DIVISION OF MEDICAL IMAGING PRODUCTS
Clinical Review of NDA 203684 Supplement
Bracco Diagnostics
Characterization of Focal Liver Lesions
Medical Reviewer: Scheldon Kress, M.D.
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1. Regulatory Action

Regulatory Recommendations

Bracco Diagnostics Inc. ("Sponsor") provided clinical evidence that intravenously administered Lumason (sulfur hexafluoride lipid-type A microspheres) is an ultrasound contrast agent (b) (4) characterization of focal liver lesions (FLLs) during ultrasonography in adult and pediatric patients. This reviewer recommends approval of this application.

Bracco's proposed indication wording is as follows:

> "Lumason is an ultrasound contrast agent indicated for use in adult and pediatric patients, (b) (4) characterization of focal liver lesions"

The recommended dose for adults is 2.4 mL for the (b) (4) indication. A second 2 (b) (4) mL dose could be injected in case of (b) (4). The clinical team recommends the 0.03 ml/kg pediatric dose for liver lesion characterization in the pediatric population. Even though it may be difficult to accurately measure small doses for younger patients, the wide range of safety observed with larger doses would overcome any minor dosing errors.

Of note, this drug's trade names 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.

Efficacy Conclusions from Studies BR1-128 and BR1-130 in Adults

To support the indication for the use of Lumason in focal liver lesion characterization during ultrasonography of the liver in adults, the Sponsor has completed two identical, independently conducted Phase III clinical studies (BR1-128 and BR1-130).

Results from BR1-130 – Confirmatory Study
Both readers 2 and 3 demonstrated superiority for both sensitivity and specificity in characterization of FLLs by SonoVue-enhanced ultrasound versus unenhanced ultrasound (p-

value <0.0001). Both readers satisfied the expected superiority requirement for CE-US assessment of liver lesion nature (malignant/benign) compared to UE-US.

For reader 2, CE-US increased sensitivity to 76% compared to UE-US of 35%, and for reader 2, CE-US increased specificity to 83% compared to UE-US of 54%. For reader 3, CE-US increased sensitivity to 92% compared to UE-US of 16%, and for reader 3, CE-US increased specificity to 73% compared to UE-US of 22%.

Results from BR1-128 – Supportive Study
Only reader 2 demonstrated superiority for both sensitivity (p-value <0.0011) and specificity (p-value <0.0001) in characterization of FLLs by SonoVue-enhanced ultrasound versus unenhanced ultrasound. Reader 2 satisfied the expected superiority requirement for CE-US assessment of liver lesion nature (malignant/benign) compared to UE-US.

For reader 2, CE-US increased sensitivity to 61% compared to UE-US of 41%, and for reader 2, CE-US increased specificity to 67% compared to UE-US of 7%.

Results from the paired off-site reads served to strengthen the usefulness CE-US in characterizing FLLs. Routinely, in clinical practice, CE-US will be evaluated in conjunction with UE-US and all available clinical information and additional imaging results

Efficacy Conclusions from Pediatric Patients (Literature –Based)

There are no Bracco-sponsored studies in the pediatric population. To support the indication for the use of Lumason in focal liver lesion characterization during ultrasonography of the liver in pediatric patients, the Sponsor provided a review of the scientific literature with specific reference to efficacy and safety of liver lesion characterization in the pediatric population.

Currently there is limited Lumason-enhanced US data available on dosing among the pediatric population. Bracco has a currently outstanding PMR to evaluate the echocardiography dosing of Lumason among patients 9-17 years of age for characterization of left ventricular endocardial border. (b) (4)
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Bracco proposed two Pediatric Doses for FLL characterization in the pediatric population:

(b) (4)

2. 0.03 mL/kg for (b) (4) liver imaging

DMIP prefers utilization of the 0.03 ml/kg as the pediatric dose for liver lesion characterization in the pediatric population. This is the equivalent weight based dose for a 70 kg adult. Based on review of the limited available efficacy data (95 pediatric patients) and safety data (> 900 pediatric patients) from children ages 2 months to 18 years, the clinical team believes that the 0.03 ml/kg pediatric dose for liver lesion characterization in the pediatric population is acceptable.

DPMH questioned the adequacy of justification for a proposed pediatric dose based on available data and recommended one or more pediatric clinical studies to determine pediatric dose and obtain more safety data.

DMIP is actively obtaining consultation with 2 SGEs (pediatric radiologists) to evaluate the indication for the use of Lumason in focal liver lesion characterization during ultrasonography of the liver in pediatric patients. The consultants are being queried regarding the adequacy of the efficacy and safety data as well as the recommended dose to be included in the forthcoming prescribing label.

Benefit/Risk Assessment

Bracco provided an Updated Safety report in May 2015 with a cut-off date for data as of September 30, 2013. Administration of Lumason, similar to other intravenously administered microspheres, has the potential to be associated with the rare immediate onset of serious life-threatening anaphylactic and anaphylactoid reactions. Both serious adverse events and adverse events with a fatal outcome either related or not to Lumason administration, occurred infrequently. The data provided within this safety update did not reveal any significant increase in the incidence of intravenous Lumason-related serious life-threatening events among adults.

The safety data provided following intravenous administration of Lumason within the pediatric population is limited. Bracco did not conduct any controlled clinical pediatric studies with intravenous Lumason. Bracco provided safety data from >900 pediatric patients administered intravenous Lumason derived from the literature to support a safety pattern similar to that observed among adults.

Among the >900 reported administrations to pediatric patients, one adverse event of severe anaphylactic shock was reported in 11-year-old girl. Event was potentially life-threatening and considered to be related to the administration of SonoVue (0.6 mL). After supportive therapy all symptoms resolved by 2 hours.

The observed safety profile of Lumason use among adults and children to characterize FLLs remains consistent with the known safety profile evaluated during the 2014 NDA approval review for transthoracic echocardiography. Thus, benefit/risk assessment of intravenously administered Lumason demonstrates that the diagnostic benefits outweigh the potential risk,

2. Background

Primary Liver Cancer and other Focal Liver Disease

Primary liver cancer is the fifth most common cancer in men (7.5% of the total in 2012), the ninth most common in women (3.4% of the total in 2012) and the second most common cause of death from cancer worldwide. The American Cancer Society (ACS) estimates that, in 2015 in the USA, 35,660 new cases of cancer of liver and intrahepatic bile duct will be diagnosed with more than 24,550 deaths from the disease. Liver may also be the site of metastasis from virtually any primary cancer and represents the second most commonly involved organ in metastatic

disease. Based on autopsy studies in Japan and the USA, up to 40% of patients with an extra-hepatic primary tumor have hepatic metastases. Benign focal liver lesions are even more common than malignant tumors, occurring in more than 5% to 10% of the general population, with hemangiomas and simple cysts, to a large extent, responsible for this extremely high incidence.

Although FLLs detected in asymptomatic healthy subjects are likely to be benign whereas FLLs in patients with history of cancer have higher probability of being malignant, malignant lesions can be found in asymptomatic subjects and benign lesions can be seen in oncology patients. Therefore, an accurate and reliable assessment of the nature of FLLs is critical, not only to reassure patients with benign lesions but also, and more importantly, to ensure that malignant lesions are diagnosed correctly.

Focal liver lesions (FLLs) are not only seen in adult subjects, but can be also encountered, even if less frequently, in children and adolescents. Pathologic masses of the liver in children include primary neoplasms, metastatic lesions from distant malignancies such as neuroblastoma, lymphoma or Wilm's tumor, inflammatory masses, and cysts (congenital or acquired). Overall, primary liver tumors are rare in children, with approximately 100 to 150 new cases of primary liver tumors diagnosed in the United States annually, according to the sponsor. Malignant liver tumors account for 1.1% of all pediatric malignancies, with hepatoblastoma comprising over two-thirds of liver malignancies in children and hepatocellular carcinoma accounting for most of the remaining cases. Most patients with hepatoblastoma are younger than 4 years of age at diagnosis, while hepatocellular carcinoma occurs primarily after 10 years of age. The most common benign primary tumor is a congenital form of hepatic hemangioma (or infantile hemangioendothelioma), followed by mesenchymal hamartoma, especially in toddlers, and focal nodular hyperplasia. Hepatic adenoma is almost exclusively a disease of older children; primary hepatic teratoma is exceedingly rare.

Ultrasonography (US) is a particularly useful study in a child with a suspected abdominal mass, as there is no ionizing radiation and no need for sedation.

Lumason – Regulatory History

Lumason was approved within the USA in 2014 as an ultrasound contrast agent indicated for use in adult patients with suboptimal echocardiograms to opacify the left ventricle chamber and to improve the delineation of the left ventricular endocardial border.

Lumason is approved outside the USA for use in adults for characterization of FFLs under the brand name of SonoVue; it is not approved for use in pediatric patients in any country. Lumason is administered as an intravenous injection. Lumason does not diffuse into the extravascular compartment, but remains within the blood vessels until the gas dissolves in the blood (with maximum concentrations occurring within 1 to 2 minutes after a single administration), and is rapidly eliminated in expired air.

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 (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 x10^8 microspheres/mL with 45 mcg/mL of sulfur hexafluoride. (b) (4) of the total lipids in the suspension are associated with the microspheres.

