After initial injection and acceptance an entire run was stored in the autosampler at 2-8°C for 75 hours, then the entire run was reinjected and quantitated. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). The results met acceptance criteria and established processed extract integrity of indigo carmine at 2-8°C for 75 hours prior to analysis.		Acceptable

Parameter	Details	
Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine and 5-Sulfo-Anthranilic Acid in Human Feces by LC-MS/MS Amendment 1: Method Validation Project for the Bioanalytical Quantitation of Indigo Carmine, 5-Sulfo-Isatin, and 5-Sulfo-Anthranilic Acid in Human Feces by LC-MS/MS	
Freeze-thaw stability	Six replicates of low and high QC samples were subjected to a series of freeze-thaw cycles and subsequently analyzed. Each sample was initially frozen for at least 24 hours. Each cycle consisted of unassisted thawing on wet ice (2-8°C) and storage at -80°C for at least 12 hours. The mean concentrations after the final freeze-thaw cycle were compared to the nominal concentration to obtain the percent difference. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). Results met acceptance criteria and established that the analytes are stable in matrix after at least three freeze-thaw cycles.	Acceptable
Long-term storage	Stability was determined by comparing six replicates of the stability samples against freshly prepared calibrators and freshly prepared QC samples in the same run. The percent difference was calculated by comparing the mean back-calculated concentration with the nominal concentration. To demonstrate stability, the mean of the measured concentrations must be within ±15.0% of the nominal concentrations (100%). The results met acceptance criteria and established that the analytes are stable when stored in matrix at -80°C for at least 167 days. The results met acceptance criteria and established that the analytes are stable when stored in matrix at -20°C for at least 14 days.	Acceptable
Carry over	For both analytes in all three accuracy and precision runs, all blanks in the run were found to be free of analyte or internal standard responses.	Acceptable
Method performance in Study PVP-20IC02 An Open-Label, Randomized Study to Evaluate the Pharmacokinetics and Excretion of Two Different Doses of 3,3'-Dioxo-2,2'-bisindolylidene-5,5'-disulfonate disodium 0.8% Solution in Healthy Subjects		
Assay passing rate	100%	
Standard curve performance	Cumulative bias range: -4.4 to 1.7% Cumulative precision: ≤6.95% CV	Acceptable
QC performance	Cumulative bias range: -4.0 to 10.4% Cumulative precision: ≤7.56% CV TE: ≤ x% (LBA only) - NOT APPLICABLE	Acceptable
Method reproducibility	Incurred sample reanalysis was performed in 56% of study samples and 78% of samples met the prespecified criteria for indigo carmine.	Acceptable
Study sample analysis/ stability	The maximum feces sample storage period of 47 days was calculated from the first sample collection date (January 22, 2021) to the last extraction date (March 10, 2021). Long-term matrix stability has been established at -80°C for 167 days for both indigo carmine and 5-sulfo-anthranilic acid.	

Abbreviations: CV, coefficient of variation; LBA, liqand binding assay; LC/MS-MS, liquid chromatography/ tandem mass spectrometry; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification

Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Nonclinical Reviewer	Ronald Honchel, PhD	ORDPURM/DPTRDPURM	Sections: 5	Select one: _X_ Authored ___ Approved
	Signature: Ronald Honchel -S Digitally signed by Ronald Honchel -S Date: 2022.06.14 12:28:03 -04'00'			
Nonclinical Team Leader	Jonathan Cohen, PhD	ORDPURM/DPTRDPURM	Sections: 5	Select one: ___ Authored _X_ Approved
	Signature: Jonathan E. Cohen -S Digitally signed by Jonathan E. Cohen -S Date: 2022.06.14 12:36:25 -04'00'			
Nonclincial Division Director	Mukesh Summan, PhD	ORDPURM/DPTRDPURM	Sections: 5	Select one: ___ Authored _X_ Approved
	Signature: Mukesh Summan -S Digitally signed by Mukesh Summan -S Date: 2022.06.14 12:49:09 -04'00'			
Clinical Pharmacology Reviewer	Xiling Jiang, PhD	OCP/DCPI	Sections: Section: 6, 18.4	Select one: _x_ Authored ___ Approved
	Signature: Xiling Jiang -S Digitally signed by Xiling Jiang -S Date: 2022.06.14 17:15:30 -04'00'			
Clinical Pharmacology Team Leader	Christy John, PhD	OCP/DCPI	Section: 6, 18.4	Select one: _x_ Authored _x_ Approved
	Signature: Christy S. John -S Digitally signed by Christy S. John -S Date: 2022.06.14 16:53:35 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED / APPROVED
Clinical Pharmacology Division Director	Brian Booth, PhD	OCP/DCPI	Section: 6, 18.4	**Select one:** ___ Authored _x_ Approved
	Signature: Brian P. Booth -S Digitally signed by Brian P. Booth -S Date: 2022.06.16 16:48:15 -04'00'			
Clinical Reviewer	Joseph Rajendran, MD	OSM/DIRM	Sections: 1, 2,3,7,8,10,11,12, 13, 15	**Select one:** _X_ Authored ___ Approved
	Signature: Joseph G. Rajendran -S Digitally signed by Joseph G. Rajendran -S Date: 2022.06.17 07:36:43 -07'00'			
CDTL/Clinical Team Leader	Venkata Mattay, MD	OSM/DIRM	Sections: all sections	**Select one:** ___ Authored _X_ Approved
	Signature: Venkata S. Mattay -S Digitally signed by Venkata S. Mattay -S Date: 2022.06.17 11:14:01 -04'00'			
Primary Statistical Reviewer	Sungwon Lee, PhD	OB/DBI	Sections: 8	**Select one:** _x__ Authored ___ Approved
	Signature: Sungwon Lee -S Digitally signed by Sungwon Lee -S Date: 2022.06.15 13:56:41 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Secondary Statistical Reveiwer	Jyoti Zalkikar, PhD	OB/DBI	Sections:8	**Select one:** _x__ Authored ___ Approved
	Signature: Jyoti Zalkikar -S Digitally signed by Jyoti Zalkikar -S Date: 2022.06.15 14:19:08 -04'00'			
Deputy Division Director Supervisor	Sue-Jane Wang, PhD	OB/DBI	Sections: 8	**Select one:** ___ Authored _x__ Approved
	Signature: Suejane Wang -S Digitally signed by Suejane Wang -S Date: 2022.06.15 14:33:21 -04'00'			
Associate Director for Labeling	Younsook Kim, PharmD	OSM/DIRM	Section: 11	**Select one:** __X_ Authored ____ Approved
	Signature: Younsook Kim -S Digitally signed by Younsook Kim -S Date: 2022.06.14 13:01:26 -04'00'			
OPQ-Product Reviewer	Anne Marie Russell, PhD	OPQ/ONDP/DNDPIII	Section: 4.2	**Select one:** __X_ Authored ___ Approved
	Signature: Anne M. Russell -S Digitally signed by Anne M. Russell -S Date: 2022.06.15 12:54:38 -04'00'			
Division Director Supervisor	Danae Christodoulou, PhD	OPQ/ONDP/DNDPIII	Section: 4.2	**Select one:** ___ Authored _X__ Approved
	Signature: Danae D. Christodoulou -S Digitally signed by Danae D. Christodoulou -S Date: 2022.06.15 09:49:45 -04'00'			
Deputy Director (DIRM)	A. Alex Hofling, MD, PhD	OSM/DIRM	Sections: all sections	**Select one:** ___ Authored _X_ Approved
	Signature: August Hofling -S Digitally signed by August Hofling -S Date: 2022.06.22 10:00:54 -04'00'			

Division Director (Clinical) (DIRM)	Libero Marzella, MD, PhD	OSM/DIRM	Sections: all sections	**Select one:** ___ Authored _X_ Approved
	Signature: Libero L. Marzella -S Digitally signed by Libero L. Marzella -S Date: 2022.07.07 11:09:11 -04'00'			

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

NDA 216264 Bludigo Unireview

The Office of Specialty Medicine, Division of Imaging and Radiation Medicine's Multidisciplinary review for NDA 216264 Bludigo (Indigotindisulfonate Sodium) is complete. The purpose of this memorandum to the file is to note that in the final NDA review document section 18.4 has been removed and has been replaced with section 15.3.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

XILING JIANG
07/07/2022 08:08:15 AM

CHRISTY S JOHN
07/07/2022 08:59:00 AM

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/s/

VENKATA S MATTAY
07/08/2022 11:48:35 AM

LIBERO L MARZELLA
07/08/2022 03:04:01 PM