PAES information

Title	An Observational Study of SonoVue®/Lumason®-Enhanced Urosonography in Paediatric Subjects with Known or Suspected Vesicoureteral Reflux
Protocol version identifier	**Amendment 1: 15 January 2020**
Date of last version of protocol	**Final Version: 07 June 2019**
EU PAS register number	**EUPAS30600**
Active substance	Sulphur Hexafluoride
Medicinal product	SonoVue® (Trade name in USA: Lumason®)
Product reference	ATC code: V08DA
Procedure number	EMEA/H/C/000303/ANX/032.2
Marketing authorisation holder(s)	SonoVue®: Bracco International BV Lumason®: Bracco Diagnostics Inc.
Joint PAES	No
Research question and objectives	Primary Objective of the study: to assess subject management decision and changes during a follow-up period of 12-months among children undergoing SonoVue/Lumason-enhanced VUS (VUS group) in comparison with children undergoing VCUG (VCUG group) for assessment of VUR.
Country(-ies) of study	Germany, Greece, Italy, **France**, Slovenia, Spain, and United States of America. All countries have not been identified yet, and the list is not final
Author	Study Team at Bracco

Marketing authorisation holder(s)

Marketing authorisation holder(s)	**SonoVue®** **MAH: Bracco International BV** Strawinskylaan 3051 Amsterdam, 1077 ZX (Netherlands) **Lumason®** **MAH: Bracco Diagnostics Inc.** 259 Prospect Plains Road, Monroe NJ 08831 (USA)
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2 List of abbreviations

Abbreviations:

ACR	American College of Radiology
AE	Adverse Event
CE-VUS	Contrast-Enhanced Voiding Urosonography
CFR	Code of Federal Regulations
CRF	Case Report Form
CHMP	Committee for Medicinal Products for Human Use
CRM	Clinical Research Manager
CSR	Clinical Study Report
EC	Ethics Committee
ESPR	European Society of Paediatric Radiology
EFSUMB	Societies for Ultrasound in Medicine and Biology
ENCePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
EU	European
FDA	Food and Drug Administration
ICH	International Conference on Harmonization
IP/IMP	Investigational Product/Investigational Medicinal Product
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
PAS	Post-Authorization Study
PAES	Post-Authorization Efficacy Study
PEG	Polyethylene glycol
RNC	Radionuclide cystography
SAP	Statistical Analysis Plan
SF6	Sulfur Hexafluoride
US	Ultrasound
USA	United States of America
UTI	Urinary Tract Infection
VCUG	Voiding cystourethrography
VUR	Vesicoureteral Reflux
VUS	Voiding Urosonography

3 Responsible parties

The main responsible parties are presented in Table 1.

Table 1: List of All Main Responsible Parties

Responsible Party	**Name and Affiliation**
Sponsor	Bracco Imaging S.p.A. Via Folli 50 20134 Milan Italy *With offices at:* Via Caduti di Marcinelle 13 20134 Milan, Italy Bracco Diagnostics Inc. 259 Prospect Plains Road Monroe Township, NJ USA 08831
Principal / Coordinating Investigator	For preliminary list of Potential Investigative Sites, please refer to Annex 1

4 Abstract

Title of Study

An Observational Study of SonoVue®/Lumason®-Enhanced Urosonography in Paediatric Subjects with Known or Suspected Vesicoureteral Reflux.

Version: **Amendment 1**
Date: **15 January 2020**
Main Author: Bracco Study Team

Rationale and background

SonoVue has been recently approved in the EU for use in ultrasonography of the excretory tract in paediatric patients from newborn to 18 years to detect vesicoureteral reflux. SonoVue, under the brand name of Lumason, is also approved in the United States for use in ultrasonography of the urinary tract for the evaluation of suspected or known vesicoureteral reflux in paediatric patients. This post-authorization study is being conducted as per agreement with the Committee of Medicinal Products for Human Use (CHMP) to further assess the clinical impact of patient management decisions taken after SonoVue/Lumason-enhanced VUS through long term follow-up.

Research question and objectives

Primary Objective

- To assess subject management decision and changes during a follow-up period of 12-months among children undergoing SonoVue/Lumason-enhanced VUS (VUS group) in comparison with children undergoing VCUG (VCUG group) for assessment of VUR.

Secondary Objectives

- To describe the severity (grading) of VUR and the type of treatment (conservative or surgery) among subjects in the VUS and the VCUG groups with positive findings.
- To determine the incidence of recurrent UTIs or breakthrough UTIs during the follow-up period among subjects in the VUS and VCUG groups.
- To estimate the proportion of technically inadequate imaging procedures for both VUS and VCUG groups among the population of subjects screened for inclusion in the study

Results in the VUS group will be compared to the results in the VCUG group. The primary hypothesis of the study is that patient management and treatment decision based on VUS is not different from patient management and treatment decision based on VCUG; therefore, the clinical impact of VUS on patient management decision is not different from that of VCUG.

Study design

This is a post-authorization observational, retrospective, comparative, study in paediatric subjects assessed with SonoVue/Lumason-enhanced VUS (VUS group) or VCUG (VCUG group) for evaluation of known or suspected VUR, as part of their standard of care.

Subjects will be enrolled at sites performing VUS and/or VCUG for evaluation of VUR.

Subjects in whom SonoVue/Lumason-enhanced VUS or VCUG examination for assessment of VUR was performed at least 12 months prior to enrollment, and who meet the eligibility criteria will be enrolled in the study.

Data will be collected for the initial assessment of VUR and patient management decision as well as for the follow-up period of 12 months after the baseline VUS/VCUG exam. A documented follow-up at **12 ± 1 month** after the exam should be **used. If a documented follow-up at 12 months±1 month is** not available, an intermediate follow up (e.g. 9, 6, 3 months) should be used; the **documented intermediate follow-up should be the closest available to 12 months. If no documented follow-up is available during the 12 months after the baseline exam, the patient will be considered a screen failure and will be documented in the screening log. (See Section 9.3.1 for details)**

Population

The study will be conducted in subjects below 18 years of age who had undergone **contrast enhanced VUS with** SonoVue/Lumason or VCUG as part of their standard of care at least 12 **months** prior to enrollment **and have a documented follow-up during the 12 months after the baseline exam**.

A total of approximately 400 subjects will be enrolled in order to have at least 100 VUR positive cases and at least 100 VUR negative cases in each group (VUS and VCUG).

Enrollment will continue until at least 100 subjects with positive VUR imaging findings and at least 100 subjects with negative VUR imaging findings will be included for VUS and VCUG groups. The number of subjects with positive or negative VUR imaging findings will be tracked operationally based on results of the baseline VUS or VCUG exam, to ensure that we have the required number of subjects with negative and positive VUR imaging findings in each study group. It is expected that approximately 20 study centers will participate in the study and will be selected in order to include the majority of subjects in Europe. Each site may enroll up to 50 subjects in this study.