Rationale for Use of Lumason in the Characterization of Focal Liver Lesions

Lumason microspheres have a mean diameter of about 2.5 µm, with (b) (4)% having a diameter less than 6 µm and (b) (4)% having a diameter less than 11 µm. Due to their size, they can cross the lung bed to produce systemic enhancement after intravenous injection. The interface between the SF_6 microsphere and the blood acts as a reflector of the ultrasound beam, thus enhancing blood echogenicity; as Lumason microspheres cannot diffuse out of the circulation, they selectively increase contrast between the blood and the surrounding tissues and allow depiction of liver masses and/or liver parenchyma.

The intensity of the reflected signal is dependent on concentration of the microbubbles and frequency of the ultrasound beam. The response of the Lumason microspheres to an ultrasound beam is complex because gases are much more compressible than soft tissue and therefore, when exposed to the compression–rarefaction sequence of an ultrasonic pulse, they undergo alternate contraction and expansion. They vibrate most readily at a particular frequency, their resonance frequency, and for those <7 µm in diameter ((b) (4)% of the Lumason microspheres), this corresponds to the frequencies actually used in diagnostic ultrasound (2–10 MHz); as a result, they return very much stronger signals than similarly-sized tissue reflectors such as red blood cells.

With low acoustic powers, symmetrical oscillations occur and the frequency of the scattered signals is the same as the transmitted pulse. However, at higher acoustic powers, the expansion and contraction phases become unequal because the microspheres resist compression more strongly than expansion. This response is said to be "non-linear" and the returning signals contain multiples of the insonating frequency. These higher frequency components are known as

harmonics since the phenomenon is identical to the overtones produced by a musical instrument.

Harmonics may be used to image US contrast agents by passing the signals through a low pass filter that removes the fundamental signals. However, tissues also produce harmonics (formed in a different way, as the ultrasound beam propagates), especially when higher acoustic powers are used, and there is the need to distinguish between them.

Lumason harmonics can be completely separated from tissue harmonics since, with its phospholipid-shelled microspheres, harmonics can be elicited at much lower acoustic powers than are necessary to generate tissue harmonics. Thus, only the microspheres produce harmonics, and these can be separated from the tissue signals using contrast-specific techniques. For instance, using phase inversion mode, a pair of pulses is transmitted along each scan line, the second being phase-inverted. The returning echoes are summed, thus cancelling the linear echoes because they are out of phase, leaving only the non-linear signals from microsphere harmonics to form the image.

The normal liver parenchyma has a dual blood supply, from the hepatic artery and from the portal vein; liver tumors get most of their blood supply only from the hepatic artery. Following intravenous injection, the Lumason microbubbles cross the lung bed, move into the systemic circulation and reach the hepatic artery vascular supply first and then the portal venous circulation. The SF_6 microspheres cannot diffuse out of the circulation and selectively enhance blood echogenicity.

Due to the dual blood supply, the major phases of vascular enhancement can be observed and used to improve characterization of FLLs (**Table 1**):

Table 1: Vascular Supply of Normal Liver

Vascular Phases	Arterial	Portal Venous	Kupffer Phase Late Portal Washout
Starting	20 seconds	30-45 Seconds	
Duration	10-15 Seconds	2 Minutes	4-6 Minutes
Blood Supply	25%–30%	70%–75%	

In both adults and pediatric patients, contrast-specific ultrasound techniques have been used to exploit the above described nonlinear acoustic effects of the Lumason microspheres and provide high resolution images of tissue vascularization as liver tumors show characteristic and specific vascular patterns during the three phases of liver perfusion, from the early arterial phase to the late-portal-washout phase. **Table 2** demonstrates the typical distinguishing visual characteristics of Lumason microspheres during CE-US arterial, portal venous and late washout for malignant and benign FLLs.

In contrast to benign vascular patterns, malignant lesions demonstrate distorted and hyperechoic vessels during the arterial phase and hypoechoic late phase vascularity due to the destruction of Kupffer cells (macrophages) lining the sinusoids. The “late phase”, i.e., an extension of the

portal venous phase may be useful to characterize FLLs, since malignant lesions show a faster washout of contrast and become hypointense whereas benign lesions tend to appear isoechoic or slightly hyperechoic in the late phase (increased macrophage activity).

Table 2 - CE-US Vascular Supply Pattern of Focal Liver Lesions

Vascular Phases	Arterial	Portal Venous	Kupffer Phase Late Washout
Starting	20 seconds	30-45 Seconds	
Duration	10-15 Seconds	2 Minutes	4-6 Minutes
Blood Supply	25%–30%	70%–75%	
Benign	O O O O O O O O O O O O O O O	O O O O O O O O O O O O O O O	O O O O O O O O O O O O O O O O O O O
Malignant	O O O O O O O O O O O O O O O O O O	O O O O O O O O O O O O O O	O O O O

Clinical Usefulness of CE-US for Imaging Liver Lesions

Characterization of focal liver lesions (FLLs) is a common problem in everyday clinical practice, since liver can be the site of both malignant and benign lesions in patients with a history of cancer or in patients with chronic liver disease as well as in patients who are asymptomatic.

Characterization of FLLs at imaging is based on the assessment of morphology and vascularity characteristics of the lesion. To this aim, CT and MRI examinations of the liver are performed with contrast and with a multiphasic imaging acquisition (i.e. repeated acquisition of images at predefined interval times after the administration of the contrast agent), in order to capture the enhancement of the lesion during the hepatic arterial, portal venous, and late phases.

Characterization of FLLs and differentiation between benign and malignant lesions is then performed based on the degree of vascularity and the enhancement pattern of the lesion relative to the liver parenchyma. In the presence of typical imaging findings, CT and MRI show high sensitivity and specificity for FLLs characterization. Appropriate imaging acquisition protocols with rapid scanning and accurate selection of the acquisition timing are key factors for optimizing FLLs characterization with CT and MRI.

The clinical usefulness of CE-US is acknowledged by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) in its guidelines and recommendations on the use of contrast-enhanced ultrasound in the liver which were originally issued in 2004 and subsequently updated in 2008 and 2012. The 2012 update was jointly prepared by EFSUMB and the World Federation for Ultrasound in Medicine (WFUMB) and saw the participation of representatives of other scientific societies including the American Institute of Ultrasound in

Medicine (AIUM). These guidelines and recommendations were developed with the aim of providing standard protocols for the use and administration of ultrasound contrast agents in liver applications on an international basis and improving the management of patients worldwide.

According to the above reported recommendations, CE-US represents the first imaging modality in the diagnostic work-up of FLLs, when unenhanced ultrasound is not conclusive, for diagnosis of lesions or suspected lesions identified on a background of chronic hepatitis and liver cirrhosis or in patients with a known history of malignancy, as well as for diagnosis of incidental findings; CE-US is also recommended as an alternative to CE-CT or CE-MRI in patients with contra-indications to CE-CT or CE-MRI.

Ultrasound is the most readily available technique for liver imaging and is commonly performed in patients with known or suspected hepatic disease because of its relative low cost, safety, and patient acceptance. Gray-scale unenhanced ultrasound and color Doppler imaging, although useful for differentiating solid from cystic lesions, have a limited role in the characterization of FLLs because of the similar appearance and vascular architecture of malignant and benign lesions. In general, the accuracy of unenhanced ultrasound for FLL characterization is poor (sensitivity range is 28-60%; specificity range is 32-37%) and patients are frequently referred for further imaging procedures such as CT or MRI.

Contrast-enhanced ultrasound overcomes the limitations of unenhanced ultrasound by providing real-time assessment of dynamic enhancement and vascularity of FLLs during the triphasic evaluation - arterial, portal venous, and late phase. Similar to contrast-enhanced CT and MRI, the portal and late venous phases provide information about the washout of the contrast agent from the lesion compared to surrounding liver tissue, and are particularly useful for characterization of malignant versus benign lesions. A progressive contrast washout during the portal phase leading to hypo-enhancement of the lesion in the late phase characterizes malignant lesions, whereas solid benign lesions typically show persistent enhancement in the portal and late phase with hyper- or iso- echoic appearance relative to the adjacent liver. The hypoechoic late phase vascularity observed with malignant lesions results from the destruction of Kupffer cells (macrophages) lining the hepatic sinusoids.

The introduction of contrast-enhanced ultrasound may offer improved diagnostic performance of ultrasonography in characterization of FLLs comparable to those of contrast-enhanced CT and contrast-enhanced MRI. When compared to CT and MRI, contrast-enhanced ultrasound has the major advantage of allowing continuous and real-time imaging of lesion vascularity; this may be useful to visualize a very early or late enhancement of the liver lesion which may be missed at CT or MRI because of the predefined timing of acquisition. Contrast-enhanced ultrasound also has the potential advantage of shortening the diagnostic workup of patients with a newly detected focal liver lesion by avoiding the need for re-scheduling the patients for additional examinations. Considering that the majority of FLLs incidentally identified in asymptomatic subjects are benign, this could avoid unnecessary extensions of the diagnostic workup, further reducing waiting times or length of hospital stay and, consequently, costs.

To aid in the assessment of ultrasound images of FLLs, the readers were provided with detailed algorithms to assist in distinguishing malignant and benign FLL patterns for both UE-US and CE-US images. These differential charts are provided in **APPENDIX I**.

