

DIVISION OF MEDICAL IMAGING PRODUCTS
Clinical Review of NDA 203684 Supplement # 93
Bracco Diagnostics
Lumason for Ultrasonography of Urinary Tract in
Pediatric Patients to Evaluate Vesicoureteral Reflux
Priority Review
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Submission June 29, 2016
December 14, 2016

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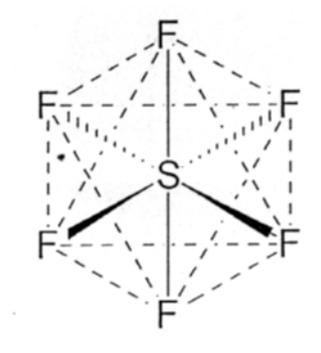
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Lumason - Regulatory Action

Lumason - Regulatory Recommendations

Bracco Diagnostics Inc. (“Sponsor”) provided clinical evidence to support the intravesicular administration of Lumason (sulfur hexafluoride lipid-type A microspheres) as an ultrasound contrast agent to improve the assessment of the excretory urinary tract for suspected or known vesicoureteral reflux (VUR) in pediatric patients. Lumason is an ultrasound contrast agent (USCA) developed by Bracco and is characterized by a microsphere structure, consisting of a low solubility gas, sulfur hexafluoride (SF₆), stabilized by a phospholipid shell.



Whereas this new imaging modality can be performed without radiation exposure it represents an unmet medical need, this NDA supplement was granted priority review as noted in the filing letter dated September 8, 2016.

This reviewer recommends approval of this application. Bracco has provided adequate evidence both for efficacy and safety of Lumason’s use for assessing the presence of VUR in the pediatric population. Approval of this ultrasound agent for this indication supports the current effort to reduce the radiation exposure associated with diagnostic radiologic imaging, particularly in children.

Of note, this drug’s trade name Lumason (as currently approved in this country) and SonoVue (as used earlier in drug development and as currently approved in Europe) are used interchangeably throughout this review.

Lumason - Regulatory History

Lumason® (sulfur hexafluoride lipid-type A microspheres) was initially approved by the United States Food and Drug Administration (US FDA) in October 2014 for use in adult patients with

suboptimal echocardiograms to opacify the left ventricular chamber and to improve the delineation of the left ventricular endocardial border (2.0 mL)

In March 2016, Lumason received FDA approval for use with ultrasound of the liver in adult and pediatric patients to characterize focal liver lesions (2.4 mL adult patients and 0.03 mL/kg pediatric patients).

Bracco Diagnostics Inc. (“the Sponsor”) submitted this supplemental New Drug Application (sNDA) to NDA 203864, seeking an additional indication for use of Lumason during ultrasonography of the (b) (4) urinary tract in pediatric patients with known or suspected vesicoureteral reflux (VUR). Bracco’s proposed wording for the new indication is as follows (bold font). Lumason is an ultrasound contrast agent indicated for use:

“Lumason is indicated for use in ultrasonography of the (b) (4) urinary tract in pediatric patients (b) (4) vesicoureteral reflux.”

The DMIP recommended the following indication statement for Lumason:

“Lumason is indicated for use in ultrasonography of the urinary tract for the evaluation of suspected or known vesicoureteral reflux in pediatric patients.”

Prescribing information for Lumason is amended to include the indication for use in ultrasonography of the (b) (4) urinary tract in pediatric patients. Draft proposed labeling conforms to the content and format regulations found at 21 CFR 201.56(a) and (d) and 201.57. Labeling is provided per PLR and has also been provided in SPL format. No labeling changes are proposed to any of the other labeling components.

The recommended dose of Lumason for use in ultrasonography of the (b) (4) urinary tract in children is 1 mL administered into the bladder through a sterile catheter. The bladder should be first emptied and then partially prefilled with saline before injection of Lumason. After Lumason administration, saline is used to continue filling the bladder until the child has the urge to micturate or there is the first slight sign of back pressure to the infusion. After baseline non-contrast ultrasound examination of the kidney and bladder, the scanner is switched to low mechanical index (≤ 0.4) for contrast specific imaging. Continuous ultrasound imaging is performed of the bladder, ureters, and kidneys during filling and voiding of the bladder. Immediately following the first voiding, the bladder may be refilled with normal saline for a second cycle of voiding and imaging, without the need of a second Lumason administration.

Diagnostic Imaging for Diagnosis of VUR

Diagnostic imaging plays a central role in diagnosis of VUR and decisions about therapeutic options. The following Terminology List is useful in understanding the diagnostic tests utilized for management. (Diagnostic tests **BOLDED**)

Terminology List

CE-VUS	Contrast-enhanced voiding urosonography (bladder instillation)
FVCUG (VCUG)	Fluoroscopic voiding cysto-urethrography (bladder instillation)
KUU	Kidney ureter units
PUU	Pelvic-ureter units
RNC	Radionuclide cystography (IV administration)
US-CA	Ultrasound contrast agent
UTI	Urinary tract infection
VUR	Vesicoureteral reflux
VUS	Voiding urosonography (no bladder instillation)

FDA Requests for Information

DMIP required that the submission include the following information:

- Off-label use and safety of Lumason or other USCAs in VUS in USA and other countries
- Justification of dose - Based on body weight or body size, not on age of pediatric patients
- Selection for assessing severity of reflux
- Imaging parameters (window and acquisition parameters) for the new indication
- Directions for administration
- Inclusion in the application of all available literature (if any) regarding the appearance of SF6 in systemic circulation following intravesical administration to human subjects or, if no data are available, a discussion from scientific principles regarding the potential for absorption, and the safety implications

Bracco provided acceptable responses to the pre-submission requests for information and these additional information requests (responses are denoted in *Italics*):

- 1. For the 4 publications you provide in support of this sNDA, please provide patient level data regarding safety and efficacy. If you cannot provide this data, please provide an explanation.**

"Patient level data for safety and efficacy were retrieved for one of the four pivotal studies. Patient level data for efficacy is forthcoming and safety was provided"

- 1. "Definitions for the computation of diagnostic performance of VUS at the ureter level:**
 - *True negative (TN) – diagnosed with no VUR (negative) according to both VUS*

and VCUG

- True positive (TP) – diagnosed with VUR (positive) according to both VUS and VCUG
- False negative (FN) – diagnosed with no VUR (negative) according to VUS, but diagnosed with VUR (positive) according to VCUG
- False positive (FP) – diagnosed with VUR (positive) according to VUS, but diagnosed with no VUR (negative) according to VCUG.
- Sensitivity – $TP/(TP+FN)$
- Specificity – $TN/(TN+FP)$ ”

2. Reading Methodology in the Four Pivotal Clinical Studies Comparing VUS and VCUG.

“In all 4 pivotal studies, reading of VUS or VCUG was performed on site by independent readers who were blinded to the results of the other imaging modality. In 3 of the 4 studies, more than one reader assessed the VUS or the VCUG images and, in case of discordant results within each imaging modality, a final diagnosis was reached by consensus of the readers.”

3. Justification that the 1 mL dose is optimal for all children (minimum dose to be effective), rather than basing the dosage upon body weight, body size or age.

“Bladder capacity in children has been shown to correlate with age. Although different formulas have been proposed to estimate the bladder capacity in children, one of the most commonly used equation is: bladder capacity = [(age in years + 2) x 30] mL.

Using such age-related formula for prediction of bladder capacity, a Lumason dose of 1 mL would range from 1.3% to 0.4% of the bladder capacity in pediatric patients between 6 months and 6 years of age, which is the patient population that would most benefit from the use of SonoVue [Lumason] for the detection of VUR. If, as proposed by an expert, a dose of Lumason equal to 1% of bladder filling capacity should be used in individual patients, then doses of Lumason in the same age group should range between 0.75 mL and 2.4 mL for the diagnosis of VUR.

Considering:

- *The good diagnostic performance obtained with the use of a dose equal or close to 1.0 mL of the product in pediatric patients of all ages included in the selected studies;*
- *The good safety observed after intravesical administration of 1.0 mL of Lumason;*
- *The fact that most of the administered Lumason is eliminated at the end of the procedure;*
- *The difficulty to withdraw Lumason doses <1 mL following reconstitution of the*

product;

- *The difficulty to calculate and use doses of Lumason precise to the level of decimals in individual patients,*

Bracco believes that the proposed Lumason dose of 1 mL in all pediatric patients is supported by evidence and does not seem to cause any harm to patients.

If, instead, the FDA believes that Lumason doses should be based on bladder capacity, and a dose of Lumason equal to 1% of estimated bladder filling capacity should be used, then doses of Lumason between 0.6 mL (neonates) and 5.7 mL (17 years) may be the amount needed in pediatric patients for voiding urosonography."

- 4. Please summarize how the predicted bladder capacity was used to perform partial filling in each study. This is one possible sequence:**
 - 1. Calculate predicted bladder capacity**
 - 2. Administer a volume of saline equal to 50% of the predicted capacity**
 - 3. Administer 1 mL of Lumason**
 - 4. Administer additional saline until the patient reports an urge to micturate.**

Was the above sequence used for all patients in all four studies? If not, describe what alternative(s) was used.

*"A description of the saline/Lumason administration scheme, as reported in each of the four pivotal studies is provided below in tabular format (**Table 1**):*

**Table 1: Saline/Lumason Administration Scheme in 4 Primary Studies
(Table provided by Sponsor within submission)**

	Predicted bladder capacity calculation	Saline to pre-fill bladder / Volume administered	Lumason administered	Additional saline administered
Wong et al ⁶	[(age in years + 2) x 30] mL	Yes Up to 1/3 of predicted maximum bladder capacity	1 mL	Yes Until dripping stopped when the child started to void
Kljucevsek et al ⁷	10mL/kg body weight	Yes Up to 1/2 of predicted bladder volume	1 mL	Yes
Kis et al ⁸	[(age in years + 2) x 30] mL	No After catheterization, some urine was left in the bladder (volume was not specified) and the contrast agent was administered before additional administration of saline	1 mL	Yes Until the child had to micturate or there was the first slight back pressure to the infusion
Papadopoulou et al ⁹	[(age in years + 2) x 30] mL	Yes Up to 1/3 of predicted bladder volume	1 mL	Yes* Until the child started to void
* This information was not included in the published paper but was provided directly by the authors to Bracco				

Predicted bladder capacity. The predicted bladder capacity was determined using the same age-based formula (i.e. [(age in years + 2) x 30] mL) in 3 of the 4 pivotal studies in the fourth study, the predicted bladder capacity was calculated according to the formula: bladder capacity = 10 mL/kg body weight.

Bladder pre-filling. Before administration of Lumason, saline was used to partially pre-fill the bladder up to 1/3 of the predicted capacity in 2 studies and up to half of the predicted capacity in a third study.⁷ In one study, no bladder pre-filling with saline was reported; however, a small amount of urine was left in the bladder, after which Lumason was administered.

Lumason administration. In all studies, Lumason was administered at the dose of 1.0 mL into a bladder that was partially filled (with saline in 3 studies or urine in one study).

Additional administration of saline. In all studies, the administration of Lumason was followed by the administration of an additional volume of saline until the child had the urge to micturate or there was the first slight sign of back pressure to the infusion.

Overall, the proposed sequence of events: 1. Calculate predicted bladder capacity; 2. Administer a volume of saline equal to 50% of the predicted capacity; 3. Administer 1 mL of Lumason; 4. Administer additional saline until the patient reports an urge to micturate; was followed in all 4 studies, although a few differences were shown among the studies in terms of predicted bladder capacity calculation and bladder prefilling.

In the proposed Package Insert included in the sNDA, Bracco has proposed to use the age-related formula for calculation of the estimated bladder capacity based on the following considerations:

- *the above reported age-related formula is used to predict bladder capacity for other imaging procedures commonly performed for assessment of VUR in children such as radionuclide cystography and voiding cystourethrography, as reported in the Society of Nuclear Medicine and the American College of Radiology guidelines;*
- *when making reference to weight-for-age charts and considering a child in the 50th percentile, the values for the predicted bladder capacity obtained with the weight-based formula are almost superimposable on those obtained with the age-based formula. For example, at 1 year of age, the predicted bladder capacity with the 2 formulas would be:*
 - *(age in years + 2) x 30 mL = (1+2) x 30 mL = 90 mL*
 - *10 mL/kg body weight = 10 mL/9 kg = 90 mL (girl) OR 10 mL/9.6 kg = 96 mL (boy);*
- *the age-related formula was used in the majority of patients enrolled in the pivotal studies (442 out of 508).*

Bracco also proposed a slightly different wording for step #2 of the sequence of events reported above consistent with the clinical evidence to date, as follows:

2. [REDACTED] (b) (4)
[REDACTED] ”

5. If data is available that supports that drainage is “almost immediate and complete”, please submit it. Alternatively, please confirm that no data is available.