Variables

Demographic characteristics, urinary medical history, safety assessments in terms of AEs, imaging procedures, patient management information based on baseline VUS or VCUG exam, and i**nformation for** the follow-up period will be collected.

Data sources

Source documentation for this retrospective study**, from here after referred to as "medical records"** may consist of one or more elements of the following for each subject: Medical Records, Clinical Charts, Diagnostic Reports associated with Medical Images, Surgery/Endoscopy Reports, Administrative Data Records, Flow sheets, Progress Notes, Laboratory Reports.

A documented follow-up at **12 ± 1 month** after the **baseline** exam should be **used. If a documented follow-up at 12 ± 1 month is** not available, an intermediate follow up (e.g. 9, 6, 3 months) should be used; the **documented intermediate** follow-up **should be** the **closest available to 12 months. If no documented follow-up is available during the 12 months after the baseline exam, the patient will be considered a screen failure and will be documented in the screening log.**

Study size

The sample size included in the study is based on the recommendations in "Joint Rapporteurs day 150 critical assessment report" dated 24May2017: at least 100 negative subjects and 100 positive subjects based on results of technically adequate SonoVue/Lumason-enhanced VUS will need to be enrolled. In addition, a control group of approximately 100 subjects was recommended in the "Assessment Report for the Post-Authorisation Measure 032" dated 15Nov2017. Based on the above reported recommendations, a total of approximately 400 subjects will be enrolled in order to have at least 100 VUR positive and 100 VUR negative subjects in each study group (VUS and VCUG).

As the sample size of 400 subjects equally distributed between the two groups and 50% VUR positive subjects is already agreed to as presented above, sample size calculations are provided below to determine the equivalence margin the study could exclude, to address CHMP's request as stated in "Final CHMP Assessment Report for the Post-Authorisation Measure ANX 032.2" dated 24 Jan 2019.The primary endpoint in the study is the need for patient management changes during a follow-up period of 12-months among children undergoing SonoVue/Lumason-enhanced VUS or VCUG for assessment of VUR. The study will perform an equivalence test with a pre-specified equivalence margin of ±12.5%. Based on preliminary discussions with expert investigators, the expected proportion of subjects who would require a change in management during the 12-month follow-up would not exceed 25% for both VCUG and VUS groups. Given this estimate, with 200 subjects in each group (total of 400 subjects in the study), the observed two-sided 95% confidence interval of the difference in the proportion of patient management change will be expected to contain the equivalence margin of ±12.5%

with approximately 80% power based on 2000 simulations using the Wilson score interval method to construct the confidence interval[10]. Equivalence can be concluded if the 2-sided 95% CI for the difference in the proportion of patient management change is within ±12.5%.

Data analysis

Primary Efficacy Analysis:

The primary objective is to assess subject management decision and changes during the follow-up period of 12 months among children undergoing VUS in comparison with children undergoing VCUG for assessment of VUR.

The study will perform an equivalence test with ± 12.5 % of pre-specified equivalence margin.

For the primary endpoint, changes in patient management/treatment in the follow-up period will be summarized and presented with frequency and percentage for each study group, as well as for the difference between two study groups. The 95% confidence interval for the difference in the proportions of patient management change will be estimated using Wilson score confidence interval method. The equivalence of two groups can be concluded if the observed 2-sided 95% confidence interval for the difference in the proportion of patients with change in patient management is within the pre-specified equivalent margin of ±12.5%. Furthermore, the difference in patient management change between two study groups will be tested by Chi-squared test or Fisher's exact test.

The other data collected for the follow-up period will also be summarized for each category with frequency and percentage for each study group.

Secondary Efficacy Analysis:

Among subjects with positive findings from VUS or VCUG as assessed at the time of initial patient management, the grading of VUR and the type of treatment will be summarized using descriptive statistics. Difference between the VUS and VCUG groups will be examined by Chi-squared test or Fisher's exact test.

The incidence rate of recurrent UTIs or breakthrough UTIs as collected during the follow-up period will be calculated along with 95% confidence intervals for each study group if available.

The proportion of technically inadequate imaging procedures for both VUS and VCUG groups among the population of subjects screened for inclusion in the study will be calculated for each group.

Milestones

Milestone	Planned date
Start of data collection	Four months after protocol approval
End of data collection	Eight months after the start of data collection
Registration in EU PAS register	Before the start of the data collection
Final Report of study results	Six months after the end of data collection (database lock: 2 months after last data collection; statistical analysis: 2 months after database lock; report writing: 2 months)

5 Amendments and updates

Amendment Number 1	
Change	**Reason**
Throughout the document, removed the requirement of follow-up of "at least" 12 months	**The protocol allows for the use of documented intermediate follow-up prior to 12 months, in case a documented follow-up at 12 months is not available**
Throughout the document, clarified the use of "intermediate" follow-up	**In order to clarify that the intermediate follow-up "closest" to 12 months should be used**
Throughout the document, removed the 12 month follow-up phone call	**During the site selection process, sites informed that the standard of care is for patients to have scheduled follow-up visits during the 12 month period, even if initial assessment for VUR with VUS or VCUG is negative. Furthermore, the possibility to collect follow-up data at intermediate timepoints will reduce the need for the phone call at 12 months. Finally, based on the feedback from sites, IRB approval of retrospective study which includes a phone call with the patient/guardian would be difficult to obtain and would reduce the willingness of the sites to participate in the study.**
Throughout the document, period for screening changed to 12 months before the	**There is potential for sites to obtain EC/IRB approval at a certain date, but be initiated**

SIV instead of the EC/IRB approval date	**several months later**

6 Milestones

Milestone	**Planned date**
Start of data collection	Four months after protocol approval
End of data collection	Eight months after the start of data collection
Registration in EU PAS register	Before the start of the data collection
Final Report of study results	Six months after the end of data collection (database lock: 2 months after last data collection; statistical analysis: 2 months after database lock; report writing: 2 months)

7 Rationale and background

Vesicoureteral reflux (VUR) 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. 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 urinary tract infection (UTI) with potential for nephrovascular hypertension and renal failure[1,2,3]. 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[4]. The prevalence of VUR in children with UTIs is 30% to 40% and increases in children with recurrent UTIs[3,5].

As reported in the guidelines of the European Association of Urology (EAU), imaging plays a central role in the diagnosis of VUR and decision for therapeutic options[1]. Commonly performed imaging procedures include fluoroscopic voiding cystourethrography (VCUG) and direct radionuclide cystography (RNC) with reported comparable sensitivity and specificity for diagnosis of VUR[1,2,3,6]. However, both VCUG and RNC require exposure to ionizing radiation, a concern which 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.