3. Clinical Development Program

Clinical Efficacy Supportive Studies

Bracco Diagnostics Inc. ("Sponsor") provided clinical efficacy studies in support of an indication for Lumason™ (sulfur hexafluoride lipid-type A microspheres) to improve characterization of focal liver lesions (FLLs) during ultrasonography of the liver. Bracco's proposed indication wording is as follows:

> "Lumason is an ultrasound contrast agent indicated for use in adult and pediatric patients, (b) (4) characterization of focal liver lesions"

To support the indication for the use of Lumason in focal liver lesion characterization during ultrasonography of the liver in adults, the Sponsor has completed two identical, independently conducted Phase III clinical studies (BR1-128 and BR1-130). Both studies are titled:

"Characterization of Focal Liver Lesions with SonoVue-Enhanced Ultrasound Imaging: A Phase III, Intra-patient Comparative Study versus Unenhanced Ultrasound Imaging Using Histology or Combined Imaging/Clinical Data as Truth Standard."

The two clinical studies were conducted in a total of 677 patients at 34 clinical sites in the United States and Europe between September 2009 and July 2013.

Overview of Clinical Efficacy Supportive Evidence

This Efficacy Supplement Summary presents supportive evidence for the use of Lumason in focal liver lesion characterization in adults:

- A description of the critical study features of and efficacy results from the two well controlled clinical studies, BR1-128 and BR1-130;
- Efficacy results within subpopulations in BR1-128 and BR1-130 by demographic variables for each of the two studies and for the integrated population from the two studies;
- Supportive Bracco-sponsored studies of SonoVue in the characterization of focal liver lesions in adults
- A review of the peer-reviewed literature reporting studies of SonoVue, not conducted by the Sponsor, that address the proposed indication;
- An overview of the efficacy of SonoVue in the pediatric population based on reported literature studies of SonoVue.

The two phase III studies conducted in adults in support of this application were designed and

conducted based on guidance given by the FDA Division of Medical Imaging Products ("the Division") to Bracco Diagnostics, Inc. ("the Sponsor"). The primary efficacy endpoint of the two studies, i.e., the characterization of lesions as benign (specificity) or malignant (sensitivity), was prospectively defined and agreed upon with the FDA.

Reference is made to the following communications with FDA about the Sponsor's plan to file a regulatory submission for use of Lumason for a liver indication based on the results from the two Phase III studies:

- Meeting package submitted 26 September 2014 by the Sponsor to the DMIP in advance of a meeting scheduled for 28 October 2014. The purpose of the meeting was to discuss and receive feedback on the results of the two completed Phase III studies and the appropriateness of documentation to support a regulatory submission for the use of Lumason in focal liver lesion (FLL) characterization during ultrasonography of liver.
- Meeting preliminary comments from the DMIP received by the Sponsor on 23 October 2014.
- The Agency's comments and input were addressed, including the additional analyses requested and submission of site level listings requested relevant to Bioresearch Monitoring Program (BIMO) inspections.

Clinical Confirmatory Studies in the Characterization of Focal Liver Lesions

Primary Efficacy Endpoints - Intent-to-Diagnose [ITD]
Characterization of Lesions as Malignant

1) Sensitivity of SonoVue-enhanced ultrasound is superior as compared to unenhanced ultrasound for at least 2 of the 3 off-site assessors analyzing their data separately.
2) Specificity of SonoVue-enhanced ultrasound is superior as compared to unenhanced ultrasound for at least 2 of the 3 off-site assessors analyzing their data separately.
3) Sensitivity and specificity are both superior in the same reader.

Study BR1-128 was conducted in clinical sites in the USA (225 patients) and Italy (15 patients) in patients at least 18 years of age with at least 1 focal liver lesion (target lesion) requiring work-up for characterization. In total, 337 patients received SonoVue; this included 74 patients enrolled in the training phase of the study, and 263 enrolled in the efficacy phase of the study. As prospectively defined, the 263 patients enrolled in the efficacy phase were included in the blinded read. Among these 263 patients, 240 comprised the ITD population, i.e., had off-site ultrasound evaluations available and a definite final diagnosis from truth standard; 124/240 (52%) patients had malignant lesions and 116/240 (48%) had benign lesions.

Study BR1-130 was conducted in clinical sites in the USA (132 patients), Canada (34 patients), France (1 patient), and Germany (92 patients) in patients at least 18 years of age with at least 1 focal liver lesion (target lesion) requiring work-up for characterization. In total, 340 patients received SonoVue; this included 67 patients who were enrolled in the training phase, and 273 enrolled in the efficacy phase of the study. As prospectively defined, these 273 patients were included in the blinded read. Among the 273 patients, 259 comprised the ITD population. Based

on the truth standard, 119/259 (46%) patients had malignant lesions and 140/259 (54%) had benign lesions.

Phase III Clinical Studies in the Characterization of Focal Liver Lesions

Study Objectives

Primary Objective of both studies BR1-128 and BR1-130 was to demonstrate that the sensitivity and specificity of SonoVue-enhanced ultrasound for the characterization of benign versus malignant focal liver lesions (FLLs) are superior to sensitivity and specificity of unenhanced ultrasound, using final diagnosis based on histology or combined imaging (contrast-enhanced computed tomography [CE-CT] and/or contrast-enhanced magnetic resonance imaging [CE-MRI]/clinical data as truth standard).

Secondary Objectives of the studies were:

- to evaluate the accuracy and other performance parameters (positive predictive value [PPV], negative predictive value [NPV]) of SonoVue-enhanced ultrasound for characterization of benign versus malignant FLLs in comparison to unenhanced ultrasound;
- to evaluate the ability of SonoVue-enhanced ultrasound to obtain a specific diagnosis of FLLs in comparison to unenhanced ultrasound;
- to evaluate the inter-reader agreement in ultrasound image assessment (unenhanced and SonoVue-enhanced separately); and to provide evidence of the safety and tolerability of intravenously administered SonoVue in subjects with focal liver disease.

Ethical and Regulatory Aspects

Both studies were in strict compliance with regulatory guidance and recommendations of current regulatory agencies and professional advisory bodies for clinical studies in liver disease.
Both studies were designed and conducted in accordance with the ethical principles and the scientific quality standards as outlined in the International Conference on Harmonization (ICH) E6 Good Clinical Practice: Consolidated Guideline, the recommendations from the American Institute of Ultrasound in Medicine (AIUM) and Part 3: Design, Analysis and Interpretation of Clinical Studies from the FDA Guidance for Industry: Developing Medical Imaging Drug and Biological Products. This included:

- Institutional Review Board (IRB) or Ethics Committee (EC) approval of the protocol was obtained from each site prior to the initiation of each study.
- Written informed consent was obtained from each subject before any study procedures were performed.
- The two studies were designed as within-subject comparisons in which Lumason enhanced and unenhanced images were assessed in the same subject.
- Subjects included in the studies were representative of the population in which the diagnostic agent is intended to be used, i.e., subjects who required further work-up for liver lesion characterization, but who may not have been necessarily selected based on a screening ultrasound.

- All ultrasound examinations in the study, both unenhanced and contrast-enhanced, were performed by qualified personnel (sonographer/MD) experienced in liver ultrasound.
- A predetermined randomization order was used for the presentation of ultrasound images for evaluation by the blinded readers.
- The diagnosis obtained with each of the ultrasound procedures by each of the 3 off-site assessors in each study were compared with the final diagnosis based on a truth standard;
- The contrast agent used for the CE-CT and CE-MRI truth standards was an FDA approved contrast agent for liver imaging for the modality.
- The unenhanced and SonoVue-enhanced examinations were performed in the same session, using commercially available (FDA-approved) ultrasound systems with appropriate contrast-specific capabilities.

Statistical principles for the clinical trials were planned and performed in full accord with the ICH Topic E9, Note for Guidance on Statistical Principles for Clinical Trials, CPMP/ICH/363/96 and structure and content of the final clinical study reports are in accord with ICH Topic E3, Note for Guidance on Structure and Content of Clinical Study Reports, CPMP/ICH/137/95.

Study Design

Both studies were designed as Phase III, multicenter studies to assess the sensitivity and specificity of contrast-enhanced ultrasound with SonoVue for characterization of focal liver lesions in comparison with unenhanced ultrasound. The design is in accordance with the recommendations from the American Institute of Ultrasound in Medicine (AIUM)23 for clinical trials assessing the efficacy of contrast-enhanced ultrasound in liver imaging.
Each study was to be conducted at approximately 15 investigational sites. Before study initiation at each site, the sonographer/MD for the study in each center was to undergo specific training, including the performance of contrast-enhanced ultrasound examination for characterization of FLLs in up to 4 enrolled patients. These patients enrolled in the training phase of the study were to undergo all safety assessments planned in the study and were to be included in the safety population, but would not be included in the efficacy analyses.

Only those subjects enrolled in the efficacy phase (i.e., after completion of the training phase at each site) were included in the blinded read. Subjects who enrolled in the training phase would not be included in the blinded read. The blinded reads were conducted according to a prospectively defined methodology at an independent core laboratory (b) (4)). Blinded off-site reads of images were performed for each study by 3 independent board-certified radiologists. The readers were unaffiliated with any of the 111 investigational sites for the study in which they participated and were blinded to any patient clinical information and results of other diagnostic and imaging procedures. A different set of blinded readers was used in each of the two studies. The CT/MRI reader for each study was board-certified, was not affiliated with the study centers and was blinded to any clinical information about the subject or to the diagnosis obtained with CE-US.