“Voiding is a substantial part of the VUS procedure, and is a prerequisite for a technically successful VUS examination. The pivotal studies consisted of such technically adequate exams. Elimination of the intravesically applied contrast agent occurs via voiding. Therefore, although no precise measurements of timing or degree of completion of voiding were provided, it is assumed that the examined children emptied their bladder upon conclusion of the procedure and “almost immediate” drainage seems to be an adequate description.

Studies have demonstrated that post-voiding residual volume is almost zero in children. Thus, since normal voiding results in an almost completely empty bladder, the term “complete” also seems accurate.

In one study assessing the safety of intravesical administration of SonoVue during VUS in 1,010 children, it was reported that 965 patients were able to void directly at the end of the ultrasound procedure and 45 (4.4%) patients were not. In the same study, significant post-void residual urine volume (defined as a residual volume >10% of the actual bladder volume) was present in 148 (14.6%) patients. No adverse events related to the administration of Lumason were reported. It should be noted that, in case of

incomplete voiding, any potential residual volume within the bladder can be easily detected with ultrasound during the procedure and can be removed through the intravesical catheter.”

Supplement Application

This supplemental NDA is being submitted as a 505(b)(2) application because the safety and efficacy data presented in support are exclusively from published literature and from Bracco post-marketing surveillance database. Bracco has not conducted clinical trials for this indication, nor does Bracco have right of reference to the raw data that is reported in the published literature used in support of this application. Product labeling for this proposed indication relies on information from peer-reviewed literature and guidelines published by internationally recognized medical societies.

Bracco provided to FDA the Information Package (26 August 2015) submitted for the Type B Meeting scheduled for 29 September 2015. On 25 September 2015, FDA provided preliminary meeting comments and, based on the responses provided, the meeting was cancelled. That Type B Meeting package stated that only those modules and sections that are relevant to this 505(b)(2) submission should be included in the eCTD submission.

In support of the proposed new indication language, Bracco has conducted a broad literature search and identified several published trials assessing the effectiveness and safety of Lumason use (under the brand name “SonoVue”) during VUS in pediatric patients with known or suspected VUR.

Limits for the literature search were: published through February 19, 2015; and use of the search terms (urosonography OR vesicoureteral reflux OR (voiding AND (ultrasonography OR ultrasound))) AND (contrast or enhanced or microbubbles). In addition to a scientific review of all identified articles for efficacy data to support the proposed indication language, a comprehensive meta-analysis of controlled studies in the literature comparing VUS with VCUG as the standard of truth has been performed. The purpose of this meta-analysis is to provide an evidence-based estimation of diagnostic performance of VUS for detection of VUR.

Bracco's review of the literature also involves a review of publications reporting safety information for intravesical administration of Lumason in pediatric patients. In addition, the Bracco pharmacovigilance database has been reviewed for any spontaneous report of adverse events that occurred following intravesical administration of Lumason in pediatric patients.

Bracco's review of the literature also involves a review of publications reporting safety information for intravesical administration of Lumason in pediatric patients. In addition, the Bracco pharmacovigilance database has been reviewed for any spontaneous report of adverse events that occurred following intravesical administration of Lumason in pediatric patients.

Lumason is not approved for use in VUS (Voiding urosonography) in or outside the USA. However, the product has been used off-label in pediatric patients with medical need for

assessment of VUR, as documented in scientific studies reported for a decade in the peer-reviewed literature.

Bracco is not requesting a waiver for this indication for any age group.

2. Efficacy and Safety Conclusions

Overview of Efficacy in the Published Literature

Four Published Key Clinical Publications report efficacy data for clinical trials with use of 1.0 mL of Lumason in CE-VUS, an examination that eliminates exposure to ionizing radiation in pediatric patients. Findings from these studies conducted in the target clinical population in which Lumason is intended to be used for CE-VUS demonstrated:

- Sensitivity values for detection of VUR between 80% and 100%;
- Specificity values between 77% and 86%; and
- Moderate to good agreement between Lumason-enhanced VUS and VCUG for evaluation of the grading of VUR 105/151 (70%).

Passage of the contrast agent (radiopaque or microbubble) from the bladder through the vesicourethral valve into the upper urinary tract (ureters and kidneys) is referred to as vesicoureteral reflux (VUR). Within the 4 key clinical studies, 357 VUR positive ureter units were detected from among 1,023 possible ureter units (35%).

- 167 (47%) VUR positive ureter units were detected by both VCUG and CE-VUS.
- 195 (55%) VUR positive ureter units were detected by VCUG.
- 329 (92%) VUR positive ureter units were detected by CE-VUS.
- 162 (45%) VUR positive ureter units were detected by Lumason alone and not by the reference standard.
- 28 (8%) VUR positive ureter units were detected by VCUG alone.

Notably, a significant number of VUR positive ureter units in these four studies were detected by Lumason alone and not by the reference standard. Utilizing strict statistical principles, these cases would be classified as false positives (demonstration of the presence of disease when disease was not detected by the reference standard) potentially leading to underestimation of specificity. Clinically, demonstration of sonography-induced echogenic signals from microbubbles in the upper urinary tract during bladder filling and voiding phases appears to meet the diagnostic definition for vesicoureteral reflux. Likewise, Lumason has demonstrated one

higher grade of reflux in 24/151 units (16%), than the reference standard. Thus nominally, contrast-enhanced microbubbles may have performed better than the reference standard. This reviewer is providing the following possible explanations:

1. Contrast-enhanced microbubbles permitted continuous monitoring of filling and voiding, while the reference standard required “intermittent snapshots” (spot films) to minimize radiation exposure. Thus, the intermittent timing for “snapshot radiographic images” may have missed the occurrence of reflux.
2. Contrast-enhanced microbubble usage permits repeating bladder filing and a longer examination time
3. Contrast- enhanced microbubbles may have greater sensitivity than the reference standard.

Results of a **Sponsor-Conducted Meta-analysis** of CE-VUS performance from the 4 key studies demonstrated:

- Pooled sensitivity of 89% (95% CI: 80% to 97%) and pooled specificity of 81% (95% CI:76% to 86%) in the total of 508 pediatric patients (1,023 ureter units); age range between 2 days and 13 years.

Eight (8) **Additional Published Supportive Studies** reporting on the use of Lumason confirmed the utility of Lumason for the evaluation of VUR in children were retrieved in the literature search performed by Bracco. These studies included 1,645 patients and 3,306 UUs. Although they did not meet the stricter criteria (controlled study of Lumason in VUS, VCUG truth standard, sensitivity/specificity endpoints, consistent Lumason dose and administration scheme) required for the key publications, they nevertheless directly support the use of Lumason in VUS.

Overview of Safety in the Published Literature

No safety data are available from Bracco-sponsored clinical trials with use of Lumason after intravesical administration. Among the papers retrieved from the literature search, 13 manuscripts reported safety information in approximately 6,000 children exposed to Lumason after intravesical administration.

- No complications or adverse reactions were reported in 12 publications (**2,153 patients**) presenting results of individual clinical studies for the evaluation or treatment of VUR in pediatric patients ranging in age from 2 days to 13 years. (N=**508 patients** in 4 Key studies and **1,645 patients** in 8 supportive studies)
- Minor adverse events were reported in 37 of **1,010** children ranging in age from 15 days to 17.6 years in one large prospective safety study. None were related to Lumason

administration, but were considered related to the catheterization procedure.

- No side effects were reported after intravesical administration of Lumason in one large survey conducted by Uroradiology Task Force of the European Society of Paediatric Radiology (ESPR) and the Paediatric Work Group of the European Society of Urogenital Radiology (ESUR) in **4,131** examinations in children ranging in age from newborn to 18 years.

Based on available efficacy and safety information presented, sufficient data exists to support the application of Lumason for assessment of VUR in children. Contrast-enhanced vesicular ultrasound (CE-VUS) demonstrates significant diagnostic performance in assessing VUR when compared to VCUG (fluoroscopic voiding cystourethrography). Bladder instillation of Lumason for the CE-VUS procedure has demonstrated an excellent safety record, few adverse reactions have been observed, all have been minor and all were related to the catheterization procedure not to Lumason.

Lumason-enhanced VUS has major advantages over both VCUG and RNC for evaluations of VUR as it provides clinical evidence of efficacy and does not involve the exposure of children to potentially harmful ionizing radiation. Ionizing radiation associated VCUG is approximately 0.4 to 0.9 mSv. RNC is associated with lower gonadal radiation (approximately 0.005 to 0.01 mGy & testes less) and poor anatomic resolution and inability to study the urethra.

Benefit/Risk Assessment

Diagnostic imaging plays a central role in the diagnosis of VUR and decisions about therapeutic options. The reference standard test for VUR is VCUG, in which x-ray imaging of the bladder and urethra is performed while the bladder fills and empties. The major disadvantage of VCUG is the associated exposure to ionizing radiation, which remains substantial even when using a digital technique or intermittent fluoroscopic imaging. The radiation exposure concern is particularly relevant in children because of their ongoing development, greater cell turnover, and increased lifetime risk of cancer based on a greater life expectancy when compared with an adult.

RNC is also used for diagnosis of VUR. The RNC procedure is similar to VCUG except that rather than a radiopaque contrast material a radiopharmaceutical is instilled into the bladder. When compared to VCUG, radionuclide imaging is characterized by lower radiation exposure and comparable sensitivity and specificity for detection of VUR; however, radionuclide voiding cystography is limited by poor anatomic resolution and inability to study the urethra.

As presented in the sNDA for Lumason, 4 prospective publications report efficacy data for clinical trials with use of 1.0 mL of Lumason in VUS, an examination that eliminates exposure to ionizing radiation in pediatric patients. Findings from these studies conducted in the target clinical population in which Lumason is intended to be used for VUS showed:

- Sensitivity values for detection of VUR between 80% and 100%;
- Specificity values between 77% and 86%; and

- Moderate to good agreement between VUS with Lumason and VCUG for evaluation of the grading of VUR.

Results of a Sponsor-conducted meta-analysis of VUS performance from the 4 pivotal studies showed:

- pooled sensitivity of 89% (95% CI: 80% to 97%) and pooled specificity of 81% (95% CI: 76% to 86%) in the total of 508 pediatric patients (1,023 ureter units); age range between 2 days and 13 years.

The efficacy of VUS with Lumason for detection and grading of VUR is further supported by results of the 8 supportive studies.

Among the papers retrieved from the literature search, 12 studies reported safety information in over 6,000 children exposed to Lumason after intravesical administration. Minor non-serious adverse events were reported in 37 children in one single study; none was related to Lumason administration. These findings are comparable to the reported safety of Levovist in the published literature.

Based on the available efficacy and safety information as presented, Bracco believes that there are sufficient data to support the application of Lumason for assessment of VUR in children. The low risk of the use of Lumason in the VUS procedure, combined with the high diagnostic performance of this exam as compared to VCUG, indicate that a contrast-enhanced VUS exam may have advantages over the reference methods for evaluations of VUR, which involve the exposure of children to potentially harmful ionizing radiation.

Approximately 110,000 pediatric patients had VCUG in USA in 2015. Estimated number of VUR diagnoses in 2015 was approximately 24,000.

Bracco proposed the following risk minimization measures for intravesically administered Lumason that include routine pharmacovigilance surveillance with the following activities:

- Systematic collection of adverse events from multiple sources (including cases that originate from literature)
- Expeditious and periodic medical assessments of single and aggregate reports
- Identification of potential safety signals
- Evaluation of the risk-benefit balance of the product through its life cycle.

3. Background

Lumason – European Regulatory History

Lumason has been commercialized in Europe since 2001 under the brand name SonoVue®. SonoVue is currently approved for intravenous use in more than 35 countries throughout the world, outside the United States of America (USA), and is marketed in more than 25 countries. Outside the USA, SonoVue is approved for use in adults in the following indications:

- Echocardiography, in patients with suspected or established cardiovascular disease to provide opacification of cardiac chambers and enhance left ventricular endocardial border delineation;
- Doppler of macrovasculature to increase the accuracy in detection or exclusion of abnormalities in cerebral arteries and extracranial carotid or peripheral arteries by improving the Doppler signal-to-noise ratio;
- Doppler of microvasculature to increase the quality of the Doppler flow image and the duration of clinically useful signal enhancement in portal vein assessment;
- Doppler of microvasculature to improve display of the vascularity of liver and breast lesions during Doppler sonography, leading to more specific lesion characterization.