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 diagnosis of VUR is low[1,2,3.] Contrast-enhanced voiding urosonography (CE-VUS) encompasses evaluation of the urinary tract after intravesical administration of an ultrasound contrast agent and has been increasingly used in the clinical assessment of pediatric patients with known or suspected VUR. As in VCUG or RNC, the contrast agent is administered through a catheter into the bladder and imaging is acquired during filling of the bladder and voiding. The diagnosis of VUR using VUS is straightforward:

when the microbubbles are administered intravesically, 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[7]. As for US, VUS does not require exposure to ionizing radiation and has the advantage of higher patient comfort, when compared to VCUG; as a matter of fact, VUS can be performed with the patient voiding in a normal/physiological position and parents may stay with the patient for the whole duration of the procedure which makes a big difference in young children.

The clinical usefulness of 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 ultrasound[8] and by the European Society of Paediatric Radiology (ESPR) uroradiology task force[9]. Similarly, the American College of Radiology (ACR) recognizes that echo-enhanced cystography is a non-ionizing, safe, and reliable method to evaluate for VUR[3].

SonoVue has been recently approved in the EU for use in ultrasonography of the excretory tract in paediatric patients from newborn to 18 years to detect vesicoureteral reflux. SonoVue, under the brand name of Lumason, is also approved in the United States for use in ultrasonography of the urinary tract for the evaluation of suspected or known vesicoureteral reflux in paediatric patients. This post-authorization study is being conducted as per agreement with the Committee of Medicinal Products for Human Use (CHMP) to further assess the clinical impact of patient management decisions taken after SonoVue/Lumason-enhanced VUS through long term follow-up.

8 Research question and objectives

Primary Objective

- To assess subject management decision and changes during a follow-up period of 12-months among children undergoing SonoVue/Lumason-enhanced VUS (VUS group) in comparison with children undergoing VCUG (VCUG group) for assessment of VUR.

Secondary Objectives

- To describe the severity (grading) of VUR and the type of treatment (conservative or surgery) among subjects in the VUS and the VCUG groups with positive findings.
- To determine the incidence of recurrent UTIs or breakthrough UTIs during the follow-up period among subjects in the VUS and VCUG groups.
- To estimate the proportion of technically inadequate imaging procedures for both VUS and VCUG groups among the population of subjects screened for inclusion in the study.

9 Research methods

9.1 Study design

This is a post-authorization observational, retrospective, comparative study in paediatric subjects assessed with SonoVue/Lumason-enhanced VUS (VUS group) or VCUG (VCUG group) for evaluation of known or suspected VUR, as part of their standard of care.

Subjects will be enrolled at sites performing VUS **and**/or VCUG for evaluation of VUR.

Subjects in whom SonoVue/Lumason-enhanced VUS or VCUG examination for assessment of VUR was performed at least 12 months prior to enrollment, and who meet the eligibility criteria will be enrolled in the study.

Data will be collected for the initial assessment of VUR and patient management decision as well as for the follow-up period of 12 months after the baseline VUS/VCUG exam. Data collection will be conducted through review of subjects' medical records. A documented follow-up at **12± 1 month** after the **baseline** exam should be **used. If a documented follow-up at 12 ± 1 month is** not available, an intermediate follow up (**e.g.** 9, 6, 3 months) should be used; the **documented intermediate** follow-up **should be the closest available to 12 months. If no documented follow-up is available during the 12 months after the baseline exam, the patient will be considered a screen failure and will be documented in the screening log.**

Results in the VUS group will be compared to the results in the VCUG group. The primary hypothesis of the study is that patient management and treatment decision based on VUS is not different from patient management and treatment decision based on VCUG; therefore, the clinical impact of VUS on patient management decision is not different from that of VCUG.

9.1.1 Discussion of the Study Design

This post-authorization study is designed as an observational, retrospective, comparative study to further assess the clinical impact of patient management decisions taken after SonoVue/Lumason-enhanced VUS through a follow-up of 12 months, as compared to a control group of subjects in whom management decision was taken on the basis of VCUG.

To this aim, subjects with known or suspected VUR who had undergone a technically adequate **contrast**-enhanced VUS **with SonoVue/Lumason** (VUS group) or VCUG (VCUG group) as part of their standard of care at least 12 months **prior to enrollment and have a documented follow-up during the 12 months** after **the** baseline **exam**, will be considered for enrollment.

A documented follow-up at **12 ± 1 month** after the **baseline** exam should be **used. If a documented follow-up at 12 ± 1 month is** not available, an intermediate follow up (**e.g.** 9, 6, 3 months) should be used; the **documented intermediate** follow-up **should be the closest available to 12 months. If no documented follow-up is available during the 12 months after the baseline exam, the patient will be considered a screen failure and will be documented in the screening log.**

In order to avoid selection biases, all consecutive subjects who were assessed for VUR at each site will be evaluated for enrollment starting from 12 months prior to the date of **site initiation** and moving

backwards chronologically up to September 1, 2017. This date is selected as an anchor date to have a consistent time period for enrollment and data collection among the sites and among the two study groups (VUS and VCUG). September 1, 2017 was selected on the **basis** of the approval date of VUR indication for SonoVue in EU (August 2017).

All subjects who meet the prospectively defined inclusion/exclusion criteria will be enrolled in the study and will comprise the subject population for data collection. The detailed reason(s) for any exclusion of each subject screened will be documented in the Subject Screening/Enrollment Log. In case of exclusion for technical inadequacy of the VUS/VCUG exam, the reason for exclusion will also be documented (e.g. motion artifacts, equipment failure, etc.).

Enrollment will continue in each study group until at least 100 subjects with positive VUR imaging findings and at least 100 subjects with negative VUR imaging findings are included. The number of subjects with positive or negative VUR imaging findings will be tracked operationally based on results of the baseline VUS or VCUG exam, to ensure that there are the required number of subjects with negative and positive VUR imaging findings in each study group.

As the study will enroll subjects who required a VUS exam with SonoVue/Lumason or VCUG as part of their standard of care for assessment of a known or suspected VUR, inclusion in the present clinical study will not add any risk to the subject.

For subjects enrolled in the study, the following information will be collected through a review of medical records:

- data relative to the baseline VUS or VCUG exam and patient management decision; **this includes the information about additional diagnostic imaging procedures performed after the baseline exam and before subject treatment decision was made;**
- data relative to the follow-up period (such as any additional diagnostic procedure performed after patient management decision, any change in treatment, occurrence of UTI during treatment).