Prior to the start of the blinded review sessions, the blinded readers underwent training for both the study-specific review of images and correct use of the computer applications used for the

readings. Proficiency testing was not required. In general, on-site and off-site data were analyzed separately and for the off-site assessments of the three blinded readers, each reader's assessment was analyzed separately.

Dose and Mode of SonoVue Administration

SonoVue, 2.4 mL dose, was administered intravenously as bolus injection into an upper extremity vein using a 20 gauge catheter or through a central venous catheter (internal jugular vein, subclavian vein) without an IV filter. Immediately following the SonoVue injection, 5 mL to 10 mL of saline was administered to flush the IV line of any remaining contrast agent.

A second 2.4 mL dose could be injected in case of technical failure of the first bolus (including but not limited to, e.g., malfunctioning of ultrasound machinery, needle with gauge smaller than required per protocol, wrong needle positioning, contrast medium extravasation, SonoVue administration rate too slow, incorrect image acquisition). An interval of 30 minutes was to follow a first SonoVue administration before administration of a second SonoVue dose.
A maximum of 2 injections of 2.4 mL of SonoVue was allowed. Two injections were utilized in 12 % of patients in Study 128 and 8 % of patients in Study 130.

The 2.4 mL dose is the recommended dose for the microvasculature indication in all countries where SonoVue is registered and it is the dose most commonly used in published clinical experience in this indication.

Patient Population

The patients enrolled in the two studies are representative of patients who would benefit from contrast-enhanced liver ultrasound and of those receiving ultrasound contrast in current clinical practice, i.e., patients with an indeterminate liver lesion requiring further work-up for characterization.

Enrollment Criteria

- Patients (>18 years of age)
- With an (one) indeterminate Liver Lesion (FLL)
- Scheduled for surgical removal or biopsy (24 hours to 30 days)
- Scheduled for CE-CT and/or CE-MRI (alternate)
- Unenhanced target lesion imaged at low MI (<0.4)
- Unenhanced target lesion located and mapped (Couinaud)
- CE-US performed immediately following U-US
- No controls utilized (no approved contrast agent)
- No placebo control utilized (saline not a valid imaging procedure)

These subjects were not necessarily selected based on a screening ultrasound, but had lesions that may have been incidentally detected, or had chronic hepatitis or liver cirrhosis, or a history of malignancy. Individuals with an acoustic window insufficient for adequate ultrasound

examination of the liver or an FLL that could not be identified with unenhanced ultrasound were not eligible for participation.

Ultrasound Image Acquisition

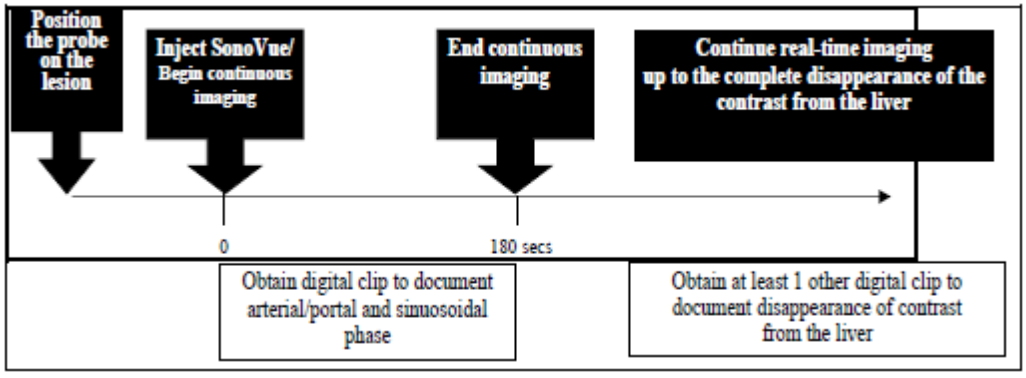
During the training phase at each center, a defined number of subjects were to undergo contrast-enhanced ultrasound for focal lesion characterization. At the end of the training phase, the trainer certified the technical qualification of the center and of the sonographer/MD at the center for participation in the study.

The target lesion was to be located and imaged using predefined liver maps to ensure that the same lesion was consistently examined as the target lesion on unenhanced and SonoVue-enhanced ultrasound (lesion tracking). Prior to SonoVue administration, gray scale and Doppler (color or power imaging) ultrasound investigations of the target lesion were to be performed using standard techniques (B-mode or harmonic imaging) to study the anatomy of the target lesion and surrounding parenchyma.

To guarantee that imaging acquisition and documentation were maintained at the predefined quality standards during the entire period in each study, qualified personnel selected by the Sponsor monitored participating centers on a regular basis. Any noncompliant center was excluded from further participation in the study [Note: no site was noncompliant in either study.]

Immediately after the unenhanced ultrasound evaluation, SonoVue-enhanced ultrasound was to be performed using contrast-specific imaging modes operating at a low mechanical index (MI) i.e., lower than 0.4. The contrast-enhanced ultrasound examination was to consist of the complete dynamic real-time assessment of the contrast enhancement profile of the lesion in comparison with surrounding liver parenchyma. During the contrast-enhanced ultrasound examination, the probe was positioned so as to provide optimal visualization over the target lesion and was kept in the same position for at least 180 seconds (**Figure 1**). Real-time imaging was used to periodically check for the complete disappearance of contrast from the liver.

Figure 1: SonoVue Imaging Methodology



In order to standardize the procedure as much as possible, all centers using the same model of ultrasound commercial system were to use the same release-of-contrast dedicated platform and

probe; the machine presets were to be defined and kept constant and used for all subjects tested. It was anticipated that several commercially available platforms made by several different manufacturers would be employed during this trial. A technical manual was provided to each study center, describing the procedure to be followed for obtaining an image, and noting that it was to be as standardized as possible. Unenhanced ultrasound and SonoVue-enhanced ultrasound images were recorded and sent to a central lab (b) (4) for storage and preparation for off-site assessment by 3 independent readers.

Ultrasound Image Review and Assessment

Unenhanced ultrasound and SonoVue-enhanced ultrasound images were assessed by both the onsite Investigator and independently by 3 off-site independent readers. In both on-site and off-site assessments, unenhanced and SonoVue-enhanced images were evaluated separately in one session and in matched pairs (unenhanced and SonoVue-enhanced images viewed together) when the separate image assessment was completed.

For the off-site evaluations, the images were presented to the readers by following the provided randomization order. As prospectively defined, only those subjects enrolled in the efficacy phase (i.e., after completion of the training phase) at each site were included in the blinded read; subjects who enrolled in the training phase were not to be included in the blinded read.

For unenhanced images, the on-site Investigators and off-site readers provided assessments of the same parameters, except where noted. These included:

- technical adequacy of the images
- lesion location identification as region of interest (ROI) (off-site only)
- lesion characteristics, including size and depth (on-site only), border definition, shape, echogenicity and vascularity (e.g., intra-lesional flow, peripheral flow) of the single target lesion
- Diagnostic Conclusion (i.e., benign, malignant or indeterminate) for the target lesion
- Detailed Diagnosis for the type of lesion: for *malignant*, the types were hepatocellular hemangioma, focal nodular hyperplasia (FNH), focal fatty sparing or change, regenerating nodule, simple cyst, adenoma or abscess or other/unable to determine Diagnostic Confidence as high (no other exam needed) or low (other exams needed).
- For SonoVue-enhanced images, the on-site Investigators and off-site readers provided Assessments of the same parameters, except where noted. These included:

    - technical adequacy of the images
    - lesion location identification as ROI (off-site only)
    - patterns of enhancement of contrast distribution during the 3 vascular phases
    - a diagnostic conclusion (i.e., benign, malignant or indeterminate) for the target lesion
    - a Detailed Diagnosis for the type of lesion: *for malignant*, the types were hepatocellular carcinoma (HCC), hypo- or hypervascular metastases, cystic metastases or cholangiocarcinoma or other/unable to determine; *for benign*, the types were hemangioma, focal nodular hyperplasia (FNH), focal fatty sparing or

change, regenerating nodule, simple cyst, adenoma or abscess or other/unable to determine
- Diagnostic Confidence.

The on-site Investigators also evaluated paired unenhanced and SonoVue-enhanced images for each subject and provided an assessment of the diagnostic quality of enhancement over unenhanced images (scores from 0 = no feature provided to help lesion characterization through 3=only contrast provided features to allow lesion characterization).

CE-CT/MR Image Acquisition

For subjects in which the final diagnosis was based on liver imaging CE-CT and/or CE-MRI as the truth standard, a contrast-enhanced multi-detector computed tomography (CE-MDCT) examination and /or CE-MRI using a gadolinium-based contrast agent was recommended. It was required that the contrast agent used for the CE-CT and CE-MRI be an FDA-approved contrast agent for liver imaging for that modality and the exam had to be performed within 30 days to 48 hours prior to or 24 hours to 30 days after the SonoVue-enhanced ultrasound examination. MRI acquisition was to include fast T2-weighted and T1-weighted gradient echo sequences. CE-CT and /or CE-MR images were to be acquired with at least arterial and portal venous phases. An equilibrium phase was to be acquired if indicated to make a diagnosis (e.g., hemangiomas). The examinations were to be recorded and sent to the central lab (b) (4)) for storage.