During market use (April 01, 2001 through September 30, 2015), an estimated (b) (4) patients have been exposed to Lumason.

Worldwide, SonoVue, Definity and Sonazoid, all suspensions of gas-filled microspheres, are approved in many countries for CE-US characterization of liver lesions.

Lumason Formulation

Lumason is for diagnostic use with ultrasound imaging to enhance microsphere echogenicity which results in an improved signal-to-noise ratio between the microspheres and their surrounding tissues. Lumason (sulfur hexafluoride lipid-Type A microspheres) is supplied within a kit containing the following:

- a clear glass vial labeled as Lumason (sulfur hexafluoride lipid-type A microspheres) for injectable suspension, containing 25 mg of lyophilized powder lipid-type A and headspace filled with 60.7 mg sulfur hexafluoride gas,
- a prefilled syringe with 5 mL Sodium Chloride 0.9% Injection, USP, (Diluent),
- a Mini-Spike.

Lumason is reconstituted by injecting the prefilled syringe contents (5 mL Sodium Chloride 0.9% Injection, USP) into the Lumason vial. Following reconstitution with the provided diluent, Lumason suspension contains 1.5 to 5.6×10^8 microspheres/mL with 45 mcg/mL of sulfur hexafluoride. Fifteen (15) to 23% of the total lipids in the suspension are associated with the microspheres. Mean diameter of Lumason microspheres is 2.5 μm .

Lumason Dosing for VUR Evaluation

The recommended dose of Lumason for use in ultrasonography of the (b) (4) urinary tract in children is 1 mL administered into the bladder through a sterile catheter. Due to the intravesical route of administration of Lumason for the VUS, systemic absorption of Lumason is unlikely. Concern was raised regarding use of a single dose for all children, rather than basing the dosage upon body weight, body size or age. Justification of dose was supported by response to IR referred to earlier.

PREA Requirements

Pediatric information in support of the vesicoureteral reflux (VUR) indication was provided. No Bracco sponsored studies have been conducted in support of this indication. Included in this submission are:

- Supportive efficacy data from published literature for this indication
- Confirmation of the safety of SonoVue after intravesical administration in pediatric patients in numerous clinical trials reported in the peer-reviewed literature.
- Bracco did not request a waiver for this indication for any age group.

Pediatric Formulation Development

PREA requires pediatric assessments to be gathered using appropriate formulations for each age group for which the assessment is required (section 505B(a)(2)(A) of the Act).

In the case of SF₆ microspheres, the formulation is a lyophilized powder, which is reconstituted with a solvent (0.9% Na Cl solution) to produce an aqueous suspension of microspheres. This suspension is then administered intravesicularly by a healthcare professional (physician, nurse or sonography technician) during the course of an ultrasound examination.

Because of the nature of the product, the intravesical route and method of administration, a specific formulation was not required for assessment of the excretory urinary tract in the pediatric population.

4. VUR (VESICOURETERAL REFLUX)

Overview of the VUR in the Pediatric Population

VUR represents a common clinical condition in childhood, although its exact prevalence is unknown since invasive diagnostic procedures are performed only when clinically indicated. The estimated prevalence of VUR in “well” children is between 0.4 and 1.8%; the prevalence of disease is higher (up to 16.2%) among infants with hydronephrosis detected on antenatal ultrasound and in siblings of children with VUR (11%-67% in a review of 10 papers). Case series of children with UTI who underwent fluoroscopic voiding cystourethrography (VCUG) have reported an incidence of VUR between 25% and 50%. Considering that the cumulative incidence of UTI in children is 6%, it is estimated that 1.5% to 2.4% of all children will be diagnosed with VUR after UTI.

The clinical importance of VUR derives from the observation that it represents a common cause of non-obstructive chronic nephropathy in children and it is associated with an increased risk of renal scarring after UTI with potential for nephrovascular hypertension and renal failure.

Diagnostic Imaging for Diagnosis of VUR

The reference standard test for VUR is **VCUG** (Fluoroscopic voiding cysto-urethrography) which provides anatomic details and accurate grading of the severity of VUR, for which the standardized International Reflux Study system is commonly used. X-ray imaging of the bladder and urethra is performed while the bladder fills and empties. To help distinguish the contents of the urinary bladder, a radiopaque iodinated contrast agent is instilled into the bladder via a transurethral catheter. The presence of opacification of the upper urinary tract with radiographic contrast during bladder filling and voiding phases is diagnostic of VUR. VCUG also provides precise anatomic details and optimal assessment of the urethra. The major disadvantage of VCUG is the associated exposure to ionizing radiation, which remains substantial even when using a digital technique or intermittent “snap shot” fluoroscopic imaging. The standard mean effective dose of VCUG is approximately 0.4 to 0.9 mSv. The radiation exposure concern is particularly relevant in children because of their ongoing development, greater cell turnover, and increased lifetime risk of cancer based on a greater life expectancy when compared with an adult.

RNC (Radionuclide voiding cystography) is also used for diagnosis of VUR. The RNC procedure is similar to VCUG except that rather than a radiopaque contrast material instilled into the bladder, a radiopharmaceutical is administered intravenously. When compared to VCUG, radionuclide imaging is characterized by comparable sensitivity and specificity for detection of VUR with lower radiation exposure. Ionizing radiation with VCUG is approximately 0.4 to 0.9 mSv. RNC carries the advantage of lower gonadal radiation dose. The estimated dose to the ovary is 0.005 to 0.01 mGy, and even smaller dose to the testis. However, RNC is limited by poor anatomic resolution and inability to study the urethra.

Ultrasound (US) is a noninvasive imaging method that eliminates the risk of ionizing radiation and is readily available. It can detect urinary tract anomalies such as pyeloureteral dilatation, duplex renal system, and ureterocele which may raise the suspicion of VUR; however, the sensitivity of US for detecting VUR is low. In a retrospective analysis of 493 infants and children, renal US was compared to VCUG for assessing the presence of VUR. Among the 272 kidneys with VUR, 201 (74%) showed normal findings at US; 28% of the missed refluxing kidneys had grade III or higher reflux.

CE-VUS (Contrast-enhanced voiding urosonography) encompasses evaluation of the urinary tract after intravesical administration of a microsphere ultrasound contrast agent for diagnosis of VUR and for assessment of urethra in pediatric patients. Administration of the microspheres through a catheter into the bladder is followed by continuous examination of the urinary tract during filling and voiding phases (bladder, both ureters and kidneys). The diagnosis of VUR is determined by the presence of moving echogenic (bright) microspheres from the bladder into the upper urinary tract. Any detection of microbubbles in the upper urinary tract (ureter, renal pelvis) indicates the presence and severity of reflux. Results of *in vitro* testing suggest that microbubbles in the ureter do not ascend passively and that reflux pressure is necessary for propagation; this latter is even more relevant *in vivo* because of a constant counter-flow of urine from the renal pelvis to the bladder. The severity of VUR is based on height of reflux up the bladder, and degree of dilatation/ tortuosity of the ureters, pelvis and calyces. CE- VUS does not require exposure to ionizing radiation and has been reported to have diagnostic

performance (sensitivity and specificity for the detection/exclusion of VUR) similar to that of VCUG and RNC. Results of *in vitro* testing suggest that microbubbles in the ureter do not ascend passively and that reflux pressure is necessary for propagation; this latter is even more relevant *in vivo* because of a constant counter-flow of urine from the renal pelvis to the bladder,

Rationale for Use of Lumason in Characterization of VUR

Since 1997, the American Urology Association stated the necessity for a “less traumatic methods of determining whether reflux is present as well as techniques of voiding cystourethrography that results in less radiation exposure.”¹ The clinical usefulness of CE-VUS in pediatric patients is acknowledged by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) in the 2011 update of guidelines and recommendations on the clinical practice of contrast-enhanced ultrasound² and by the European Society of Paediatric Radiology (ESPR) urology task force.³ Similarly, the American College of Radiology (ACR) recognizes that echo-enhanced cystography is a non-ionizing, safe, and reliable method to evaluate for VUR.⁴

Summary of Rationale for the Use of Lumason in VUS

- CE-VUS is technically analogous to conventional VCUG (radio-opaque liquid), in that an ultrasound contrast agent is administered intravesically via the urinary catheter
- Instillation is followed by continuous, alternate examination of the urinary tract (bladder, ureters, kidneys and urethra) during bladder filling and voiding phases
- Diagnosis of VUR is determined by the presence of moving echogenic (bright) microspheres from the bladder into the upper urinary tract.
- Severity of VUR is based on height of reflux above the bladder, and degree of dilatation/tortuosity of the ureters, pelvis and calyces.

¹ American Urological Association report on the management of primary vesicoureteral reflux in children. Available at: http://www.auanet.org/gudelines/main_reports/vesi_reflux.pdf. Accessed June 3, 2016

² Piscaglia F, Nolsøe C, Dietrich CF, Cosgrove DO, Gilja OH, Bachmann Nielsen M, et al. The EFSUMB guidelines and recommendations on the clinical practice of contrast enhanced ultrasound (CEUS): update 2011 on non-hepatic applications. *Ultraschall Med.* 2012; 33:33-59.

³ Riccabona M, Vivier P-H, Ntoulia A, Darge K, Avni F, Papadopoulou F, Damasio B, et al. ESPR urology task force imaging recommendations in paediatric urology, part VII: standardised terminology, impact of existing recommendations, and update on contrast enhanced ultrasound of the paediatric urogenital tract. *Pediatr Radiol.* 2014; 44:1478-84.

⁴ Karmazyn B, Coley BD, Binkovitz LA, Dempsey-Robertson ME, Dillman JR, Dory CE, et al. American College of Radiology ACR Appropriateness Criteria® Urinary Tract Infection - Child. [online publication]. Reston (VA): American College of Radiology (ACR), 1995. Updated 2012. Available at: <https://acsearch.acr.org/docs/69444/Narrative/>

Based on the published literature and the recommendations issued by the scientific societies, CE-VUS has been increasingly used instead of VCUG in the clinical assessment of pediatric patients with known or suspected VUR. In Europe, the Uroradiology Task Force of the European Society of Pediatric Radiology, together with the Paediatric Working Group of the European Society of Urogenital Radiology conducted a survey to collect information on extent of off-label use and safety of Lumason in pediatric patients.⁵ The questionnaire was sent out in 2010, and asked for specific information on number of pediatric applications, mean age and gender, on the individual application, and on all observed adverse events associated with Lumason. Forty-five centers from all over Europe reported on pediatric applications of Lumason, documenting 5,079 examinations in total, of which approximately 2,000 in 2010 alone, showing progressive increase in off-label use of Lumason in the pediatric population. The majority of use was in VUS (4,131/5,079 or 81%). Predominant use in VUS may also explain the mean age (2.9 yrs) of patients exposed to Lumason. Use of Lumason in pediatric patients was mainly reported by centers with more extensive experience and use of contrast in ultrasound procedures.

Basis for Considering VUR to Be a Serious Pediatric Condition

VUR (vesicoureteral reflux) is a common urinary tract abnormality in children characterized by retrograde flow of urine from the bladder into the ureter and toward the kidney, secondary to a dysfunctional vesicoureteral junction. This junction usually acts like a one-way valve which allows urine flow from the ureter into the bladder and closes during micturition, thus preventing back flow. Several pathologic conditions, either congenital or acquired, may be responsible for an ineffective valve function of the vesico-ureteral junction. VUR is detected most commonly during voiding, when intravesical pressure rises, but may occur at any time in the voiding cycle, particularly when bladder function is abnormal.

VUR represents a common cause of non-obstructive chronic nephropathy in children. The presence of VUR is associated with an increased risk of renal scarring after urinary tract infection (UTI) with potential for nephrovascular hypertension and renal failure.

UTI is the most frequent serious bacterial infection during childhood, affecting approximately 2% of boys and 8% of girls by the age of 7 years, and represents a frequent indication for diagnostic imaging in children. The prevalence of VUR in children with UTIs is 30% to 40% and increases in children with recurrent UTIs.

Although VUR is common in childhood, its exact prevalence is unknown since invasive diagnostic procedures are performed only when clinically indicated. The estimated prevalence of VUR in “well” children is between 0.4 and 1.8%; the prevalence of disease is higher (up to 16.2%) among infants with hydronephrosis detected on antenatal ultrasound and in siblings of children with VUR (11%-67% in a review of 10 papers). Case series of children with UTI who underwent VCUG have reported an incidence of VUR between 25% and 50%. Considering the cumulative incidence of UTI in children is 6%, it is estimated that 1.5% to 2.4% of all children will be diagnosed with VUR after UTI.