The primary outcome of the study is the need for change in patient management/treatment during the follow-up period of 12 months. The change in patient management is defined as the occurrence of any of the following conditions:

- change from conservative to surgical or endoscopic treatment that was not initially planned;
- change from no treatment to treatment or change from treatment to no treatment that was not initially planned;
- results of additional diagnostic procedures during follow-up not confirming findings of baseline VUS or VCUG exam that are not justified by changes of VUR condition due to surgical/endoscopic correction or spontaneous improvement/resolution;
- surgical/endoscopic findings not confirming imaging findings of baseline VUS or VCUG exam.

The secondary outcomes of the study include:

- the distribution of severity (grading) of VUR among subjects with positive results at VUS or VCUG exam (baseline or follow-up);

- the incidence of the different treatment decisions (conservative, endoscopy, surgery) in children with positive results at VUS or VCUG (baseline or follow-up);
- the incidence of recurrent UTIs during the follow-up period;
- the incidence of febrile breakthrough UTIs during the follow-up period;
- the proportion of technically inadequate imaging procedures for both VUS and VCUG groups among the population of subjects screened for inclusion in the study.

Subjects will be enrolled at sites which have implemented VUS in their routine clinical practice for assessment of subjects with known or suspected VUR (VUS group) and sites which primarily use VCUG for assessment of VUR (VCUG group). Based on the observational nature of the study, it is expected that patient management decisions at each site after the baseline VUS or VCUG exam will follow the clinical protocols and algorithms in place at the site. Particular attention will be taken to match suitable VUS and VCUG sites in order to minimize selection biases in terms of subject population and treatment algorithm. This will require a proper assessment of local procedures at each site (such as characteristics of children undergoing VUS or VCUG for assessment of VUR, use of additional confirmatory procedures before treatment start, clinical algorithm for patient management) before inclusion of the site in the study.

9.2 Setting

9.2.1 Study population

The study will be conducted in subjects below 18 years of age who had undergone **contrast enhanced VUS with** SonoVue/Lumason or VCUG as part of their standard of care at least 12 **months** prior to enrollment **and have a documented follow-up during the 12 months after the baseline exam.**

A total of approximately 400 subjects will be enrolled in order to have at least 100 VUR positive cases and at least 100 VUR negative cases in each group (VUS and VCUG).

It is expected that approximately 20 study centers will participate in the study and will be selected in order to include the majority of subjects in Europe. Each site may enroll up to 50 subjects in this study.

In order to avoid selection biases, all consecutive subjects who were assessed for VUR at each site will be screened starting from 12 months prior to **site initiation** and moving backwards up to September 1, 2017; all subjects who meet the prospectively defined inclusion/exclusion criteria will be enrolled in the study and will comprise the subject population for data collection. The detailed reason(s) for any exclusion of each subject screened will be documented in the Subject Screening/Enrollment Log. In case of exclusion for technical inadequacy of the images, the reason for exclusion will also be documented (e.g. motion artifacts, equipment failure, etc.).

Enrollment will continue until at least 100 subjects with positive VUR imaging findings and at least 100 subjects with negative VUR imaging findings will be included for VUS and VCUG groups. The number of subjects with positive or negative VUR imaging findings will be tracked operationally based on results of the baseline VUS or VCUG exam, to ensure that we have the required number of subjects with negative and positive VUR imaging findings in each study group.

The Study Flow is presented in Figure 1.

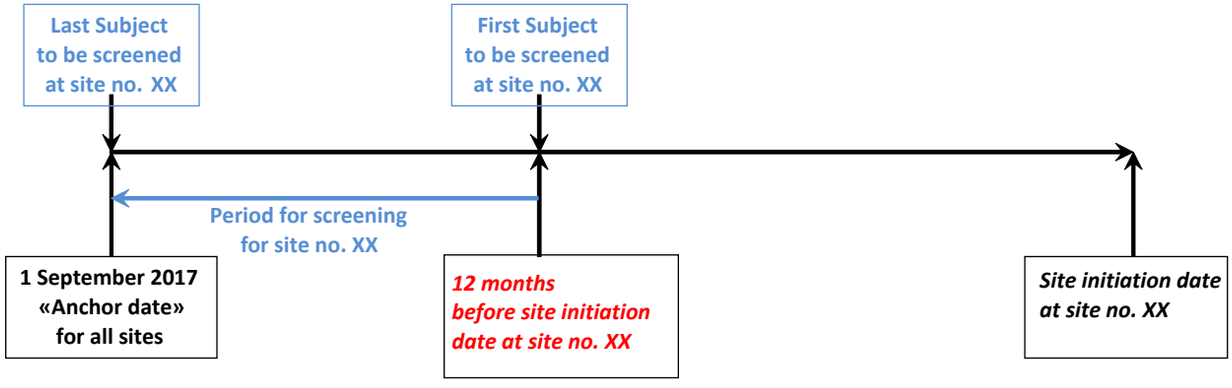

Figure 1: Study Flow

9.2.2 Study Duration

All consecutive subjects who were assessed for VUR at each site will be considered for enrollment starting from the date of the **site initiation.**

The period for screening will be from 12 months before the date of **site initiation** at each site and will go backwards chronologically until September 1, 2017.

A subject will be enrolled at least 12 months after a technically adequate SonoVue/Lumason-enhanced VUS (VUS group) or VCUG (VCUG group) exam for known or suspected VUR not already surgically or endoscopically treated.

A subject's participation will consist of the collection of data from the review of medical records.

9.2.3 Inclusion Criteria

Enroll a subject in this study if the subject meets the following inclusion criteria:

- Is less than 18 years of age at the time of the baseline VUS or VCUG examination;
- Had undergone a technically adequate SonoVue/Lumason-enhanced VUS (VUS group) or VCUG (VCUG group) exam for known or suspected VUR not already surgically or endoscopically treated, as part of his/her standard of care, at least 12 months prior to enrollment and not before September 1, 2017;
- Has follow-up data available for the period of 12 months after baseline VUS or VCUG based on medical records.

9.2.4 Exclusion Criteria

Exclude a subject from this study if the subject does not fulfill the inclusion criteria, or if the subject has been already enrolled in this study.

9.2.5 Discontinuation Criteria

There are no discontinuation criteria for this study due to its observational nature.

9.2.6 Investigational Product/Investigational Medicinal Product

9.2.6.1 Description and Labeling

SonoVue/Lumason is formulated as a 25-mg sterile, non-pyrogenic lyophilized powder in a septum-sealed vial. The ingredients in each vial of SonoVue/Lumason, after reconstitution, are listed in Table A.