CE-CT/CE-MRI Image Assessment

For those cases in which CT/MR imaging was used as truth standard, the contrast-enhanced CT/MR images were to be assessed by the on-site Investigator and also off-site by an experienced independent reader not affiliated with the study centers and blinded to any clinical information about the subject or to the diagnosis obtained with contrast-enhanced ultrasound (CE-US). The CT/MR images were assessed for technical adequacy and the reader was to provide a diagnosis (malignant or benign), lesion type, and diagnostic confidence (high or low; only for on-site). If both CE-CT and CE-MR images were available, the blinded CT/MRI reader was to read them together.

Truth Standard

To measure the diagnostic performance of test procedures, the diagnosis obtained with each ultrasound technique (unenhanced ultrasound and SonoVue-enhanced ultrasound) was matched against the final diagnosis based on the truth standard.

In subjects with suspected HCC, consistent with the recommendations of the AASLD6, the truth standard was to be based on the following:

- For an FLL ≤1 cm in maximum diameter: only histology accepted.
- For an FLL of 1 to 2 cm in diameter: final diagnosis based on 2 imaging procedures (CE-CT and CE-MRI) performed within ±30 days of the SonoVue administration, showing

coincidental typical vascular pattern.

- For an FLL of >2 cm in maximum diameter: final diagnosis based on 1 imaging procedure (CE-CT or CE-MRI) provided that findings are typical for HCC and the procedure is performed within ±30 days of the SonoVue administration.

If the above criteria for truth standard were not met to provide a final diagnosis, the lesion was to be biopsied or, if biopsy was not performed, proof of malignancy at any time within the 6 months after SonoVue administration to show disease progression by CE-MRI or CE-CT would fulfill the requirement for truth standard. In addition, if tissue pathology/histology of the target lesion was obtained at any time during the 6 month follow-up window, this also fulfilled the requirement for truth standard. Subjects without proven malignancy had to be followed for a period of 6 months, at which time the lack of disease progression had to be documented by one of the approved modalities (i.e., surgical resection, biopsy, CE-MRI or CE-CT).

The final diagnosis from the truth standard was used to measure the diagnostic performance of ultrasound for both onsite and offsite data analyses.

Statistical Methods

Efficacy Analysis

General Methodology

Statistical significance was defined as $p < 0.05$ (two-tailed). In general, summary statistics (N, mean, median, standard deviation [SD], and range) were provided for continuous variables and the number and percentage of patients in each category were provided for categorical data. In general, on-site and off-site data were analyzed separately. In case of off-site assessments of the three blinded readers, each reader's assessment was analyzed separately. The unit of analysis was the lesion, equivalent to the subject, since each subject had a single lesion that was to be characterized. Statistical analyses were conducted using SAS® Version 9.2.

Analysis Populations

Analyses of study data were based on the following populations: safety, intent-to diagnose (ITD), per protocol and sensitivity analysis populations.

- All subjects who received SonoVue and enrolled during the training phase or efficacy phase are included in the safety population.
- The efficacy analysis population (intent-to-diagnose [ITD]) includes all subjects who received SonoVue and enrolled in the efficacy phase (i.e., after the end of the training phase), had a definite final diagnosis (benign or malignant) from the truth standard and had unenhanced and SonoVue-enhanced ultrasonography available.
- The per-protocol population includes ITD subjects without protocol violations.
- For the purposes of the sensitivity analysis, all subjects who received SonoVue and enrolled in the efficacy phase (i.e. after the end of the training phase) and had a definite diagnosis from the truth standard were included, and any missing ultrasound diagnosis

due to missing images was imputed as false negative (FN) for the positive truth standard diagnosis or false positive (FP) for the negative truth standard diagnosis.

Demographic and baseline characteristics, medical history and concomitant medications were presented for the safety population. Safety analysis was done for the safety population. Unless otherwise specified, all efficacy analyses were based on data from the ITD population.

Diagnostic Performance of Ultrasound

The diagnostic performance of ultrasound (unenhanced ultrasound, SonoVue-enhanced ultrasound, and paired unenhanced and SonoVue-enhanced ultrasound) was derived based on the ultrasound diagnosis and final diagnosis from the truth standard (**Table 3**).

All subjects with technically inadequate or indeterminate ultrasound images were considered as false positive (FP) or false negative (FN), based on the diagnosis from the truth standard.

Table 3: Cross Tabulation of Focal Liver Lesion Diagnosis: Truth Standard versus Ultrasonography

	Ultrasonography		
Truth Standard	**Benign**	**Indeterminate or Technically Inadequate**	**Malignant**
Benign	True Negative (TN)	False Positive (FP)	False Positive (FP)
Malignant	False Negative (FN)	False Negative (FN)	True Positive (TP)

- True positive (TP) is defined as a subject with the target lesion characterized as malignant based on both ultrasonography and the truth standard.
- True negative (TN) is defined as a subject with the target lesion characterized as benign based on both ultrasonography and the truth standard.
- False positive (FP) is defined as a subject with the target lesion characterized as benign by truth standard but either malignant or indeterminate based on ultrasonography.
- False negative (FN) is defined as a subject with the target lesion characterized as malignant by truth standard but either benign or indeterminate based on ultrasonography.

Based on the above definitions, the following diagnostic performance parameters were calculated:

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{TN}{TN+FP}$$

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN}$$

$$\text{Negative Predictive Value (NPV)} = \frac{TN}{TN+FN}$$

$$\text{Positive Predictive Value (PPV)} = \frac{TP}{TP+FP}$$

Primary Efficacy Endpoints

Characterization of Lesions as Benign or Malignant –Off-site Reads
FLLs = Indeterminant Lesions

The primary analysis was based on a comparison of the sensitivity and specificity of SonoVue enhanced versus unenhanced ultrasound, using diagnoses provided by the 3 off-site assessors. It was prospectively defined that the study would meet its primary endpoint if:

1) the sensitivity of SonoVue-enhanced ultrasound is superior as compared to unenhanced ultrasound for at least 2 of the 3 off-site assessors analyzing their data separately;
2) the specificity of SonoVue-enhanced ultrasound is superior as compared to unenhanced ultrasound for at least 2 of the 3 off-site assessors analyzing their data separately;
3) sensitivity and specificity are both superior in the same reader.

The difference in sensitivity/specificity was tested using McNemar's chi-square 2-sided test. The 95% 2-sided confidence intervals (CIs) were also calculated for sensitivity and specificity. In addition, a secondary analysis of the blinded read data compared, in the same manner, the sensitivity and specificity of the "paired" assessment (unenhanced and SonoVue enhanced images evaluated together) versus the unenhanced images alone.

Characterization of Lesions as Benign or Malignant –On-site Reads

Once the decision was made that subject met study qualification criteria, study team administered the SonoVue. Investigator evaluated images for technical adequacy and indeterminacy of lesion. UE-US and CE-US images were read separately and then in matched pairs together. Investigator read images at site selected by investigator. On-site reader's performance was better because they utilized standard clinical methods of diagnosis – on-site reader, in some cases, had access to patient's medical history, clinical information and prior imaging results. Finally, the on-site reader was required to score the added diagnostic value derived from the CE-US images.

Secondary Efficacy Endpoints

Accuracy
Based on the blinded read assessments, the accuracy of each reader in the characterization of lesions as malignant or as benign was tested using McNemar's chi-square 2-sided test. In addition, the 95% 2-sided CIs of accuracy were calculated relying on normal approximation.

Positive Predictive Value (PPV)
The difference in PPV was tested using the Wald test for the Global Evaluation of Efficacy (GEE) coefficients. In addition, the 95% 2-sided CIs of PPV were calculated relying on normal approximation.

Negative Predictive Value (NPV)
The difference in NPV was tested using the Wald test for GEE coefficients. In addition, the 95% 2-sided CIs were calculated relying on normal approximation.

Morphology, Vascularity and Echogenicity of the Lesion
Descriptive statistics were presented for lesion size, by lesion type, and across lesion types. Lesion shape, depth, vascularity, echogenicity, and border definition were summarized.

Inter-reader Agreement
For each study, the inter-reader agreement about diagnosis of a lesion as malignant or benign was measured by Kappa statistic. A Kappa statistic was based on assessment of malignant or benign by unenhanced and SonoVue-enhanced ultrasonography separately. In addition, the inter-reader agreement among readers in each study was computed using the percentage agreement at two categories: "3 out of 3 readers agree" and "2 out of 3 readers agree".

Per protocol Analysis
For the primary efficacy objective, the same analysis as described above was to be performed for the per protocol population.

Sensitivity Analysis
Sensitivity analysis was to be performed for the primary and secondary efficacy objectives using the sensitivity analysis population if the difference between the ITD population and the sensitivity analysis population was deemed to be significant.

NOTE: In both studies, the sensitivity analysis population was the same as the ITD population, i.e., no patients with definite final diagnosis (benign or malignant) from truth standard had missing ultrasound images and were excluded; therefore, in both studies, results for the sensitivity analysis population were identical to those for the ITD population.

Analysis of Specific Diagnoses
The accuracy of ultrasound in terms of correctly characterizing the specific lesion type of each lesion (HCC, metastasis, focal nodular hyperplasia, or any other major lesion types) relative to the final diagnosis/characterization of truth standard was determined for the unenhanced, the contrast-enhanced, and the unenhanced plus contrast-enhanced (UE+CE) assessments. For any lesion with a diagnosis from the truth standard as "other", a medical review was conducted to categorize the specific lesion type for this analysis.