⁵ Riccabona M. Application of a second-generation US contrast agent in infants and children—a European questionnaire-based survey. *Pediatr Radiol.* 2012;42:1471-80.

Why Is VUR Recognition Important?

The complications from untreated VUR include repeated urinary infections, potential destruction of kidneys and ultimately renal failure. Less severe reflux (i.e., grades 1 and 2) tend to resolve with expectant, non-interventional management. Treatment of more severe VUR consists of prophylactic antibiotics, endoscopic injection of bulking agents at the uretero-vesicular junction and/or ureteral re-implantation surgery. Ureteral re-implantation, particularly for grade 5, is performed either by open surgery or more often today by robotic surgery.

Frequently the VUR is found to be associated with perpendicular implantation of the ureter into the bladder wall instead of preferable diagonally tunneled implantation. Corrective implantation surgery, tunnels the terminal portion of the ureter into the bladder wall so that when the bladder contracts to empty, the terminal ureter is squeezed shut during micturition – preventing reflux and destructive recurrent infections. Frequently, repeat reflux examinations are performed post-operatively for assessment of quality of surgical repairs.

History of Diagnostic Methodology for VUR

The first direct means used to diagnose VUR required instilling different substances intravesically. The most frequently administered fluid was physiological saline solution. Ballooning of the renal pelvis during the filling of the bladder was the criterion for diagnosis of VUR. Application of air bubbles, by shaking the normal saline before administration or adding carbon dioxide, was also tried. US studies were also carried out, in which the empty bladder was solely filled with air. In addition to low diagnostic accuracy, all the above methods had major procedural drawbacks making them impractical for widespread integration into routine imaging.

The first intravesical use of ultrasound contrast agents was made in mid-1990s and consisted of sonicated albumin or water-soluble α -D-galactose based microspheres, both containing room air. The short imaging window of approximately 5 min provided by these agents, however, prevented their larger use in VUS. In the late 1990s, the introduction of an improved room-air ultrasound contrast agent, Levovist®, allowed for longer sonographic assessment times thus improving depiction of fluid propagation from the bladder into the pelvi-caliceal system and, therefore, the diagnosis of VUR. Enhancement with the Levovist microspheres was best detected with dedicated technologies using high mechanical index (MI) resulting in destruction of microspheres and depicting the microsphere-destruction signature as a color overlay with the possibility of visualizing the grey-scale image alone, the grey-scale image together with the microspheres with color overlay, or just the microspheres with color overlay alone contrast imaging with multiple display options.

Lumason is a second-generation contrast agent characterized by a microsphere structure consisting of a low-solubility gas, sulfur hexafluoride, stabilized by a phospholipid shell. The interface between the Lumason microsphere and the surroundings acts as a reflector of the ultrasound beam, thus enhancing echogenicity; when Lumason microspheres are injected into the bladder they selectively increase the echogenicity of the urine, thus

facilitating the detection of reflux into the ureters. A freshly prepared suspension of Lumason is stable over 3 hours.

Although it is possible to use all imaging techniques with Lumason (e.g., fundamental imaging, harmonic imaging, Doppler US, high-MI imaging), the highest contrast difference between tissue and microspheres seems to be achieved with low-MI imaging, in which the tissue is suppressed and the microspheres become more conspicuous.

VUS is increasingly used to detect VUR in children. As the use of Lumason in VUS has been extensively tested in clinical studies, and in view of the off-label use of the product in routine clinical practice, Bracco is seeking approval of a VUS indication for the product in the United States.

Pharmacokinetics of Intravesicular Lumason in Pediatric Patients

For the proposed indication of use of Lumason during VUS, the contrast agent is administered through a sterile catheter into the bladder and ultrasound imaging is acquired during filling of the bladder and voiding; therefore, almost immediate and complete drainage of the contrast agent is obtained upon conclusion of the procedure. No pharmacokinetic studies have been performed after intravesical administration of Lumason; however, considering the anatomical characteristics of the urinary bladder and the technique of the ultrasound examination, it is unlikely that Lumason would be absorbed and have a systemic effect following its intravesical administration during VUS:

- The examination technique used for VUS is very similar to VCUG, a radiographic imaging procedure that uses iodinated contrast agents to opacify the bladder and the urethra following the intravesical administration of the contrast agent. VCUG is performed with acquisition of radiographic images with the bladder at full capacity and while the patient is voiding. Because of the non-intravascular route of administration, and the almost immediate drainage of the contrast agent through the urethra, systemic absorption of the iodinated contrast agent during VCUG is unlikely. Similar to VCUG, during VUS, the intravesical administration of the ultrasound contrast agent is followed by voiding and excretion of contrast at the end of the procedure, making absorption of microspheres through the urinary tract or bladder wall during VUS unlikely. Furthermore, the mean diameter of the administered microspheres is considerably larger (2.5 μm) than the iodinated contrast agent molecules applied during VCUG.
- The bladder wall is lined by the urothelium, an unperfused transitional epithelium that represents an impermeable barrier between the urine and the blood stream.⁶ The most superficial layer of urothelium is composed of large cells, termed umbrella cells, each of which cover more than one cell of the cell layer beneath and are characterized by apical, rigid membrane plaques and by tight junctions ensuring strong cellular connection during bladder filling. These unique properties of umbrella cells contribute to the barrier function

⁶ Cviktović Roić A, Roić G, Palić I, Jaklin Kekez A. Contrast-enhanced voiding urosonography (ce-VUS) as a diagnostic tool in the evaluation of vesicoureteral reflux. *Pediatr Today* 2016; 12:56-65.

of urothelium, despite large variations in urine volume during bladder filling and voiding. These anatomical characteristics of the urinary bladder represent the basis for intravesical administration of drugs with the aim of reducing systemic toxicity. For example, intravesical administration of cytotoxic agents is commonly performed in patients with superficial bladder cancer and has the advantage of optimal drug delivery near the tumor site while minimizing systemic exposure.

Taken together, these points indicate that systemic absorption of Lumason after intravesical administration is unlikely. Bracco considers development studies for bioavailability, comparative bioavailability, bioequivalence, and dissolution profile not relevant to the proposed application.

Pharmacodynamics

For ultrasonography of the excretory urinary tract in pediatric patients, the intravesically administered Lumason microspheres increase signal intensity of fluids within the urethra, bladder, ureters, and renal pelvis, and facilitate the detection of reflux of fluid from the bladder into the ureters.

5. Clinical Experience of Lumason in Pediatric Patients

Assessing Efficacy

Efficacy results are presented at the renal-ureter unit level. This may have been described as pelvi-ureter unit (PUU), kidney-ureter unit (KUU) or renal unit (RU) in the publications and is hereafter referred to as Ureter Unit or UU. Some patients have duplication of the upper urinary tract. Therefore, in several studies the number of UUs exceeds twice the number of patients in the study.

The voiding radiographic procedure, which represented the truth standard, may have been referred to as voiding cystourethrography (VCUG) or micturating cystourethrography (MCU) in the publications.

VUR (vesicoureteral reflux) was diagnosed when echogenic microspheres were detected in the upper urinary tract, namely the ureters or above;

- A. A summary and discussion of four (4) well-controlled studies in the literature supporting the use of Lumason in the proposed indication [**508 patients 1,023 kidney-ureter units**]
- B. A meta-analysis of data from the 4 well-controlled studies that presented essential diagnostic performance data and qualify based on the Quality Assessment Tool for Diagnostic Accuracy (QUADAS) guidelines [**508 patients 1,023 kidney-ureter units**]
- C. Presentation of eight (8) supportive clinical studies in the literature that assessed the effectiveness of CE-VUS with Lumason in the evaluation of VUR [**1,645 patients**]

3,306 kidney-ureter units]

Data Supporting the Efficacy and Safety of Lumason

Lumason is not approved for use in VUS in or outside the USA. However, the product is used off-label in pediatric patients with medical need for assessment of VUR, as documented in scientific studies reported in the peer-reviewed literature.

In this 505(b)(2) submission, data supporting the efficacy and safety of Lumason use during VUS for assessment of VUR in children were derived from the peer-reviewed literature of clinical studies.

An extensive literature search was performed utilizing PubMed, a service of the US National Library of Medicine® and using the following search terms (urosonography OR vesicoureteral reflux OR (voiding AND (ultrasonography OR ultrasound))) AND (contrast or enhanced or microbubbles). Limits for the literature search were: published up to December 31, 2015.

Among the 574 publications that were retrieved in the literature search, 12 unique references were identified, as described below:

- Twelve (12) peer-reviewed clinical papers, each of which reported both efficacy and safety data on the use of Lumason during VUS.
- One large multicenter retrospective safety survey conducted by the Uroradiology Task Force of the European Society of Paediatric Radiology (ESPR) and the Paediatric Work Group of the European Society of Urogenital Radiology (ESUR).

The 12 clinical papers were further categorized into:

- A** - As a result of the literature search conducted by Bracco, four (4) adequate and well controlled studies support the effectiveness and safety of Lumason use during VUS for assessment of VUR in children, by meeting the following criteria:
- ***Clinical settings***: Lumason was used in children referred for detection or exclusion of VUR following UTI, a diagnosis of pelvicalyceal dilatation, or for follow-up of a known VUR, i.e., in patients representative of the population in which VUS with Lumason is intended to be used. Only commercially available hardware and software was used for all studies;
 - ***Study design***: prospective, within-patient comparisons of VUS against VCUG, with blinded evaluation of study images;
 - ***Efficacy endpoints***: diagnostic performance endpoints, i.e. sensitivity and specificity for the detection/exclusion of VUR, measured against VCUG, used as standard of truth;
 - ***Safety endpoints***: VUS and VCUG were performed in sequence during the same catheterization procedure. Patients were monitored for incidence of adverse events (or procedural complications) following administration of Lumason and iodinated contrast agents;

- **Lumason dose:** all 4 studies used the same dose of Lumason (1.0 mL).
- B -** The 4 primary studies were included in a meta-analysis conducted by Bracco.
- C –** Eight (8) supportive publications based on the following criteria support the effectiveness and safety of Lumason use during VUS for assessment of VUR:
- included a diagnostic performance endpoint with VCUG as truth standard, but utilized different doses of Lumason, or different dosing techniques; or
 - presented a technical endpoint, such as quality of visualization.

Reflux Grading System

The severity of VUR at VUS was graded according to a five-grade scale by Darge et al in 3 of the 4 pivotal studies. The study by Kljucsevsek used a three-point grading scale, following the modified Kenda's classification. In both scales, the severity of VUR was assessed on the basis of extent of reflux in the ureter/renal pelvis as well as the presence and degree of ureteral/pelvic dilatation. While grading of the degree of reflux is important from the clinical standpoint, grading was not utilized in evaluation of the application's approvability. In the **Five-Point Scale**, grades were defined in line with the International Reflux Grading System⁷ which is commonly used for grading VUR at VCUG:

Grade I: Microspheres detected only in the ureter

Grade II: Microspheres detected in the renal pelvis; no significant renal pelvic dilatation

Grade III: Microspheres detected in the renal pelvis + significant renal pelvic dilatation + moderate calyceal dilatation

Grade IV: Microspheres detected in the renal pelvis + significant renal pelvic dilatation + significant calyceal dilatation

Grade V: Microspheres detected in the renal pelvis + significant renal pelvic dilatation + significant calyceal dilatation + loss of renal pelvis contour + dilated tortuous ureter

In the **Three-Grade Scale**, the severity of VUR was scored as follows:

Grade I: Microspheres in the ureter only

Grade II: Microspheres in mildly to moderately dilated renal pelvis (AP diameter of pelvis between 5 and 10 mm with or without calyceal dilatation, or between 10 and 15 mm without calyceal dilatation) and normal or mildly dilated ureter (up to 5 mm).

Grade III: Microspheres in significant dilated renal pelvis (AP diameter more than 10 mm) and in dilated (wider and rounded) calyces, and in dilated ureter (more

⁷ Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen-Möbius TE. International system of radiographic grading of vesicoureteric reflux. International Reflux Study in Children. *Pediatr Radiol.* 1985; 15:105-09.

than 5 mm), which can be tortuous.

Key Clinical Studies Assessing Diagnostic Performance of CE-VUS with Lumason (4)

Study design

A similar administration scheme was used in 3 studies, with initial emptying of bladder, followed by partial filling of bladder with pre-warmed saline (1/3 or 1/2 of estimated bladder volume – [(age in years + 2) x 30 mL] intravesical administration of Lumason, and then continued instillation of saline until voiding. In one study, a small amount of urine was left in the bladder, after which Lumason was administered, and then saline was instilled until urinary urge or voiding.