Table A: Description of Investigational Product

Ingredient	Concentration / Amount per Unit
Polyethylene glycol (PEG) 4000	4.91 mg/ml
Phospholipids (DSPC/DPPG 1:1 w/w)	0.075 mg/ml
Palmitic Acid	0.008 mg/ml
Sulfur Hexafluoride (SF6)	8 µL/ml*

*Contents in the microspheres/microbubbles

9.3 Variables

9.3.1 Screening/Enrollment Log – Screening/Subject Numbering

All potential subjects must be entered into the Subject Screening/Enrollment Log regardless of whether they enter the study or not. In the case of subjects who do not enter the study, the subject's screening number must be recorded together with the reason(s) why subject was not included in the study. In case of exclusion for technical inadequacy of the images, the reason for exclusion will also be documented. An effort will be made to minimize selection bias by recruiting all subjects who meet the prospectively defined inclusion/exclusion criteria moving chronologically backward from the date of the **site initiation** until September 1, 2017.

The first digit of the screening number will be a 9, the second and third digits will be the investigational site number assigned by Bracco. The fourth and fifth digits will be a number starting with 01 for the first subject screened and incrementing by "1" for each sequential subject.

Assign a four-digit subject number for each subject who qualifies for the study. The first two digits of the subject number will be the investigational site number assigned by Sponsor. For site numbers less than ten use a leading zero. The third and fourth digits will be a number starting with 01 for the first subject enrolled and incrementing by "1" for each sequential subject.

For example, at site 01, the investigative site will assign the first subject 0101; the second subject 0102, etc.

Screening and subject numbers must never be re-assigned. In the event that a subject with a screening number fails the screening evaluation, the screening number assigned to that subject is retired and the next subject receives the next screening number. Due to possible screening failures, screening and subject numbers may not directly correspond.

9.3.2 Subject Evaluations

9.3.2.1 Urinary Medical History and Demographics

The following data of the subject's urinary medical history will be recorded on the Urinary Medical History section of the CRF:

- circumcision and toilet training, if applicable;
- presence of voiding dysfunction;
- presence of prenatal hydronephrosis;
- VUR already diagnosed in siblings;
- previous/ongoing UTI (date) and treatment (type, date) including occurrence of breakthrough febrile UTI;
- previous evaluation of VUR (date, type of evaluation performed and results);
- previous assessment for relevant genito-urinary anatomic abnormalities (date, type of evaluation performed and results);
- previous assessment for renal cortical abnormalities (date, type of evaluation performed and results).

Obtain demographic information on each subject (including height, weight, age, sex and race).

The clinical reason for VUS or VCUG exam will also be collected in the CRF; this might include the following conditions:

- first episode of UTI;
- recurrent UTI;
- VUR diagnosed in siblings;
- renal pelvis dilatation at pre-natal/neonatal ultrasound;
- follow-up of a known condition of VUR not already surgically or endoscopically treated.

9.3.2.2 Safety Assessments

Record in the Adverse Event section of the CRF whether the subject had any untoward medical occurrences during the timeframe associated with the SonoVue/Lumason administration for VUS group and with the iodinated contrast agent administration for VCUG group.

9.3.2.3 Imaging Procedures

VUS and VCUG exams were performed according to the clinical practice at each enrolling site. Information about the imaging procedures will be collected retrospectively.

9.3.2.4 Initial VUS or VCUG Exam and Patient Management Decision

The diagnosis of VUR, as assessed on technically adequate SonoVue/Lumason-enhanced VUS or VCUG exams will be collected in the CRF as follows:

- absence or presence of VUR;
- side and grading of VUR, if present;
- presence of any additional relevant findings observed in the kidneys, ureters, bladder, and urethra.

Additional diagnostic procedures performed **after the baseline exam and** before **making** a decision on subject treatment will be collected in the CRF, together with the procedure results and the reason for the additional procedure.

The patient management and treatment decision will be collected as follows:

- no treatment
- antibiotic prophylaxis (type and planned duration)
- surgery (type)
- endoscopic treatment (type)
- continue/change ongoing treatment (type), for subjects with known VUR already on a treatment scheme
- other

9.3.2.5 Follow-up Period

A documented follow-up at **12 ± 1 month after** the baseline exam should be **used. If a documented follow-up at 12 months is** not available, an intermediate follow up (**e.g.** 9, 6, 3 months) should be used; the **documented intermediate follow-up should be the closest available to 12 months. If no documented follow-up is available during the 12 months after the baseline exam, the patient will be considered a screen failure and will be documented in the screening log.**

The following information will be collected:

- surgery/endoscopic treatment performed (type, date, surgical/endoscopic findings confirm/do not confirm baseline VUS/VCUG findings)
- antibiotic prophylaxis or other non-surgical treatment (type and duration)
  - compliance with antibiotic prophylaxis or other non-surgical treatment (yes/no)
- occurrence of febrile breakthrough UTI (date, treatment)
- occurrence of recurrent UTI (date, treatment)
- change in patient management/treatment (reason, type of change, date), as previously defined in section 9.1.1

- imaging procedures/tests performed during follow-up, i.e. after initial patient management decision (reason, type, date, results, additional imaging findings confirm/do not confirm baseline VUS/VCUG findings).
- any other new information about presence/absence and grading of VUR, if available

9.4 Data sources

Source documentation for this retrospective study may consist of one or more elements of the following for each subject:

Medical Records

Clinical Charts

Diagnostic Reports associated with Medical Images

Surgery/Endoscopy Reports

Administrative Data Records

Flow sheets

Progress Notes

Laboratory Reports

9.5 Study Size

The sample size included in the study is based on the recommendations in "Joint Rapporteurs day 150 critical assessment report" dated 24May2017: at least 100 negative subjects and 100 positive subjects based on results of technically adequate SonoVue/Lumason-enhanced VUS will need to be enrolled. In addition, a control group of approximately 100 subjects was recommended in the "Assessment Report for the Post- Authorisation Measure 032" dated 15Nov2017. Based on the above reported recommendations, a total of approximately 400 subjects will be enrolled in order to have at least 100 VUR positive and 100 VUR negative subjects in each study group (VUS and VCUG).

As the sample size of 400 subjects equally distributed between the two groups and 50% VUR positive subjects is already agreed to as presented above, sample size calculations are provided below to determine the equivalence margin the study could exclude, to address CHMP's request as stated in "Final CHMP Assessment Report for the Post-Authorisation Measure ANX 032.2" dated 24 Jan 2019.The primary endpoint in the study is the patient management changes during a follow-up period of 12-months among children undergoing SonoVue/Lumason-enhanced VUS or VCUG for assessment of VUR. The study will perform an equivalence test with a pre-specified equivalence margin of ±12.5%. Based on preliminary discussions with expert investigators, the expected proportion of subjects who would require a change in management during the 12-month follow-up would not exceed 25% for both VCUG and VUS groups. Given this estimate, with 200 subjects in each group (total of 400 subjects in the study), the observed two-sided 95% confidence interval of the difference in the proportion of patient management change will be expected to contain the equivalence margin of ±12.5% with approximately 80% power based on 2000 simulations using the Wilson score interval method to construct the

confidence interval[10]. Equivalence can be concluded if the 2-sided 95% CI for the difference in the proportion of patient management change is within ±12.5%.