Diagnostic Confidence
Diagnostic confidence was collected from on-site and off-site ultrasound assessment and the on-site assessment from truth standard procedures (including histology, CE-CT and CE-MRI).

Sample Size Calculation
In each study, a total of 222 evaluable subjects was required (111 with malignant lesions and 111 with benign lesions) to test the hypothesis of superiority in sensitivity and specificity of SonoVue-enhanced ultrasound versus unenhanced ultrasound, based on the assumption of 20%

superiority in sensitivity and 20% in specificity were expected. The number of subjects planned for enrollment was 246 to ensure at least 222 subjects evaluable for efficacy.

4. Clinical Efficacy Analyses

Diagnostic Performance in the Characterization of Lesions as Malignant or Benign

Analysis of Efficacy for Studies BR1-128 and BR1-130

The efficacy analysis was performed for the subgroup populations using the same methods as those used for the total population. The subgroup analyses were performed for the ITD population within each study.

Primary Efficacy Analysis

Sensitivity and specificity of unenhanced and SonoVue-enhanced ultrasound were estimated together with 95% CIs, and the differences in sensitivity and specificity between unenhanced and SonoVue-enhanced ultrasound were tested using McNemar's 2-sided Chi-square test.

BR1-128: Diagnostic Performance in Characterization of FLLs

For subjects with technically adequate ultrasound images, the diagnosis based on the ultrasound assessment could be indeterminate, benign or malignant. For off-site readers 1, 2, and 3 and the on-site Investigator, 128, 160, 54 and 167 subjects, respectively, had an indeterminate diagnosis from UE-US, and 27, 25, 6 and 11 subjects, respectively, had an indeterminate diagnosis from CE-US. This indicates that CE-US was able to provide more patients with a definite diagnosis (benign or malignant) than was UE-US.

The diagnostic performance of ultrasound was derived based on the ultrasound diagnosis of malignant or benign from the 3 readers and the final diagnosis provided from the truth standard. Results of the analysis of diagnostic performance from the off-site blinded readers and on-site ultrasound assessment relative to the final diagnosis from the truth standard are summarized in **Table 4**.

PrimaryAnalysis: For the subjects with a final diagnosis of malignant FLL based on the truth standard (N=124), CE-US correctly diagnosed more subjects (i.e., more True Positives) than UE-US as determined by off-site Readers 1 and 2. The sensitivity of UE-US (correct diagnosis of malignant) was from 41% to 66%, and the sensitivity of CE-US was between 47% and 65% for the 3 off-site readers. The sensitivity from CE-US was higher than that from UE-US for off-site readers 1 and 2, and the difference was statistically significant for off-site Reader 2 in favor of CE-US. However, for off-site Reader 3, sensitivity of UE-US was significantly greater than that of CE-US.

For subjects with benign FLL according to the truth standard (N=116), all 3 off-site readers were able to correctly diagnose more subjects (i.e. more True Negative subjects) from CE-US images than from UE-US images. The specificity for UE-US (correct diagnosis of benign) ranged from 7% to 59%, while the specificity for CE-US was between 67% and 88% for the 3

off-site readers. The difference in specificity between CE-US and UE-US in correctly diagnosing a lesion as benign was significant, in favor of CE-US, for each off-site reader.

However, for sensitivity, all 3 off-site readers failed to achieve the 20% superiority expected with CE-US, but did satisfy the 20% superiority expected for specificity with CE-US. Thus, in this study, CE-US performed better at detecting benign (True Negatives) than malignant FLLs.

Table 4 : Diagnostic Performance of Off-site and On-site Ultrasound Assessment- ITD Population Study BR1-128

	Off-site Reader 1 N = 240		Off-site Reader 2 N = 240		Off-site Reader 3 N = 240		On-site N = 240	
Parameter	UE-US	CE-US	UE-US	CE-US	UE-US	CE-US	UE-US	CE-US
Sensitivity (%)	53.2	64.5[a]	41.1	60.5[b]	66.1	46.8[b]	33.9	87.9[b]
Specificity (%)	24.1	71.6[b]	6.9	67.2[b]	58.6	87.9[b]	24.1	90.5[b]
Accuracy (%)	39.2	67.9[b]	24.6	63.8[b]	62.5	66.7	29.2	89.2[b]
PPV (%)	42.9	70.8[c]	32.1	66.4[c]	63.1	80.6[c]	32.3	90.8
NPV (%)	32.6	65.4[c]	9.9	61.4[c]	61.8	60.7	25.5	87.5
True Positive	66	80	51	75	82	58	42	109
True Negative	28	83	8	78	68	102	28	105
False Positive	88	33	108	38	48	14	88	11
False Negative	58	44	73	49	42	66	82	15

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose; PPV, positive predictive value; NPV, negative predictive value; GEE, Global Evaluation of Efficacy.
[a] Based on McNemar's test of difference between CE-US and UE-US, p=0.0754.
[b] Statistically significant difference from UE-US ($p<0.05$ based on McNemar's test).
[c] Statistically significant difference from UE-US ($p<0.05$ based on Wald Test from GEE model).
Data source: Module 5, Section 5.3.5.2 Clinical Trial Report BR1-128

Secondary Endpoint Results

Secondary Analysis: In the analysis of the difference between UE-US and CE-US in accuracy of diagnosis (malignant or benign), and in NPV, all off-site readers except reader 3 provided significantly higher values from CE-US than from UE-US; for PPV, all 3 off-site readers agreed that CE-US had significantly higher values than UE-US. Results from the paired off-site assessment (UE-US + CE-US) were generally similar to the CE-US results.

Results from the image assessment by the on-site Investigators showed superiority of SonoVue enhanced ultrasound over unenhanced ultrasound with statistically significant differences in the sensitivity (88% vs 34%), specificity (91% vs 24%), and accuracy (89% vs 29%) for the characterization of lesions as malignant or benign (p-value <0.0001 for each).

Study BR1-128 - Accuracy of Off-Site US Assessment of Malignant Lesions

The malignant FLLs in the 124 subjects were further characterized from the truth standard into lesion types; 117 FLLs had specific lesion type assigned, including:

- 84 classified as HCC
- 31 classified as metastasis
- 2 classified as cholangiocarcinoma.

The remaining 7 malignant FLLs were characterized as "other" lesion types.

For the 84 subjects with HCC as the specific lesion type diagnosed from the truth standard, CE-US correctly characterized HCC FLLs in 16%,, 25% and 39% of subjects, across the 3 off-site readers. Similar results were obtained for UE-US (**Table 5**).

For the 31 subjects with metastasis as the specific lesion type, accuracy from CE-US ranged

Malignant FLL	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]

FLLs - ITD Population (Study BR1-128)

	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
	UE-US	CE-US	UE-US	CE-US	UE-US	CE-US

FLLs - ITD Population (Study BR1-128)

Malignant FLL	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]
HCC	29/84 (34.5)	33/84 (39.3)	12/84 (14.3)	13/84 (15.5)	21/84 (25.0)	20/84 (23.8)
Cholangiocarcinoma	0/2	0/2	0/2	0/2	0/2	1/2 (50.0)
Metastasis	14/31 (45.2)	20/31 (64.5)	16/31 (51.6)	17/31 (54.8)	19/31 (61.3)	21/31 (67.7)

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose; FLL, focal liver lesion; HCC, hepatocellular carcinoma.

[a] For each category, N is the total number of FLLs characterized by Truth Standard, and n is the total number of FLLs correctly characterized by UE-US or CE-US.

Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-128.

Performance as measured by improved accuracy for detection of malignant HCCs by CE-US when compared to UE-US by off-site readers was poor. The best performance by a single reader was improvement from 35 % to 39%. Performance as measured for detection of metastases was slightly better for all three readers by CE-US when compared to UE-US, improvement by 3%, 7% and 20%, but less than desirable.

Study BR1-128 - Accuracy of Off-Site US Assessment of Benign Lesions

Truth standard provided a specific lesion type to all benign FLLs with the exception of lesions in 19 subjects: 13 were characterized as "Other" and 6 as "Unable To Determine." Hemangioma was the major benign lesion type for FLLs. All 3 off-site readers correctly characterized more of these lesions based on CE-US than on UE-US; the accuracy for hemangioma identification with CE-US ranged from 63% to 78%. The same trend was also observed for FNH, with a much higher accuracy detected for CE-US than for UE-US across all 3 readers. (**Table 6**)

Performance as measured by improved accuracy for detection of benign FLLs was significantly better for all three readers by CE-US when compared to UE-US. Improvement

for hemangiomas (22%, 40% and 60%) and for focal nodular hyperplasia (40%, 44% and 48%) was significant.