The three studies which used similar administration scheme of saline and Lumason also used similar VUS imaging technique, i.e., low-MI (between 0.06 and 0.16) harmonic imaging, while the remaining study used harmonic imaging and higher MI (0.4-0.6).

The four (4) well-controlled studies in the literature supporting the use of Lumason in the proposed indication contain data of 508 patients and 1,023 kidney-ureter units (***Table 2 and Table 3***).

**Table 2: Key Clinical Studies Assessing Diagnostic Performance of VUS with Lumason
(Table provided by Sponsor within submission)**

Reference	<ul style="list-style-type: none"> Patients M/F Age range 	<ul style="list-style-type: none"> Study Design Truth Standard Image evaluation VUS imaging protocol 	<ul style="list-style-type: none"> Sample size Endpoints 	<ul style="list-style-type: none"> Lumason dose Administration scheme 	Results
Wong et al., Eur J Pediatr. 2014 ¹	<ul style="list-style-type: none"> 31 pts with UTI and suspected VUR 23 M/8 F 2-48 months 	<ul style="list-style-type: none"> Prospective, within-patient VCUG On-site, blinded, independent Low-MI (operated at 0.05-0.07) harmonic imaging 	<ul style="list-style-type: none"> 62 pelvi-ureter units (2 per patient) Sensitivity/specificity for detection of VUR Agreement VUS/VCUG in grading VUR Assessment of urethral abnormalities Monitoring for adverse events 	<ul style="list-style-type: none"> 1.0 mL Pre-filling of bladder with saline (1/3 maximum bladder capacity), followed by Lumason injection, followed by continuous instillation of saline until voiding 	<ul style="list-style-type: none"> Sensitivity: 100% Specificity: 84% 100% agreement in grading of VUR severity No urethral abnormalities detected with either VUS or VCUG No adverse events or procedural complications
Ključevšek et al., Acta Paediatr. 2012 ²	<ul style="list-style-type: none"> 66 pts with UTI or bacteriuria 35 M/ 31 F 5 days-1 year 	<ul style="list-style-type: none"> Prospective, within-patient VCUG On-site, blinded, independent (2 readers) for detection of VUR Low-MI (operated at 0.06-0.1) harmonic imaging 	<ul style="list-style-type: none"> 132 renal units (2 per patient) Sensitivity/specificity for detection of VUR Agreement in grading VUR Monitoring for adverse events 	<ul style="list-style-type: none"> 1.0 mL Pre-filling of bladder with saline (1/2 maximum bladder capacity), followed by Lumason injection, followed by continuous instillation of saline until voiding 	<ul style="list-style-type: none"> Sensitivity: 100% Specificity: 77.5% No adverse events or procedural complications

Table 3: Key Clinical Studies Assessing Diagnostic Performance of VUS with Lumason
(Table provided by Sponsor within submission)

Reference	<ul style="list-style-type: none"> • Patients • M/F • Age range 	<ul style="list-style-type: none"> • Study Design • Truth Standard • Image evaluation • VUS imaging protocol 	<ul style="list-style-type: none"> • Sample size • Endpoints 	<ul style="list-style-type: none"> • Lumason dose • Administration scheme 	Results
Kis et al., <i>Pediatr Nephrol.</i> 2010 ³	<ul style="list-style-type: none"> • 183 pts with UTI, pelvicalyceal dilatation or follow-up of known VUR • 94 M/89 F • 2 days to 44 months 	<ul style="list-style-type: none"> • Prospective, within-patient • VCUG • On-site, blinded, independent (2 readers) for detection of VUR • Harmonic imaging, MI: 0.4-0.6 	<ul style="list-style-type: none"> • 366 kidney-ureter units (2 per patient) • Sensitivity/specificity for detection of VUR • Agreement in grading VUR • Monitoring for adverse events 	<ul style="list-style-type: none"> • 1.0 mL • With some urine left in the bladder, Lumason injection, followed by continuous instillation of saline until voiding 	<ul style="list-style-type: none"> • Sensitivity: 86% • Specificity: 86% • Moderate agreement in grading of VUR severity • No adverse events or procedural complications
Papadopoulou et al., <i>Pediatr Radiol.</i> 2009 ⁴	<ul style="list-style-type: none"> • 228 pts with UTI, follow-up of known VUR, antenatal urinary tract dilatation, or siblings of pts with VUR • 123 M/105 F • 6 days to 13 years 	<ul style="list-style-type: none"> • Prospective, within-patient • VCUG • On-site, blinded, independent (2 readers) for detection of VUR (discordant cases reassessed by consensus read) • Low-MI (operated at 0.08-0.16) harmonic imaging 	<ul style="list-style-type: none"> • 463 kidney-ureter units • Sensitivity/specificity for detection of VUR • Monitoring for adverse events 	<ul style="list-style-type: none"> • 1.0 mL • Pre-filling of bladder with saline (1/3 maximum bladder capacity), followed by Lumason injection, followed by continuous instillation of saline until voiding 	<ul style="list-style-type: none"> • Sensitivity: 80% • Specificity: 77% • No adverse events or procedural complications

Studies and Population Characteristics

Overall, 508 pediatric patients, for a total of 1,023 ureter units, were included in the 4 studies; patient age range was between 2 days and 13 years, and more than half of the patients were male (N=275, 54%). The study populations included in these studies were representative of the clinical settings in routine clinical practice CE-VUS:

- The first peak of UTI is in the first year of life, and VUR is more prevalent in younger children. All studies included patients in their first year of life, even after their first episode of febrile UTI;
- The second peak of UTI occurs between the ages of 2 to 4 during toilet training, and three studies focused on children below 5 years of age, including patients with UTI, patients with imaging ultrasound findings suspected for VUR, and patients on follow-up for known VUR;

- The prevalence of VUR in children with UTIs decreases with age, and after the age of 6 years UTIs are infrequent; however, UTIs are often associated with dysfunctional elimination in older children. One study included patients older than 6 years of age.

Table 4 summarizes the four key publications for use of Lumason for VUS in children.

Table 4: Table: Summary of Key Publications for Use of Lumason for VUS in Children
(Table provided by Sponsor within submission)

Study	Patients (N) M (%) / F (%) Ureter Units	Age Range / Mean	Population	Lumason Volume (mL)
Wong et al., Eur J Pediatr. 2014 ²⁴	31 pts M: 23 (74%) / F: 8 (26%) 62 units	2 to 48 months / mean not reported	Children <5 years of age, after their 1st episode of urinary tract infection	1.0
Kljucevsek et al., Acta Paediatr. 2012 ²⁵	66 pts M: 35 (53%) / F: 31 (47%) 132 units	5 days to 1 year / 5.06 months	Children with proven febrile urinary tract infection (39), bacteriuria (8), isolated abnormal urinary tract ultrasound (19)	1.0
Kis et al., Pediatr Nephrol. 2010 ²⁶	183 pts M: 94 (51%) / F: 89 (49%) 366 units	2 days to 44 months / 7.6 months	Children with urinary tract infection (112), dilatation of the uretero- pelviccalyceal system (47), follow-up of VUR (24)	1.0
Papadopoulou et al., Pediatr Radiol. 2009 ²⁷	228 pts M: 123 (54%) / F: 105 (46%) 463 units	6 days to 13 years / 17.6±23.1 months	Children with urinary tract infection (164), follow-up of VUR (40), antenatal urinary tract dilatation (15), sibling of a child with VUR (9)	1.0

Diagnostic Performance of Individual Studies (Tables 5 -8)

Wong et al., 2014

A summary of the diagnostic performance results of VUS from the study by Wong et al. is provided in **Table 5**. Concordance between the 2 imaging modalities for confirming or excluding the presence of VUR was 86% (53/62 UU).

There was also good agreement between VCUG and VUS with Lumason for grading VUR in the 5 diseased UU detected by both methods. All 5 reflux units (grade III [n=1], grade IV [n=4]) were graded concordantly. Grading of reflux for the 9 UU in 7 patients with VUR detected at VUS only was grade I (n=1), grade II (n=4), grade III (n=2) and grade IV (n=2). Among these 9 reflux units, 4 units occurred in 2 patients who had bilateral reflux, with grades II and III in 1 patient and grades III and IV in another.

There was perfect inter-observer agreement in diagnosing VUR at VUS; Cohen's kappa statistics was 1.00 (p<0.001).

Table 5: Wong et al., 2014:

**Summary of Diagnostic Performance Results for
Detection of VUR in Children on a Per UU Basis
(Table provided by Sponsor within submission)**

	VUS +	VUS -	Total
VCUG +	5	0	5
VCUG -	9*	48	57
Total	14	48	62

* 1 VUR Grade I, 4 VUR Grade II, 2 VUR Grade III, 2 VUR Grade IV

Kljucevsek et al., 2012

A summary of the diagnostic performance results VUS from the study by Kljucevsek et al. is provided in [Table 6](#).

VCUG identified VUR in 16/132 (12%) of UU examined, while VUS detected VUR in 42/132 (32%) UU, including all the UU with reflux identified at VCUG. Using VCUG as the reference standard, the sensitivity of VUS with Lumason for detection of VUR was 100%, with a specificity of 78%.

Table 6: Kljucevsek et al., 2012:

**Summary of Diagnostic Performance Results for
Detection of VUR in Children on a Per UU Basis
(Table provided by Sponsor within submission)**

	VUS +	VUS -	Total
VCUG +	16	0	16
VCUG -	26*	90	116
Total	42	90	132

* 19 VUR Grade II and 7 Grade III, based on the 3 point scale of Kenda.¹³

Kis et al., 2010

A summary of the diagnostic performance results of VUS from the study by Kis et al. is provided in [Table 7](#).

VCUG identified VUR in 103/366 UU (28%), while VUS identified VUR in 126/366 UU

(34%). VUR was identified by both methods in 89 UU, by VCUG alone in 14 UU and by VUS alone in 37 UU. Using the results of VCUG as the reference standard, the sensitivity of VUS with Lumason for detection of VUR was 86% and the specificity was 86%.

Table 7: Kis et al., 2010:

**Summary of Diagnostic Performance Results for
Detection of VUR in Children on a Per UU Basis
(Table provided by Sponsor within submission)**

	VUS +	VUS -	Total
VCUG +	89	14#	103
VCUG -	37*	226	263
Total	126	240	366

4 VUR Grade I, 10 VUR Grade II

* 2 VUR Grade I, 26 VUR Grade II, 2 VUR Grade III, 7 VUR Grade IV

Papadopoulou et al., 2009

A summary of the diagnostic performance results of VUS from the study by Papadopoulou et al. is provided in [Table 8](#).

Concordance between the two exams for the presence or absence of VUR was 77.5% (359/463 UU; $\kappa=0.40$). Overall of the 161 UU with reflux identified on either or both exams, 90 (56%) were detected only at VUS. Reflux that was missed by VCUG tended to be of higher grades [Grade I (2), Grade II (65), Grade III (19), Grade IV (4)] than reflux missed by VUS [Grade I (8), Grade II (5), Grade III (1)].

Table 8: Papadopoulou et al., 2009

**Summary of Diagnostic Performance Results for
Detection of VUR in Children on a Per Ureter Basis
(Table provided by Sponsor within submission)**

	VUS +	VUS -	Total
VCUG +	57	14#	71
VCUG -	90*	302	392
Total	147	316	463

8 VUR Grade I, 5 VUR Grade II, 1 VUR Grade III

* 2 VUR Grade I, 65 VUR Grade II, 19 VUR Grade III, 4 VUR Grades IV or V

Table 9 summarizes the performance of Lumason for VUS in children in these four studies.

Table 9: Summary of Efficacy and Safety Performance of Lumason for VUS in Children (Table provided by Sponsor within submission)

Publication	Patients/ Renal units (units with VUR ^a)	Age Range	Diagnostic Performance		Safety	
			Sensitivity %	Specificity %	Assessment	Result
Wong LS et al, Eur J Pediatr. 2014 ¹⁸	31 patients (23 M/ 8 F)/ 62 units (5)	2 to 48 months	100	84	Adverse reactions to contrast immediately and up to 2-days after the procedure	No immediate or delayed complications observed; no incidents related to contrast allergy, infection or catheterization
Kljucevsek D et al, Acta Paediatr. 2012 ¹⁷	66 patients (35 M/ 31 F)/ 132 units (16)	5 days to 1 year;	100	78	Adverse events related to Lumason administration up to 48 hours	No adverse events related to Lumason administration were reported
Kis E et al, Pediatr Nephrol. 2010 ¹⁶	183 patients (94 M/ 89 F)/ 366 units (103)	2 days to 44 months	86	86	Adverse event during the 6-hour clinic period or at 24-hour follow-up	No adverse reactions were reported up to 24 hour after the procedure
Papadopoulou E et al, Pediatr Radiol. 2009 ¹⁵	228 patients (123 M/ 105 F) / 463 units (71)	6 days to 13 years	80	77	Adverse event during the 6-hour clinic period or at 24-hour follow-up	All examinations were well tolerated. No adverse events related to Lumason administration up to 24 hour after the procedure

^a Based on voiding cystourethrography.