9.6 Data Management

All data collected will be entered into the database and displayed in the data listings and/or tables. Details of the data handling procedures will be specified in the SAP.

9.7 Data analysis

In general, summary statistics (mean, median, standard deviation, minimum, and maximum) will be provided for continuous variables, and the number and percentage of each category will be provided for categorical data. Unless otherwise specified, the analysis will be provided by study group, i.e. VUS and VCUG, and the statistical tests will be 2-sided at 0.05 level of significance.

No interim analysis is planned. Missing data will not be imputed in general.

Any changes in the original statistical methodology will be documented in the statistical analysis plan.

All statistical analyses will be performed using SAS® software.

9.7.1 Subject Disposition and Demographic and Baseline Characteristics

Summary tables will be provided for the number of subjects who have been enrolled and completed the study according to the protocol.

Summary of the screening failure will be provided by the reason for any exclusion (e.g., technical inadequacy of VUS/VCUG) based on the Subject Screening/Enrollment Log.

Summary tables will be provided for demographic and baseline characteristics, including age, sex, race, height, weight, clinical reason for SonoVue/Lumason-enhanced VUS or VCUG exam, and relevant urinary medical history.

9.7.2 Analysis Population

Analysis population will include all subjects of this study who underwent technically adequate SonoVue/Lumason-enhanced VUS or VCUG exams at least 12 months prior to enrollment and who have **documented** follow-up **during** the 12-months **after the baseline exam**.

9.7.3 Safety Analysis

The safety data will be summarized. All adverse events will be coded using MedDRA and summarized by system organ class and preferred term, by intensity and by causal relationship to the SonoVue/Lumason.

Only those collected adverse events that occur after VUS or VCUG will be summarized.

9.7.4 Efficacy Analysis

9.7.4.1 Primary Efficacy Analysis

The primary objective is to assess subject management decision and changes during the follow-up period among children undergoing VUS in comparison with children undergoing VCUG for assessment of VUR.

The study will perform an equivalence test with ± 12.5 % of pre-specified equivalence margin.

For the primary endpoint, changes in patient management/treatment in the follow-up period will be summarized and presented with frequency and percentage for each study group, as well as for the difference between two study groups. The 95% confidence interval for the difference in the proportions of patient management change will be estimated using Wilson score confidence interval method. The equivalence of two groups can be concluded if the observed 2-sided 95% confidence interval for the difference in the proportion of patients with change in patient management is within the pre-specified equivalent margin of ±12.5%. Furthermore, the difference in patient management change between two study groups will be tested by Chi-squared test or Fisher's exact test.

The other data collected for the follow-up period will also be summarized for each category with frequency and percentage for each study group.

9.7.4.2 Secondary Efficacy Analysis

Among subjects with positive findings from VUS or VCUG (baseline or follow-up), the grading of VUR and the type of treatment will be summarized using descriptive statistics. Difference between the VUS and VCUG groups will be examined by Chi-squared test or Fisher's exact test.

The incidence rate of recurrent UTIs or breakthrough UTIs as collected during the follow-up period will be calculated along with 95% confidence intervals for each study group if available.

The proportion of technically inadequate imaging procedures for both VUS and VCUG groups among the population of subjects screened for inclusion in the study will be calculated for each group.

9.8 Quality control

9.8.1 Regulatory Requirements–Sponsor/Investigator Obligations

This study will be conducted in accordance with the Declaration of Helsinki, ICH E6 Guideline, and as applicable, Title 21 CFR 312.50 through 312.70. To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation by authorized individuals, and regulatory authorities.

9.8.2 Study Monitoring

An appropriate representative of Sponsor (Study Monitor) will maintain contact with the Investigator and will visit the study site for the purpose of discussing and/or retrieving data and verify the rights, safety and well-being of subjects are protected, that the reported data are reliable and robust, and that the conduct of the clinical trial is in compliance with the applicable regulatory requirements.

An initiation visit will be made by the Study Monitor to discuss the protocol and the obligations of both the Sponsor and the Investigator. The Investigator must allow the Study Monitor to perform periodic, interim monitoring visits. The purposes of these visits are to:

- assess the progress of the study;
- review the compliance with the study protocol;
- determine whether all adverse events were appropriately collected in the CRF;
- determine whether the Investigator is maintaining the essential documents;
- discuss any emergent problem;
- check the Case Report Forms for legibility, accuracy and completeness;
- validate the contents of the Case Report Forms against source documents;

All data required by the protocol must be reported accurately on the Case Report Form and must be consistent with the source documents. Source documents are original documents, data and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, administration data records, recorded data from automated instruments, copies or transcription certified after verification as being accurate copies, microfiches, photographic negatives, microfilms or magnetic media, X-rays or other diagnostic images, subject files, laboratory records). The Investigator will make available the source documents for inspection and auditing purposes. This information will be considered confidential.

The Study Monitor will perform a close-out visit at the conclusion of the Investigator's involvement in the study.

9.8.3 Case Report Form

The Sponsor will provide a Case Report Form for each subject. The Investigator must record all data on the Case Report Form and archive a copy of the completed Case Report Form at the investigational site. Case Report Forms must be completed for all subjects for whom signed Informed Consent and Assent (when appropriate) are obtained even if the subject fails to complete the study.

If requested, copies of the Case Report Forms are to be made available to the appropriate Regulatory Authorities.

9.8.4 Inspection and Auditing

The Investigator/Institution will make available for direct access all trial related records including source data and documents for inspection by Regulatory Authorities, IRB/EC and for auditing by the Sponsor. This information will be considered confidential.

9.8.5 Archiving of Records

Paper and/or electronic medical files and records of the subjects will be archived in accordance with national law. ***For non EU sites*: The retention time period must be at least two years after the last approval of the marketing application of the investigational product in an ICH region and until there is no pending or contemplated marketing application in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational**

product. *For EU sites only*: the retention time period must be twenty five (25) years after study completion.

No study document should be destroyed without prior written agreement between Sponsor and the Investigator.

The content of the Trial Master File owned by the Sponsor and of the Investigator's File shall be archived in a way that ensures that it is readily available and accessible, upon request, to the Regulatory Authorities and auditors.

Access to the Sponsor/Investigator archive will be restricted to those appointed individuals authorized to access.

The media used to archive the content of the Trial Master File/Investigator's File shall be such that the content remains complete and legible throughout the period referred to in this Section of the protocol.