Table 6: Accuracy of Off-Site Ultrasound Assessments in Characterizing Benign FLLs – ITD Population (Study BR1-128)

	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
Benign Characterization	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]
Hemangioma	21/64 (32.8)	47/64 (73.4)	2/64 (3.1)	40/64 (62.5)	36/64 (56.3)	50/64 (78.1)
Focal nodular hyperplasia	0/25	10/25 (40.0)	2/25 (8.0)	13/25 (52.0)	0/25	12/25 (48.0)
Focal fatty sparing	1/1 (100.0)	0/1	1/1 (100.0)	0/1	1/1 (100.0)	1/1 (100.0)
Focal fatty change	0/7	0/7	0/7	0/7	1/7 (14.3)	1/7 (14.3)
Regenerating nodule	0/8	1/8 (12.5)	0/8	0/8	0/8	0/8
Simple cyst	1/5 (20.0)	1/5 (20.0)	1/5 (20.0)	1/5 (20.0)	1/5 (20.0)	1/5 (20.0)
Adenoma	0/1	0/1	0/1	0/1	0/1	0/1

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to diagnose; FLL, focal liver lesion
[a] For each category, N is the total number of FLLs characterized by Truth Standard, and n is the total number of FLLs correctly characterized by UE-US or CE-US.
Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-128.

Study BR1-128 - Comparison of Unpaired and Paired Reads -

Results from the paired off-site reads (**Table 7**) serve to strengthen the usefulness CE-US in characterizing FLLs. Routinely, in clinical practice, CE-US will be evaluated in conjunction with UE-US and all available clinical information and additional imaging results.

Table 7: Study BR1-128 - Diagnostic Performance of Off-site US Assessments Unpaired and Paired Reads – ITD Population

	Off-site Reader #1			Off-site Reader #2			Off-site Reader #3		
	UE	CE	UE+CE	UE	CE	UE+CE	UE	CE	UE+CE
Total subjects	240	240	240	240	240	240	240	240	240
T P	66 28%	80 33%	70 29%	51 21%	75 31%	79 33%	82 34%	58 24%	69 29%
T N	28 12%	83 35%	89 37%	8 3%	78 33%	92 38%	68 28%	102 43%	103 43%
F P	88 37%	33 14%	27 12%	108 45%	38 16%	24 10%	48 20%	14 6%	13 5%
F N	58 24%	44 18%	54 23%	73 30%	49 20%	45 19%	42 18%	66 28%	55 23%
Sensitivity	53% (44,62)	65% (56,73)	57% (48,65)	41% (33,50)	61% (52,69)	64% (55,72)	66% (58,75)	47% (38,56)	56% (47,64)
Specificity	24% (16,32)	72% (63,80)	77% (69,84)	7% (2,12)	67% (59,76)	79% (72,87)	59% (50,68)	88% (82,94)	89% (83,95)
Accuracy	39% (33,45)	68% (62,74)	66% (60,72)	25% (19,30)	64% (58,70)	71% (66,77)	63% (56,69)	67% (61,73)	72% (66,77)
PPV	43% (35,51)	71% (62,79)	72% (63,81)	32% (25,39)	66% (58,75)	77% (69,85)	63% (55,71)	81% (71,90)	84% (76,92)
NPV	33% (23,43)	65% (57,74)	62% (54,70)	10% (3,16)	61% (53,70)	67% (59,75)	62% (53,71)	61% (53,68)	65% (58,73)

BR1-128 - On-site Matched Paired Assessment Study

Investigators evaluated matched paired unenhanced vs. SonoVue-enhanced images for each subject and provided an assessment of the diagnostic quality of enhancement over unenhanced images (diagnostic value of CE-US images) using the following scores:

0 = No feature provided to help lesion characterization
1 = The contrast enhancement obtained provided some additional objective features, but none of them were additive to those obtained with unenhanced ultrasound.
2 = The contrast enhancement obtained provided diagnostic clues that are independent of and additive to those obtained with unenhanced ultrasound.
3 = Only contrast provided features to allow lesion characterization.

Results of the on-site matched read are provided in **Table 8**. Only one parameter for the paired images was evaluated by the on-site Investigator - diagnostic quality of enhancement.

Table 8: Study BR1-128 – On-Site Ultrasound Combined Matched Pair Assessment – Diagnostic Quality of Enhancement - ITD Population

Quality of Contrast Enhancement	N=240 N (%)
The contrast enhancement obtained did not provide any objective feature that could help in the characterization of the lesion	**1 0.4%**
The contrast enhancement obtained provided some additional objective features, but none of them were additive to those obtained with unenhanced ultrasound	**6 3%**
The contrast enhancement obtained provided diagnostic clues that are independent of and additive to those obtained with unenhanced ultrasound	**117 49%**
Only contrast enhancement provided objective features that allowed lesion characterization	**113 47%**

Paired reads by the on-site reader revealed that CE-US images provided additive diagnostic clues and objective features that allowed lesion characterization in 96% of reads.

BR1-130: Diagnostic Performance in Characterization of FLLs

For off-site readers 1-3 and the on-site Investigators, 73, 112, 205 and 178 subjects, respectively, had an indeterminate diagnosis from UE-US, and 0, 2, 0 and 17 subjects, respectively, had an indeterminate diagnosis from CE-US; this indicates that CE-US was able to provide more patients with a definite diagnosis (benign or malignant) than was UE-US.

The diagnostic performance of ultrasound was derived based on the ultrasound diagnosis of malignant or benign from the 3 readers and the final diagnosis provided from the truth standard. Results of the analysis of diagnostic performance are summarized in **Table 9**.

PrimaryAnalysis: For subjects with a final diagnosis of malignant FLL based on truth standard (N=119), CE-US correctly diagnosed more lesions (i.e., more True Positives) than UE-US as assessed by all 3 readers. Sensitivity of UE-US (correct diagnosis of malignant) was achieved in 16%, 35% and 49% of reads, and sensitivity of CE-US was achieved in 76%, 87% and 92% by the 3 readers. The sensitivity from CE-US was significantly greater than 20% for all 3 UE-US reads.

For the subjects with benign FLL according to the final diagnosis of truth standard (N=140), All 3 off-site readers were able to correctly diagnose more lesions (i.e. more True Negative lesions) from CE-US images than from UE-US images. Specificity for UE-US (correct diagnosis of malignant) ranged from 22% to 63%, and Specificity for CE-US was between 71% and 83% across the 3 off-site readers.

Specificity from CE-US was higher than that from UE-US for all 3 readers, and the difference between CE-US and UE-US in correctly diagnosing a lesion as benign was significant for 2 of the readers.

Off-site readers #2 and #3 were successfully superior by greater than 20% for both sensitivity and specificity when comparing CE-US to UE-US. All 3 off-site readers achieved similar superiority for sensitivity.

Table 9: Diagnostic Performance of Off-site and On-site Ultrasound Assessment-ITD Population Study BR1-130

	Off-site Reader 1 N = 259		Off-site Reader 2 N = 259		Off-site Reader 3 N = 259		On-site N = 259	
Parameter	UE-US	CE-US	UE-US	CE-US	UE-US	CE-US	UE-US	CE-US
Sensitivity (%)	48.7	86.6 [a]	35.3	75.6 [a]	16.0	91.6 [a]	40.3	90.8 [a]
Specificity (%)	62.9	70.7 [b]	54.3	82.9 [a]	22.1	72.9 [a]	19.3	78.6 [a]
Accuracy (%)	56.4	78.0 [a]	45.6	79.5 [a]	19.3	81.5 [a]	29.0	84.2 [a]
PPV (%)	52.7	71.5 [c]	39.6	78.9 [c]	14.8	74.1 [c]	29.8	78.3 [c]
NPV (%)	59.1	86.1 [c]	49.7	80.0 [c]	23.7	91.1 [c]	27.6	90.9
True Positive	58	103	42	90	19	109	48	108
True Negative	88	99	76	116	31	102	27	110
False Positive	52	41	64	24	109	38	113	30
False Negative	61	16	77	29	100	10	71	11

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose; PPV, positive predictive value; NPV, negative predictive value; GEE, Global Evaluation of Efficacy.
[a] Statistically significant difference from UE-US ($p<0.05$ based on McNemar's test).
[b] Based on McNemar's test of difference between CE-US and UE-US, $p=0.1380$.
[c] Statistically significant difference from UE-US ($p<0.05$ based on Wald Test from GEE model).
Data source: Module 5, Section 5.3.5.2 Clinical Trial Report BR1-130

Secondary Endpoint Results

Secondary Analysis: In the analysis of the difference between UE-US and CE-US in accuracy of diagnosis (malignant or benign), in NPV, and in PPV, CE-US had significantly higher values than UE-US for all 3 off-site readers. Results from the off-site paired assessment (UE-US + CEUS) were generally similar to the CE-US results.

Results from the image assessment by the on-site Investigators showed superiority of Sonovue enhanced ultrasound over unenhanced ultrasound with statistically significant differences in the sensitivity (91% vs 40%), specificity (79% vs 19%), and accuracy (84% vs 29%) for the characterization of lesions as malignant or benign (p-value <0.0001 for each).

Study BR1-130 - Accuracy of Off-Site US Assessment of Malignant Lesions

The malignant FLLs in the 119 subjects were further characterized from the truth standard into lesion types; 102 FLLs had specific lesion type assigned, including:

- 47 classified as HCC
- 47 classified as metastasis
- 8 classified as cholangiocarcinoma

The remaining 17 malignant FLLs were characterized either as "other" (n=8) or "Unable To Determine" (n=9) categories.

For the 47 subjects with HCC as the specific lesion type from the truth standard, CE-US

correctly characterized HCC FLLs in 55% to 64%, across the 3 off-site readers. The accuracy from UE-US was much lower than that from CE-US for each reader.