Individual Study Results

Diagnostic performance results, with ureter unit as the analysis unit, from the 4 studies are provided in Table 10. The sensitivity ranged from 80% to 100%, and the specificity was between 77% and 86% among the 4 studies.

Table 10: Diagnostic Performance Results from the Key Individual Studies
(Table provided by Sponsor within submission)

First Author	Total of Ureter Units (N)	With Disease (N)	TP (n)	TN (n)	FP (n)	FN (n)	Sensitivity (%)	Specificity (%)
Wong LS, 2014 ¹⁸	62	5	5	48	9	0	100	84
Kljucevsek D, 2012 ¹⁷	132	16	16	90	26	0	100	78
Kis E, 2010 ¹⁶	366	103	89	226	37	14	86	86
Papadopoulou F, 2009 ¹⁵	463	71	57	302	90	14	80	77

Quality Assessment Data

Each of the 4 publications was assessed for study quality and applicability for inclusion in the met-analysis using a modified checklist based on the QUADAS guidelines; the total scores (total number of items checked Yes) ranged from 8 to 10 across the studies (Table 11).

Table 11: Quality Assessment (QUADAS Guidelines)
(Table provided by Sponsor within submission)

First Author	QUADAS Item										Total Score
	1	2	3	4	5	6	7	8	9	10	
Wong LS ¹⁸	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	9
Kljucevsek D ¹⁷	Yes	Yes	No	Yes	9						
Kis E ¹⁶	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	8
Papadopoulou F ¹⁵	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10
Item 1 = Was the population clinically relevant, defined as a group of patients covering the spectrum of disease that is likely to be encountered in the current or future use of the test? Item 2 = Was there complete verification by the reference standard? Item 3 = Was there blinded interpretation of the test results? Item 4 = Was there consecutive patient selection? Item 5 = Was there prospective enrollment of patients? Item 6 = Was there adequate description and quality of the imaging procedure? Item 7 = Was the quality of the reference test technically adequate? Item 8 = Was there adequate clinical description of the patient population? Item 9 = Was the sample size ≥ 35 patients? Item 10 = Was there adequate reporting of results, including summary and subgroup indices of accuracy?											

Table 12 compares the performance of VUCG & CE-VUS in these 4 studies: From among 1,023 possible ureter units, 167/357 (47%) positive ureteral units were proven to have VUR by both VUCG and CE-VUS. Twenty-eight/357 (8%) positive ureteral units were only detected by VUCG. CE-VUS detected 162/357(45%) positive ureteral units that were not detected by the standard comparator, VUCG. From among 357 VUR positive ureter units detected in these 4 studies, 195 (55%) were positive by VUCG and 329 (92%) were positive by CE-VUS. This Table suggests that CE-VUS may perform better than the reference standard.

Table 12: Comparison VUCG & CE-VUS Performance in Key Clinical Studies

	Both +	VUCG + Only	CE-VUS + Only	Total Units
Study #1	5	0	9	14
Study #2	16	0	26	42
Study #3	89	14	37	140
Study #4	57	14	90	161
Totals	167	28	162	357

Table 13 demonstrates the agreement between VUCG and CE-VUS with Lumason for grading of VUR on a ureter unit basis in the 4 key clinical studies using a 5 point grading scale.

Table 13: Agreement between VUCG and CE-VUS with Lumason for Grading of VUR on a Per UU Basis in the Key Clinical Studies using a 5 Point Grading Scale

		VUS with Lumason					Total
		Grade I	Grade II	Grade III	Grade IV [#]	Grade V	
VUCG	Grade I	2	6	4	2	0	14
	Grade II	1	39	11	1	1	53
	Grade III	0	5	23	4	0	32
	Grade IV [#]	1	2	2	39	3	47
	Grade V	0	0	0	3	2	5
Total		4	52	40	49	6	151

* For all UU with VUR detected by both methods.

Papadopoulou et al. used 5-point grading scales but reported agreement combining Grades IV and V together. For this presentation of the data, all Grades IV/V from the Papadopoulou study were assigned to Grade IV.

- Both VUCG and CE-VUS agree on the Grade of VUR (105/151 Units).
- CE-VUS scores the VUR one Grade higher (24/151 Units)

In 105/151 cases (70%) with evidence of VUR, both VUCG and CE-VUS agreed on the Grade of VUR. CE-VUS up-graded the VUR by one-level above that of VUCG in 24 units (16%). CE-VUS down-graded the VUR by one-level below that of VUCG in 11 units (7%). While grading

of the degree of reflux is important from the clinical standpoint, grading was not utilized in evaluation of the application's approvability.

Conclusions on the Key Studies

All 4 studies selected by the Sponsor as pivotal to support this application were performed in pediatric patients (age range: 2 days-13 years) referred for VCUG for suspected VUR, or follow-up of VUR, i.e., involved patients representative of the population in which VUS with Lumason is intended to be used:

- The first peak of UTI is in the first year of life, and VUR is more prevalent in younger children. All studies included patients in their first year of life, even after their first episode of febrile UTI;
- The second peak of UTI occurs between the ages of 2 to 4 during toilet training, and three studies focused on children below 5 years of age, including patients with UTI, patients with imaging ultrasound findings suspected for VUR, and patients on follow-up for known VUR;
- The prevalence of VUR in children with UTIs decreases with age, and after the age of 6 years UTIs are infrequent; however, UTIs are often associated with dysfunctional elimination in older children. One study included patients older than 6 years of age.

Overall, 508 pediatric patients were enrolled in the key studies.

B. Sponsor's Meta-analysis of VUS with Lumason

To further evaluate and support the efficacy of Lumason for VUS, a meta-analysis of the four pivotal studies was performed for sensitivity and specificity versus the reference standard, VCUG. Population composition, study design, analysis methods, quality of data, and reference standards were key elements considered across the studies.

The same 4 studies were available for the meta-analysis of sensitivity and specificity. Study and population characteristics of the 4 studies are displayed in **Table 14**. Overall, 508 pediatric patients, for a total of 1023 ureter units, were included in the 4 studies; patient age range was between 2 days and 13 years. More than half of the patients were male (N=275, 54%), and the majority were below 5 years of age.

A study was included in the meta-analysis if:

- It was a controlled study with prospective enrollment;
- Pediatric patients were referred for VUS for the diagnosis of VUR;
- VCUG was used as the reference standard;
- Cases were reported in absolute numbers of True Positive (TP), True Negative (TN), False Positive (FP), False Negative (FN) results, or stated data adequate to derive this information was available;

- A similar volume of Lumason was administered for VUS, with similar administration scheme of Lumason and saline.

A study was excluded from the meta-analysis if it was performed in fewer than 10 patients.

Data extraction was performed by one physician and one statistician. Inconsistencies were resolved by discussion and consensus.

The following information was extracted from each study:

- First author
- Year of publication
- Journal
- Study population
- Gender
- Mean or median or range of age, whichever available.

In addition, diagnostic performance results were extracted from the included studies.

Table 14:

**Key Study and Population Characteristics of Studies Included in the Meta-analysis
(Table provided by Sponsor within submission)**

Study	Patients (N) M (%) / F (%) Ureter Units	Age Range / Mean	Population	Lumason Volume (mL)
Wong et al., Eur J Pediatr. 2014 ¹	31 pts M: 23 (74%) / F: 8 (26%) 62 units	2 to 48 months / mean not reported	Children <5 years of age, after their 1st episode of urinary tract infection	1.0
Ključevšek et al., Acta Paediatr. 2012 ²	66 pts M: 35 (53%) / F: 31 (47%) 132 units	5 days to 1 year / 5.06 months	Children with proven febrile urinary tract infection (39), bacteriuria (8), isolated abnormal urinary tract ultrasound (19)	1.0
Kis et al., Pediatr Nephrol. 2010 ³	183 pts M: 94 (51%) / F: 89 (49%) 366 units	2 days to 44 months / 7.6 months	Children with urinary tract infection (112), dilatation of the uretero- pelvicalyceal system (47), follow-up of VUR (24)	1.0
Papadopoulou et al., Pediatr Radiol. 2009 ⁴	228 pts M: 123 (54%) / F: 105 (46%) 463 units	6 days to 13 years / 17.6±23.1 months	Children with urinary tract infection (164), follow-up of VUR (40), antenatal urinary tract dilatation (15), sibling of a child with VUR (9)	1.0

M = male; F = female

Based on the combined data the findings of the Sponsor-conducted meta-analysis of the performance of VUS with Lumason versus the reference test VCUG displayed a pooled

sensitivity of 89% (95% CI: 80% to 97%; **Figure 1**), and a pooled specificity of 81% (95% CI: 76% to 86%; **Figure 2**). (Forest Plots provided by Sponsor within submission)

Assessments of heterogeneity by Cochran’s Q indicated no significant heterogeneity among studies in sensitivity ($p = 0.0828$) and significant heterogeneity in specificity ($p=0.0196$). However, due to the small number of studies included in the meta-analysis, no subgroup analysis was performed to explore the heterogeneity in specificity.

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Figure 1. Forest Plot of Sensitivity

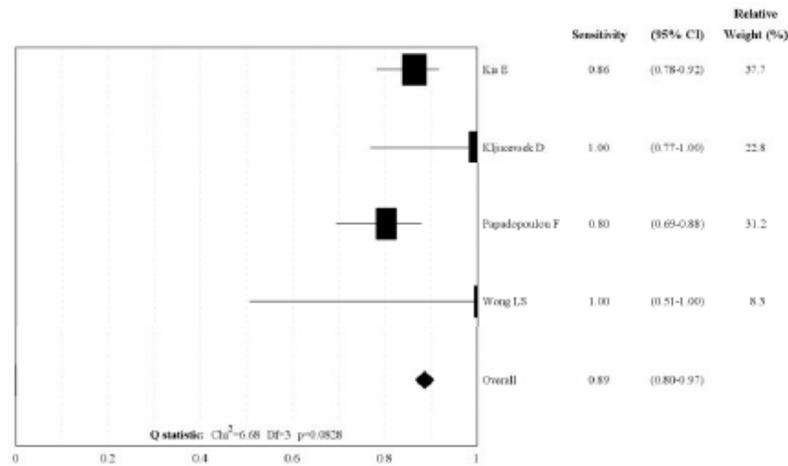
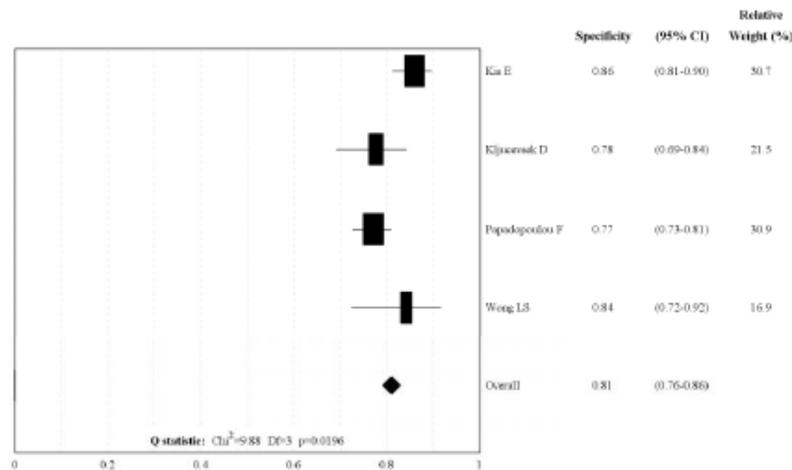


Figure 2. Forest Plot of Specificity



C. Eight (8) Supportive Clinical Studies Reported in the Literature

Although these 8 supportive studies do not meet the stricter criteria (controlled study of Lumason 1 mL in VUS, VCUG truth standard, sensitivity/specificity endpoints) required for pivotal publications, they nevertheless support the use of Lumason in VUS.

The effectiveness of CE-VUS in the evaluation of VUR with Lumason was assessed in 1,645 patients (3,306 UUs).