Any alteration to the content of the Trial Master File/Investigator's File shall be traceable.

9.8.6 Recording, Processing, Handling and Storage of Information

All clinical trial information will be recorded, processed, handled, and stored by the Sponsor and Investigator, as applicable, in such a way that it can be accurately reported, interpreted and verified while the confidentiality of records and the personal data of the subjects remain protected in accordance with the applicable law on personal data protection.

Appropriate technical and organizational measures will be adopted to protect information and personal data processed against unauthorized or unlawful access, disclosure, dissemination, alteration, or destruction or accidental loss, in particular where the processing involves the transmission over a network.

Measures to be implemented in case of a data breach will be detailed in the Monitoring Plan.

9.9 Limitations of the research methods

In order to avoid selection biases, all consecutive subjects who were assessed for VUR at each site will be evaluated for enrollment starting from 12 months prior to the date of **site initiation** and moving backwards chronologically up to September 1, 2017. This date is selected as an anchor date to have a consistent time period for enrollment and data collection among the sites and among the two study groups (VUS and VCUG). September 1, 2017 was selected on the **basis** of the approval date of VUR indication for SonoVue in EU (August 2017). The reason for any exclusion for each subject screened will be documented in the Subject Screening/Enrollment Log including cases of imaging procedure failure.

This rule for patient enrolment will be the same for all sites and therefore will ensure that the period of enrolment will be similar for both VUS and VCUG groups.

9.10 Other aspects

9.10.1 Protocol Amendment

No change to the protocol may be made without the joint agreement of both the Investigator and Sponsor. Any amendment to the original protocol will be signed by both parties and submitted to the IRB/EC/ Member States for approval or notification prior to implementation except in circumstances when it is necessary to implement urgent safety measures to remove an immediate hazard to the study subject(s).

9.10.2 Protocol Deviations, Violations and Exceptions

As a matter of policy, Bracco will not grant exceptions to protocol-specific entry criteria to allow subjects to enter a study. If investigative center personnel learn that a subject who did not meet protocol eligibility criteria was entered in the study they must immediately inform Bracco.

If a violation is serious and meets the definition of a serious breach, the CRM takes appropriate action according to the requirements and timelines stated in the Code of Federal Regulations, European Guidance and local regulations as applicable.

9.10.3 Curriculum Vitae

The Investigator and any sub-investigator(s) must provide Sponsor with current copies of their own signed and dated curriculum vitae.

9.10.4 Change in Investigator

In the event that the Investigator is unable to continue the study, another suitable person at the site will become the designated Investigator, and documentation testifying to this will be submitted to Sponsor. The new Investigator must be acceptable to both Sponsor and the IRB/EC before the study can be continued.

9.10.5 Financial Disclosure

Financial support to Investigators/Sub-investigators other than the cost of conducting the clinical study or other clinical studies will be disclosed where applicable in accordance with the EU Guidelines and Title 21 CFR 54.2 to 54.6, as well as according to requirements of FDA Physicians Payment Sunshine Act, EFPIA HCP/HCO Disclosure Code.

9.10.6 Financing

A financial agreement (separate from the protocol) will be made with the Investigator or designee. Such agreement will be archived in the relevant file.

9.10.7 Definition of the End of the Study

The end of the study is defined as the medical charts review conducted by the Investigator of the last subject who qualifies for the study.

10 Protection of human subjects

10.1 Ethical and Regulatory Compliance

The study will be conducted in accordance with the protocol, quality standards, ICH guidelines on Good Clinical Practice, FDA and EU guidelines, ethical principles that have their origin on the Declaration of Helsinki and all applicable local regulations, whichever offers greatest protection for the subject.
To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation by authorized individuals, and regulatory authorities.

10.2 Institutional Review Board/Ethics Committee/Research Ethic Board Approval

The protocol must be reviewed and approved by an appropriately constituted IRB/EC, as required in chapter 3 of the ICH E6 Guideline, and as applicable, Title 21 CFR 56.107 through 56.115. Written IRB/EC approval must be obtained by Sponsor before collecting any information on the subject.

The Investigator is committed in accordance with local requirements to inform the IRB/EC of any emergent problem and/or protocol amendments.

10.3 Written Informed Consent

Informed consent process is not feasible or practical for this study given that the research involves no risk to the subject because of the retrospective nature of the study, and that the period of subject inclusion can start from **site initiation** and can date back chronologically until September 1, 2017. However, the Sponsor won't have access to subject identifying information during the data collection process (with the exception of on-site study monitoring conducted by designated personnel). Only a de-identified case ID number will be linked to subject data. Based on this secure and confidential approach, an ICF waiver will be requested from the IRB/ EC.

Every effort will be made to protect subjects' rights to privacy in this study. Although Investigators must make data available to regulatory authorities as applicable, this information will be reviewed in the strictest of confidence and for auditing/inspection purposes only.

Should results from this research be published for scientific purposes, subjects' identities will not be disclosed.

11 Management and reporting of adverse events/adverse reactions

All adverse events which occurred during the realisation of the examination have to be reported.

12 Plans for disseminating and communicating study results

12.1 Information Material

The protocol and CRF are confidential communications of Sponsor. Acceptance constitutes the agreement by the recipient that no unpublished information herein contained will be published or disclosed without Sponsor's prior written approval except that this document may be disclosed to appropriate IRB/EC/Member State as long as they are required to keep it confidential.

12.2 Study Results

A final report of the study results will be written by Sponsor or its designee.

The Clinical Study Report (CSR) will be written within one year of the end of the notification of the study and sent to the involved ECs and Member States. It will be reviewed and approved by the Investigator when required by local authorities.

12.3 Use and Publication of Study Results

All unpublished documentation (including the Protocol and the Case Report Form) given to the Investigator is strictly confidential. All recipients must agree not to disclose the information herein contained to any person without the prior written authorization of Sponsor. The submission of these documents to the IRB/EC/Member States is expressly permitted. The Investigator agrees that Sponsor maintains the right to use the results of this study in their original form and/or in a global report for submission to governmental, competent, and regulatory authorities of any country.

The results of the study may be presented during scientific symposia or published in a scientific journal only after review by Sponsor in accordance with the guidelines set forth in the applicable publication or financial agreement.