- Patients enrolled were representative of intended pediatric VUS population
- Age range of patients enrolled in the supportive studies - 2 days to 13 years
- Dose of Lumason used during VUS varied - 0.5 mL to 4.8 mL
- In 6 of the 8 supportive studies, Lumason diluted in saline concentrations 0.2% - 10%
- Concordance between CE-VUS with Lumason and VCUG for assessment of VUR grading ranged 73% - 95%
- Results suggest that Lumason dose and administration scheme does not seem to affect diagnostic performance of CE-VUS with Lumason

All report clinical benefit of Lumason use, either as diagnostic performance with VCUG as truth standard at a Lumason dose other than 1 mL or with a different dosing technique, unique application, success as intra-operative monitoring, or a technical endpoint, such as quality of visualization, is assessed as the study endpoint.

These supportive clinical studies are summarized in **Table 15** and **Table 16**.

Table 15:

Supportive Clinical Studies Evaluating Efficacy of Lumason for Evaluation of VUR in Children

(Table provided by Sponsor within submission)

Study	Patients M / F Ureter Units (Ureter Units with VUR*)	Age Range	Lumason Volume (mL)	Diagnostic Performance of VUS vs. VCUG (if available) or Other Efficacy Findings	
				Sensitivity %	Specificity %
Ascenti et al., Pediatr Radiol. 2004 ⁵	80 pts (36 M / 44 F) 160 (49)	3 months to 5 yrs	0.5 in 4.5mL of saline; 2 [^] administrati on if needed	100	97.3
Duran et al., Pediatr Radiol. 2012 ⁶	295 pts (154 M / 141 F) 591 units	13 days- 18 yrs (mean: 27±42 months)	1.0 in 500mL of saline	High-quality images of the bladder were obtained with Lumason; the male urethra was well visualized. 137 UU with VUR by VUS.	
Woźniak et al., J Ultrasonog. 2013 ⁷	<i>Stage I:</i> 80 pts (18 M / 62 F) 161 units (60)	<i>Stage I:</i> 3 months to 17.25 yrs	2.4 in 250mL of saline(babi es and infants);- 4.8 in 500mL of saline (older children) [#]	<i>Stage I:</i> 84.5	<i>Stage I:</i> 90.0

Table 16:

Supportive Clinical Studies Evaluating Efficacy of Lumason for Evaluation of VUR in Children

(Table provided by Sponsor within submission)

Study	Patients M / F Ureter Units (Ureter Units with VUR ^a)	Age Range	Lumason Volume (mL)	Diagnostic Performance of VUS vs. VCUG (if available) or Other Efficacy Findings	
				Sensitivity %	Specificity %
Woźniak et al., J Ultrasonog. 2013 ⁷	Stage 2: 58 pts (14 M / 44 F) 116 units	Stage 2: 4 months to 10.17 yrs	2.4 in 250mL of saline(babi es and infants);- 4.8 in 500mL of saline (older children) [#]	Stage 2: VUR was detected by VUS in 23 children (39.5%) and 31 UU (26.7%). Morphologic assessment of the urethra was possible in all patients.	
Deng et al., J South Med Univ. 2013 ⁸	36 pts (23 M/ 13 F) 72 units (21)	21 days - 10 yrs	0.2 mL every 30.0 mL of saline	100	90.2
Woźniak et al., Pediatri Radiol. 2014 ⁹	17 pts (1 M / 16 F) 25 units	4 months - 15.7 yrs	2.4	Intraoperative VUS with Lumason improved overall treatment success and identified UU that would benefit from re- treatment in the same session	
Papadopoulou et al., Pediatri Radiol. 2014 ¹¹	1,010 pts 447 M / 563 F 2,043 units	Male: (15 days - 15.5 yrs (Mean: 1.6 yrs) Female: 15 days - 17.6 yrs (Mean: 2.6 yrs)	0.5	VUS successfully performed using a low dose of Lumason in conjunction with low MI techniques. VUR was detected in 351 patients (487 UU) by VUS	
Woźniak et al., Eur J Radiol. 2015 ¹⁰	69 21 M 48 F 138 units (61)	1 yr to 13.7 yrs (mean: 4.1 yrs)	2.4 in 250mL of saline (babies and infants); 4.8 in 500mL of saline (older children) [#]	95.1	87.0

Information obtained through direct contact with the lead author.

Incorrectly reported as 0.2 mL in 3 mL saline in the published study. Correct dosing information obtained through direct contact with the lead author.

Supportive Conclusions on Efficacy of Lumason in VUS (8 Studies)

Patients enrolled in the supportive studies were representative of the pediatric population in which VUS with Lumason is intended to be used. Specifically, patients were enrolled in the studies for assessment of VUR because of UTI, dilatation of the uretero-pelviccalyceal system, family history of VUR, or because of a post-treatment follow-up of a known VUR. In one study, VUS with Lumason was used to intraoperatively monitor endoscopic sub-ureteric injection of bulking agents for treatment of VUR. The age range of patients enrolled in the supportive studies was 13 days to 18 years.

The dose of Lumason used during VUS varied among the studies and ranged between 0.5 mL and 4.8 mL. In 6 of the 7 supportive studies, the selected volume of Lumason was diluted in saline in concentrations between 0.2% and 10%; the Lumason/saline solution was then administered intravesically up to the estimated bladder volume. In one study, a Lumason volume of 0.5 mL was administered after pre-filling the bladder with saline up to one third of its estimated volume; bladder filling with saline was continued after Lumason administration. No truth standard was included in this latter study.

In the 4 supportive studies in which VUS with Lumason was compared to VCUG, high sensitivity (85% to 100%) and specificity (87% to 97%) values were reported for detection of VUR. Concordance between VUS with Lumason and VCUG for assessment of VUR grading ranged between 73% and 95%. These results suggest that, independently of the Lumason dose and administration scheme, the diagnostic performance of VUS with Lumason does not seem to be affected.

- When VCUG was used as reference standard, despite variable Lumason doses and administration schemes Sensitivity 85% - 100%; Specificity 87% - 97%
- Concordance between CE-VUS with Lumason and VCUG assessment of VUR grading ranged 73% - 95%.
- Results of these studies support the feasibility of urethral imaging with CE-VUS and Lumason in terms of imaging quality and concordance with VCUG for assessment of urethra for anatomic malformation or posterior valves (performed in one pivotal study and 6 supportive studies)

Statistical Review and Evaluation of Efficacy (Office of Biostatistics)

Sponsor's co-primary endpoints were sensitivity and specificity of consensus reading of Lumason images, with the unit of analysis reported in these papers being either pelvis-ureter unit or kidney-ureter unit (referred as ureter unit or UU thereafter). Sponsor couldn't present "by reader" analysis of sensitivity and specificity due to lack of the data. Patient-level data were not provided in 3 out of 4 published studies for the FDA's assessment.

The sponsor presented the patient-level and by reader data only in one (Ključevšek et al.) study of 66 pediatric patients. There were no cases in which the two readers who assessed the VUS exams disagreed on the presence/absence of VUR; similarly, there were no cases in which the two readers who assessed the VCUG exams disagreed on the presence/absence of VUR. At the patient level, the following rules were applied for both VCUG and VUS: A patient was considered to have VUR (positive) if at least one of the patient's ureter units had VUR, and a patient was considered not to have VUR (negative) if none of the patient's ureter units had VUR.

A summary of the diagnostic performance of VUS with Lumason in this one study is presented at both the ureter level and the patient level using VCUG as the reference standard below in **Table 17**.

Table 17:

Diagnostic Performance Results at the Ureter and Patient Level in the Study By Ključevšek et al.

Unit of Analysis	Total Number	With VUR	Without VUR	Sensitivity	Specificity	True Positive	False Negative	True Negative	False Positive
Ureter	132	16	116	100.0	77.59	16	0	90	26
Patient	66	13	53	100.0	69.81	13	0	37	16

Sensitivity = TP/(TP+FN) Specificity =TN/(TN+FP)

The statistical team concluded that the cumulative information provided and analyzed in this NDA submission provides support to the proposed indication for the pediatric patient population.

Overall Conclusions on Efficacy of Lumason in VUS

Results of the clinical studies presented confirm that Lumason is effective in detecting VUR in the target clinical population in which the contrast agent is intended to be used for VUS. Whereas adequate patient-based data were not available for analysis, the cumulative ureter-based data provided adequate data for analysis and assessment of adequacy of efficacy within the pediatric patient population.

The 4 key studies included pediatric patients with UTI, patients on follow-up because of known VUR, and patients with ultrasound findings suspected for VUR, that represent the clinical conditions for which assessment of VUR is most commonly requested. The age range of patients included in the key studies was 2 days to 13 years, with the majority of children (280 patients in 3 studies) being below 5 years of age; this is in agreement with the higher incidence of VUR in younger children and with the peak incidence of UTI being in the first year of life and between the ages of 2 to 4, during toilet training.

When compared to VCUG, which is the imaging reference standard for assessment of VUR, VUS with 1.0 mL Lumason provided high sensitivity (80-100%) and specificity (77-86%) for detection of VUR in children. The pooled sensitivity and specificity of VUS with Lumason in the meta-analysis conducted by the Sponsor were 89% and 81%, respectively.

Results of the clinical studies also demonstrate the efficacy of Lumason in providing information on VUR grading during VUS. Agreement between VUS with Lumason and VCUG for assessment of VUR grade ranged from 67% to 100% in diseased UU detected by both imaging procedures.

The efficacy of VUS with Lumason for detection and grading of VUR is further supported by results of the 8 supportive studies. When VCUG was used as reference standard in the supportive studies, despite variable Lumason doses and administration schemes, sensitivity and specificity of VUS with Lumason were still well above 80% (sensitivity: 85% to 100%; specificity: 87% to 97%), and concordance between VUS with Lumason and VCUG for assessment of VUR grading ranged between 73% and 95%. This implies that VUS with Lumason is a robust imaging method for the detection and grading of VUR.

Assessment of urethra for anatomic malformation or posterior valves was performed in one key study and 6 supportive studies. Results of these studies support the feasibility of urethral imaging with VUS and Lumason in terms of imaging quality and concordance with VCUG.

Efficacy Conclusions from Pediatric Patients (Literature –Based)

As reported in the American College of Radiology (ACR) Appropriateness Criteria, Urinary Tract Infection – Child, 2012 update, imaging assessment of VUR is indicated in the diagnostic work-up of pediatric patients with recurrent urinary tract infections (UTIs). UTI is the most frequent serious bacterial infection during childhood, affecting approximately 2% of boys and 8% of girls by the age of 7 years. The prevalence of VUR in children with UTIs is 30% to 40% and increases in children with recurrent UTIs. The identification of VUR is associated with increased risk of renal scarring. 8

Appropriateness Criteria for detection and follow-up of VUR are fluoroscopic voiding cystourethrography (VCUG) and direct radionuclide cystography (RNC). Both imaging modalities require exposure to ionizing radiation. Unenhanced ultrasonography cannot reliably detect VUR. VUS with Lumason is a procedure similar to VCUG, with use of the Lumason gas-filled microspheres instead of X-ray contrast agents, and has been shown to be as accurate as VCUG; however, it does not involve any radiation exposure. At this time, no ultrasound contrast agent is approved for use in VUS in the United States.

6. Safety Findings from Market Use of Lumason for CE-VUS in Children

Clinical Safety Studies

During market use (April 01, 2001 through September 30, 2015), an estimated (b) (4) patients have been exposed to Lumason worldwide. Lumason is generally well-tolerated.

For the proposed indicated use of Lumason during VUS to evaluate suspected or known vesicoureteral reflux in pediatric patients, the product is administered directly into the bladder via a sterile catheter. Lumason is not approved for use in VUS in or outside the USA. Safety information after intravesical administration of SonoVue were reported in 13 original publications including the 4 key studies, the 8 supportive studies, and one large retrospective survey conducted by the Uroradiology Task Force of the European Society of Paediatric Radiology (ESPR) and the Paediatric Work Group of the European Society of Urogenital Radiology (ESUR).

The number of pediatric patients included in the 13 publications is over **6000**; the age range in the studies was 2 days to 18 years. In the majority of the studies, patients were monitored

⁸ Karmazyn B, Coley BD, Binkovitz LA, Dempsey-Robertson ME, Dillman JR, Dory CE, et al. American College of Radiology ACR Appropriateness Criteria® Urinary Tract Infection - Child. [online publication]. Reston (VA): American College of Radiology (ACR), 1995. Updated 2012. Available at: <https://acsearch.acr.org/docs/69444/Narrative/>

for adverse events during and immediately after the procedure and at 24-48 hour follow-up (telephone call or reports from the parents).

In one large study of 1,010 children, safety assessment included measurements of vital signs (heart rate and respiratory rate) during the examination and every 15 minutes for one hour; measurement of body temperature at the time of the exam and one hour afterward; reporting of any adverse event during the procedure; telephone call follow-up at one week after the procedure; urine analysis and culture were performed 3-5 days before VUS in all children and 24-48 hours in any patient who reported an adverse event.⁹

One large multicenter retrospective safety survey conducted by the Uroradiology Task Force of the European Society of Paediatric Radiology (ESPR) and the Paediatric Work Group of the European Society of Urogenital Radiology (ESUR).¹⁰ The safety survey involved 45 European sites and a total of 5,079 examinations with both intravascular and intracavitary administration of Lumason. The actual number of exposed patients undergoing procedures with Lumason could not be retrieved from the survey, although the number was likely lower than 5,079 since some patients may have undergone more than one contrast-enhanced ultrasound examination. Of the 5,079 ultrasound examinations reported with use of Lumason, **4,131** were performed after intracavitary administration of the contrast agent at 29 centers. According to the authors, almost all intracavitary exams involved intravesical administration of Lumason, although in a small number of cases (probably <1%) other intracavitary applications were reported.

Details of safety assessment for each of the 12 studies are provided in **Table 18** and **Table 19** that follows:

Table 18:

**Summary of Safety Findings from Use of Lumason for VUS in Children
(Table provided by Sponsor within submission)**

⁹ Papadopoulou F, Ntoulia A, Siomou E, Darge K. Contrast-enhanced voiding urosonography with intravesical administration of a second-generation ultrasound contrast agent for diagnosis of vesicoureteral reflux: prospective evaluation of contrast safety in 1,010 children. *Pediatr Radiol.* 2014; 44:719-28.

¹⁰ Riccabona M. Application of a second-generation US contrast agent in infants and children—a European questionnaire-based survey. *Pediatr Radiol.* 2012;42:1471-80.

Study	Patients (N) M / F	Age Range	Safety	
			Assessment	Result
Ascenti, 2004 ²⁸	80 44 / 36	3 months to 5 yrs	<ul style="list-style-type: none"> - All patients hospitalized for 12 hr after the procedure and monitored for signs of adverse events. - Parents instructed to report any symptom occurring within 24 hr of the procedure 	<ul style="list-style-type: none"> - No adverse effects during the examination - No complications reported during the 24 hr follow-up
Papadopoulou E. Pediatr Radiol. 2009 ²⁷	228 123 / 105	6 days to 13 yrs	<ul style="list-style-type: none"> - Adverse event during the 6 hr clinic period - 24 hour follow-up 	<ul style="list-style-type: none"> - All examinations were well tolerated - No adverse events related to Lumason administration up to 24 hr after the procedure
Kis E. Pediatr Nephrol. 2010 ²⁶	183 94 / 89	2 days to 44 months	<ul style="list-style-type: none"> - Adverse event during the 6 hr clinic period or at 24 hr follow-up 	<ul style="list-style-type: none"> - No adverse reactions were reported up to 24 hr after the procedure
Duran, 2012 ²⁹	295 pts 154 / 141	13 days to 18 yrs (mean age: 27.1 ± 42.5 months)	<ul style="list-style-type: none"> - Patients observed during and after the procedure. - Parents or guardians asked to report any symptoms occurring within 48 hrs of the procedure 	<ul style="list-style-type: none"> - No adverse effects during the examination - No complications or adverse effects during the 48 hr follow-up
Kljucevsek D, Acta Paediatr. 2012 ²⁵	66 pts 35 / 31	5 days to 1 yr;	<ul style="list-style-type: none"> - Adverse events related to Lumason administration up to 48 hrs 	<ul style="list-style-type: none"> - No adverse events related to Lumason administration were reported
Wozniak, 2013 ³⁰	<i>Stage 1:</i> 80 pts 18 / 62 <i>Stage 2:</i> 58 pts 14 / 44	<i>Stage 1:</i> 3 months to 17.25 yrs <i>Stage 2:</i> 4 months to 10.17 yrs	<ul style="list-style-type: none"> - Not Specified 	<ul style="list-style-type: none"> - No adverse effects related to the contrast agent were observed *

Table 19:

**Summary of Safety Findings from Use of Lumason for VUS in Children
(Table provided by Sponsor within submission)**

Study	Patients (N) M / F	Age Range	Safety	
			Assessment	Result
Deng, 2013 ³¹	36 pts 23 / 13	21 days to 10 yrs	- Monitoring of children during study for acute reactions - 24 hr follow-up to observe late adverse reactions	- No adverse reactions observed during immediate or delayed follow-up
Wozniak, 2014 ³²	17 pts 1 / 16	4 months to 15.66 yrs	- All patients were monitored for adverse reactions to the contrast agent.	- No adverse effects related to the contrast agent were observed
Wong LS, Eur J Pediatr. 2014 ²⁴	31 pts 23 / 8	2 to 48 months	- Adverse reactions to contrast immediately after the procedure - Delayed monitoring for up to 2 days after the procedure	- No immediate or delayed complications observed - no incidents related to contrast allergy, infection or catheterization
Papadopoulou, 2014 ³⁴	1,010 pts 447 / 563	Males: Mean age: 1.6 yrs (15 days to 15.5 yrs) Females: Mean age: 2.6 yrs (15 days to 17.6 yrs)	- Vital signs assessed during and for 1 hr after the study - Observation of local effects to skin or tissue - Monitoring for adverse reactions, including allergic reactions - Observation by parents with 7 day telephone follow-up	- No serious adverse events (AEs) - No AEs during or at 1 hour after the exam - Minor AEs in 37 children, most considered related to catheterization - None of the AEs could be directly related to Lumason administration
Riccabona, 2014 ¹⁹	4,131 intracavitary exams # Gender distribution: 1 male to 3 females	Infant - 18 yrs	- Not Specified	- No adverse effects attributable to the contrast agent; a few complaints reported were considered to be the result of catheterization
Wozniak, 2015 ³³	69 pts 21 / 48	1 yr to 13.7 yrs	- Not Specified	- No adverse effects related to the contrast agent were observed *

* Information obtained through direct contact with the lead author

Reported for all intracavitary administration of Lumason (most were intravesical administration)

Overall, among the 13 publications, non-serious minor adverse events were reported in 37 patients. None of the reported adverse events were considered related to Lumason, but instead were considered related to the catheterization procedure during VUS. All events were reported ~~in one study of 1,010 children.~~¹¹ In this single study, adverse events were reported in 19 males¹¹ Papadopoulou F, Ntoulia A, Siomou E, Darge K. Contrast-enhanced voiding urosonography with intravesical administration of a second-generation ultrasound contrast agent for diagnosis of vesicoureteral reflux: prospective evaluation of contrast safety in 1,010 children. *Pediatr Radiol.* 2014; 44:719-28.

(mean age: 2.8 years, range: 1 month - 8.6 years) and 18 females (mean age: 3.4 years, range: 1 month - 8.9 years), or 3.7% of the study population. Dysuria was the most frequently reported symptom, in 26 children. Other reported adverse events included abdominal pain (n=2), anxiety (n=1) and crying (n=1) during micturition, blood and mucous discharge (n=1), increased frequency of micturition (n=1), vomiting (n=1), perineal irritation (n=1), and urinary tract infection 10 days after VUS (n=1). Of the 37 adverse events, 92% occurred between 2 and 24 hours after the ultrasound procedure. All reported events were self-limiting and none required hospitalization.

In addition to the literature-based cases, Bracco has received one spontaneous report of lack of efficacy after intravesical administration of Lumason to a 22-month child; no adverse effects were associated with this case

Due to the intravesical route of administration of Lumason for the VUS, systemic absorption of Lumason is unlikely; therefore, intravesical administration of Lumason is expected to be safe. The safety of Lumason after intravesical administration in pediatric patients has been confirmed in numerous clinical trials reported in the peer-reviewed literature. No serious adverse reaction has been recorded after intravesical administration of Lumason. The minor, non-serious adverse events reported attributed to the procedure rather than to the administration of Lumason.

In summary:

- No SAEs were reported
- Non-serious AEs reported were considered related to catheterization procedure rather than Lumason [dysuria, crying, anxiety, abdominal pain, frequency, UTI, hematuria]
- Most occurred between 2-24 hours post procedure
- All AEs self-limited, none required hospitalization

Clinical Laboratory Evaluations

In one large study of 1,010 children, safety assessment included urine analysis and culture performed 3-5 days before VUS in all children and 24-48 hours in any patient who reported an adverse event.

No laboratory abnormalities after Lumason were reported in the 37 patients who experienced an adverse event and were assessed for urine analysis and culture after the administration of Lumason.

Laboratory evaluations were not reported in the remaining 11 published studies.

Vital Signs

Vital sign evaluations were not reported in the 11 of the 12 Lumason VUR studies. In one large study, safety assessment included measurements of vital signs (heart rate and respiratory rate) during the examination and every 15 minutes for one hour; measurement of body temperature at the time of the exam and one hour afterward.¹¹

No vital sign abnormalities after intravesical administration of Lumason were reported in this study.

Electrocardiograms

Electrocardiogram results were not reported in any of the 12 Lumason VUR studies.

Drug Interactions

For the proposed indication of use of Lumason during VUS, the contrast agent is administered intravesically and therefore no drug interaction is expected.

Use in Pregnancy and Lactation

For the proposed indication of use of Lumason during VUS in pediatric patients, the contrast agent is administered intravesically; therefore, systemic absorption which might potentially be harmful during pregnancy and lactation is unlikely.

Overdose

Based on the intravesical route of administration, there is no safety concern.

Drug Abuse

For this indication, Lumason is only administered intravesically by medical personnel; the product is not available outside of healthcare facilities, such as hospitals or imaging centers. Therefore, the risk of incidental ingestion by patients, especially by children, is negligible.

Withdrawal and Rebound

Lumason is typically given as a single administration and has no discernible pharmacologic effect. Withdrawal of the agent does not present a safety hazard to the patient, and a rebound effect is not expected.

Postmarketing Surveillance

Lumason is currently marketed in 42 countries throughout the world, including the USA, with approved indications for intravenous use in adults. In the USA, Lumason has been recently approved for intravenous use in pediatric patients for characterization of focal liver lesions. Lumason is not approved for use during VUS in any country.

During market use of Lumason from April 1, 2001 through April 30, 2016, Bracco received sporadic reports pertaining to the pediatric population, for which Lumason was used off-label after intravesical administration. Most of these pediatric reports in the Bracco pharmacovigilance database were derived from the published literature and have been discussed. In addition to the literature-based cases, Bracco has received one spontaneous report of lack of efficacy after intravesical administration of Lumason to a 22-month old child; no adverse events were associated with this case.

7. Nonclinical Studies

The nonclinical pharmacology, pharmacokinetics, and toxicology of Lumason following its intravascular administration have been assessed in several in vitro and in vivo studies. These studies were included in the original NDA submission.

Use of Lumason for the proposed indication in the sNDA requires intravesical administration of the contrast agent, after sterile catheterization of the bladder.

Since this route of administration was not evaluated in previously performed non-clinical studies, a local tolerance study was performed in adult rats. In accordance with recommendations from the FDA (September 25, 2015), a two arm intravesical tolerability study evaluating both single- dose and repeated-dose administration was performed.

Macroscopic and histopathological examination of the urinary organs, including kidneys, ureters, urinary bladder and urethra, did not reveal any test item-related lesions in either the single-dose or repeated-dose arm.

Minor inflammatory changes were seen in the urethra, urinary bladder and kidneys of animals from all 3 groups, in both the single- and repeated-dose arm. These changes were considered to be related to the administration procedure (i.e., catheterization of the bladder) rather than to test items.

Based on results of the two-arm intravesical tolerability study, Lumason appears to be well tolerated in the lower urinary tract in the rat, with no significant safety concerns identified.

8. Prescribing Information

Prescribing information for Lumason is amended to include the indication for use in ultrasonography of the urinary tract in pediatric patients for the evaluation of suspected or known vesicoureteral reflux.

Draft proposed labeling conforms to the content and format regulations found at 21 CFR 201.56(a) and (d) and 201.57. Labeling is provided per PLR and has also been provided in SPL format. Labeling changes have been proposed and are currently in the process of review with the Sponsor.

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/s/

SCHELDON KRESS
12/14/2016

IRA P KREFTING
12/19/2016