13 References

1 Tekgül S, Riedmiller H, Hoebeke P, Kočvara R, Nijman RJ, Radmayr C, Stein R, Dogan HS, European Assoc. Urology. EAU guidelines on vesicoureteral reflux in children. Eur Urol. 2012; 62:534-42

2 Zimbaro G, Ascenti G, Visalli C, Bottari A, Zimbaro F, Martino N, Mazziotti S. Contrast-enhanced ultrasonography (voiding urosonography) of vesicoureteral reflux: state of the art. Radiol Med. 2007;112:1211-24

3 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:

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4 Montini G, Tullus K, Hewitt I. Febrile urinary tract infections in children. N Engl J Med. 2011; 365:239-250

5 Slabbaert K, Bogaert G. Vesicoureteric reflux (VUR) in children: where are we now? Arch Esp Urol. 2012;65:450-8

6 Unver T, Alpay H, Biyikli NK, Ones T. Comparison of direct radionuclide cystography and voiding cystourethrography in detecting vesicoureteral reflux. Pediatr Intl. 2006; 48:287-91.

7 Darge K. Voiding urosonography with ultrasound contrast agents for the diagnosis of vesicoureteral reflux in children. I. Procedure. Pediatr Radiol 2008;38:40-53

8 Piscaglia F, Nolsøe C, Dietrich CF, 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

9 Riccabona M, Vivier P-H, Ntoulia A, Darge K, Avni F, Papadopoulou F, Damasio B, et al. ESPR uroradiology task force imaging recommendations in paediatric uroradiology, 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

10 Newcombe RG (1988) Interval estimation for the difference between independent proportions: comparison of eleven methods. Statistics in Medicine 17:873-890.

Annex 1. List of stand-alone documents

Number	Document reference number	Date	Title
1	V3_05March2019	05 March 2019	Clinical Trial BR1-145 Preliminary list of potential investigative sites

Annex 2. ENCePP checklist for study protocols

Study title:
An Observational Study of SonoVue®/Lumason®-Enhanced Urosonography in Paediatric Subjects with Known or Suspected Vesicoureteral Reflux

Study reference number:
BR1-145

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[a]	☒	☐	☐	6
1.1.2 End of data collection[b]	☒	☐	☐	6
1.1.3 Study progress report(s)	☐	☐	☒	
1.1.4 Interim progress report(s)	☐	☐	☒	
1.1.5 Registration in the EU PAS register	☒	☐	☐	**1**
1.1.6 Final report of study results.	☒	☐	☐	6

Comments:

1.1.5 Registration in the EU PAS register will be obtained before the study start

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Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:				
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7
2.1.2 The objective(s) of the study?	☒	☐	☐	8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	9.2.1
2.1.4 Which hypothesis(-es) is (are) to be tested?	☒	☐	☐	9.1.1
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

None

[a] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

[b] Date from which the analytical dataset is completely available.

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, new or alternative design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☐	☐	☒	
3.3 Does the protocol specify measures of occurrence? (e.g. incidence rate, absolute risk)	☐	☐	☒	
3.4 Does the protocol specify measure(s) of association? (e.g. relative risk, odds ratio, excess risk, incidence rate ratio, hazard ratio, number needed to harm (NNH) per year)	☐	☐	☒	
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☒	☐	☐	11

Comments:

None

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	9.2.1
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period?	☒	☐	☐	9.2.2
4.2.2 Age and sex?	☒	☐	☐	9.2.1
4.2.3 Country of origin?	☐	☐	☒	
4.2.4 Disease/indication?	☒	☐	☐	9.2
4.2.5 Duration of follow-up?	☒	☐	☐	9.1
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	9.2

Comments:

None

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☐	☐	☒	
5.2 Does the protocol address the validity of the exposure				

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
measurement? (e.g. precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure classified according to time windows? (e.g. current user, former user, non-use)	☐	☐	☒	
5.4 Is exposure classified based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	

Comments:

Not applicable, PAES retrospective study.

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	9.1.1
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.1.1
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, prospective or retrospective ascertainment, use of validation sub-study)	☐	☐	☒	
6.4 Does the protocol describe specific endpoints relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease, disease management)	☐	☐	☒	

Comments:

None

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol describe how confounding will be addressed in the study?	☐	☐	☒	
7.1.1. Does the protocol address confounding by indication if applicable?	☐	☐	☒	
7.2 Does the protocol address:				
7.2.1. Selection biases (e.g. healthy user bias)	☒	☐	☐	9.2.1
7.2.2. Information biases (e.g. misclassification of exposure and endpoints, time-related bias)	☐	☐	☒	
7.3 Does the protocol address the validity of the study covariates?	☐	☐	☒	

Comments:

None

Section 8: Effect modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☐	☐	☒	

Comments:

None

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview, etc.)	☒	☐	☐	9.4
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics, etc.)	☒	☐	☐	9.4
9.1.3 Covariates?	☐	☐	☒	
9.2 Does the protocol describe the information available from the data source(s) on:				
8.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☐	☐	☒	
8.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☐	☐	☒	
8.2.3 Covariates? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, life style, etc.)	☐	☐	☒	
9.3 Is a coding system described for:				
9.3.3 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC)Classification System)	☐	☐	☒	
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD)-10, Medical Dictionary for Regulatory Activities (MedDRA))	☐	☐	☒	
9.3.3 Covariates?	☐	☐	☒	
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☐	☒	

Comments:

Source documentation for this retrospective study may consist of one or more elements of the following for each subject: Medical Records, Clinical Charts, Diagnostic Reports associated with Medical Images, Surgery/Endoscopy Reports, Administrative Data Records, Flow sheets, Progress Notes, Laboratory Reports.

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Is the choice of statistical techniques described?	☒	☐	☐	9.7

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.2 Are descriptive analyses included?	☒	☐	☐	9.7
10.3 Are stratified analyses included?	☐	☐	☒	
10.4 Does the plan describe methods for adjusting for confounding?	☐	☐	☒	
10.5 Does the plan describe methods for handling missing data?	☒	☐	☐	9.7
10.6 Is sample size and/or statistical power estimated?	☒	☐	☐	9.5

Comments:

None

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9.8.5 9.8.6
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8
11.3 Is there a system in place for independent review of study results?	☐	☐	☒	

Comments:

None

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☐	☐	☒	
12.1.2 Information bias?	☐	☐	☒	
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods)	☐	☐	☒	
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure, duration of follow-up in a cohort study, patient recruitment)	☒	☐	☐	9.1.1

Comments:

Limitations have been described together with measures to minimize potential bias in Section 9.9

Section 13: Ethical issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional	☒	☐	☐	10

Section 13: Ethical issues	**Yes**	**No**	**N/A**	**Section Number**
Review Board been described?				
13.2 Has any outcome of an ethical review procedure been addressed?	☒	☐	☐	10
13.3 Have data protection requirements been described?	☒	☐	☐	10

Comments:

None

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	9.10.1 9.10.2

Comments:

None

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	12.2
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	12.3

Comments:

None

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Global Medical & Regulatory Affairs
Bracco Imaging spa

Date:

Signature:

Annex 3. Additional information

None