For the 47 subjects with metastasis, the accuracy from CE-US ranged from 60% to 79% across the 3 readers. The accuracy from UE-US was much lower than CE-US for each reader.

Performance as measured by improved accuracy for detection of malignant HCCs by CE-US when compared to UE-US by off-site readers was significant (21%, 41% and 58%). Likewise, performance as measured by improved accuracy for detection of metastases by CE-US when compared to UE-US by off-site readers was significant (40%, 41% and 58%) (**Table 10**).

Table 10: Accuracy of Off-Site Ultrasound Assessments of Specific Lesion Types: Malignant FLLs - ITD Population (Study BR1-130)

	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
Malignant FLL	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]
HCC	16/47 (34.0)	26/47 (55.3)	10/47 (21.3)	29/47 (61.7)	3/47 (6.4)	30/47 (63.8)
Cholangiocarcinoma	1/8 (12.5)	1/8 (12.5)	0	1/8 (12.5)	1/8 (12.5)	5/8 (62.5)
Metastasis	18/47 (38.3)	37/47 (78.7)	12/47 (25.5)	31/47 (66.0)	1/47 (2.1)	28/47 (59.6)

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose; FLL, focal liver lesion; HCC, hepatocellular carcinoma.

[a] For each category, N is the total number of FLLs characterized by Truth Standard, and n is the total number of FLLs correctly characterized by UE-US or CE-US.

Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-130.

Study BR1-130 - Accuracy of Off-Site US Assessment of Benign Lesions

Truth standard provided a specific lesion type to all benign FLLs with the exception of lesions in 19 subjects: 13 were characterized as "Other" and 6 as "Unable To Determine."

Hemangioma was the major benign lesion type for FLLs in the study (**Table 11**). All 3 off-site readers correctly characterized more of these lesions based on CE-US than on UE-US; the accuracy for hemangioma identification with CE-US ranged from 73% to 83%. The same trend was also observed for focal nodular hyperplasia, again showing a much higher accuracy detected for CE-US than for UE-US across all 3 readers.

Performance as measured by improved accuracy for detection of benign FLLs was significantly better for all three readers by CE-US when compared to UE-US. Improvement for hemangiomas (19%, 25% and 50%) and focal nodular hyperplasia (20%, 35% and 41%) was beneficial.

Table 11: Accuracy of Off-Site Ultrasound Assessments of Specific Lesion Types: Benign FLLs - ITD Population (Study BR1-130)

Benign Characterization	Off-site Reader 1		Off-site Reader 2		Off-site Reader 3	
	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]	UE-US n/N (%)[a]	CE-US n/N (%)[a]
Hemangioma	28/52 (53.8)	38/52 (73.1)	30/52 (57.7)	43/52 (82.7)	12/52 (23.1)	38/52 (73.1)
Focal nodular hyperplasia	15/39 (38.5)	23/39 (59.0)	8/39 (20.5)	22/39 (56.4)	2/39 (5.1)	18/39 (46.2)
Focal Fatty Sparing	0/1	0/1	1/1 (100.0)	0/1	0/1	0/1
Focal Fatty Change	2/3 (66.7)	2/3 (66.7)	2/3 (66.7)	2/3 (66.7)	2/3 (66.7)	2/3 (66.7)
Regenerating nodule	0/3	0/3	0/3	0/3	0/3	0/3
Simple cyst	6/8 (75.0)	3/8 (37.5)	6/8 (75.0)	5/8 (62.5)	3/8 (37.5)	4/8 (50.0)
Abscess	1/7 (14.3)	0/7	0/7	0/7	0/7	2/7 (28.6)
Adenoma	0/8	2/8 (25.0)	0/8	0/8	0/8	2/8 (25.0)

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to diagnose; FLL, focal liver lesion.
[a] For each category, N is the total number of FLLs characterized by Truth Standard, and n is the total number of FLLs correctly characterized by UE-US or CE-US.
Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-130.

Comparison of Unpaired and Paired Reads - Study BR1-130

Results from the paired off-site reads serve to strengthen the usefulness CE-US in characterizing FLLs. Routinely, in clinical practice, CE-US will be evaluated in conjunction with UE-US and all available clinical information and additional imaging results (**Table 12**).

Table 12: Study BR1-130 - Diagnostic Performance of Off-site US Assessments Unpaired and Paired Reads - ITD Population

	Off-site Reader #1			Off-site Reader #2			Off-site Reader #3		
	UE	CE	UE+CE	UE	CE	UE+CE	UE	CE	UE+CE
Total subjects	259	259	259	259	259	259	259	259	259
T P	58 22%	103 40%	95 37%	42 16%	90 35%	98 38%	19 7%	109 42%	109 42%
T N	88 34%	99 38%	111 43%	76 29%	116 45%	120 46%	31 12%	102 39%	106 41%
F P	52 20%	41 16%	29 11%	64 25%	24 9%	20 8%	109 42%	38 15%	34 13%
F N	61 24%	16 6%	24 9%	77 30%	29 11%	21 8%	100 39%	10 4%	10 4%
Sensitivity	49% (40,58)	87% (80,92)	79% (73,86)	35% (27,44)	76% (68,83)	82% (76,89)	16% (9,23)	92% (87,97)	92% (87,97)
Specificity	62% (55,71)	71% (63,78)	79% (73,86)	54% (46,63)	83% (77,89)	86% (80,92)	22% (15,23)	73% (66,80)	76% (69,83)
Accuracy	56% (50,62)	78% (73,83)	80% (75,85)	46% (40,52)	80% (75,85)	84% (80,89)	19% (15,24)	82% (77,87)	83% (78,88)
PPV	53% (43,62)	72% (64,79)	77% (69,84)	40% (30,49)	79% (72,86)	83% (76,90)	15% (9,21)	74% (67,81)	76% (69,83)
NPV	59% (51,67)	86% (80,92)	82% (76,89)	50% (42,58)	80% (74,87)	85% (79,91)	24% (16,31)	91% (86,96)	91% (86,97)

On-site Matched Paired Assessment Study BR1-130

Results of the on-site matched read are provided in **Table 13**. Only one parameter for the paired images was evaluated by the on-site Investigator: diagnostic quality of enhancement.

Table 13: Study BR1-130 – On-Site Ultrasound Combined Matched Pair Assessment - Diagnostic Quality of Enhancement - ITD Population

Quality of Contrast Enhancement	N=259 N (%)
The contrast enhancement obtained did not provide any objective feature that could help in the characterization of the lesion	4 2%
The contrast enhancement obtained provided some additional objective features, but none of them were additive to those obtained with unenhanced ultrasound	15 6%
The contrast enhancement obtained provided diagnostic clues that are independent of and additive to those obtained with unenhanced ultrasound	118 46%
Only contrast enhancement provided objective features that allowed lesion characterization	120 46%

Paired reads by the on-site reader revealed that CE-US images provided additive diagnostic clues and objective features that allowed lesion characterization in 92% of reads.

Diagnostic Performance in the Characterization of FLLs

The data supporting the proposed indication across the two identical, independently conducted and adequately controlled Phase III clinical studies (BR1-128 and BR1-130) are summarized in **Table 14**. The reader findings across the two studies show that:

Table 14: Diagnostic Performance of Off-site and On-site Ultrasound Assessment - ITD Populations in Studies BR1-128 and BR1-130

BR1-128: N = 240									
	Sensitivity N=124 Malignant Lesions[a]			Specificity N=116 Benign Lesions[a]			Accuracy[a] N=240 Total Lesions[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	53.2	64.5	0.0754	24.1	71.6	<0.0001	39.2	67.9	<0.0001
Reader 2	41.1	60.5	0.0011	6.9	67.2	<0.0001	24.6	63.8	<0.0001
Reader 3	66.1	46.8	0.0016	58.6	87.9	<0.0001	62.5	66.7	0.3173
On-site	*33.9*	*87.9*	*<0.0001*	*24.1*	*90.5*	*<0.0001*	*29.2*	*89.2*	*<0.0001*
BR1-130: N = 259									
	Sensitivity N=119 Malignant Lesions[a]			Specificity N=140 Benign Lesions[a]			Accuracy N=259 Total Lesions[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	48.7	86.6	<0.0001	62.9	70.7	0.1380	56.4	78.0	<0.0001
Reader 2	35.3	75.6	<0.0001	54.3	82.9	<0.0001	45.6	79.5	<0.0001
Reader 3	16.0	91.6	<0.0001	22.1	72.9	<0.0001	19.3	81.5	<0.0001
On-site	*40.3*	*90.8*	*<0.0001*	*19.3*	*78.6*	*<0.0001*	*29.0*	*84.2*	*<0.0001*

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
[b] McNemar's test.
[c] Results from the 3 off-site readers within each study were collapsed into one result per patient following a majority rule.
Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-128 and BR1-130

- In the **primary analysis of sensitivity (characterization of lesions as malignant)**:

  In Study BR1-128, only reader 2 successfully demonstrated statistically significant higher sensitivity for SonoVue-enhanced US versus unenhanced US. Reader 3 actually performed better with UE-US than with CE-US.

  In Study BR1-130, all three readers successfully demonstrated statistically significant higher sensitivity for SonoVue-enhanced US versus unenhanced US.

- In the **primary analysis of specificity (characterization of lesions as benign)**:

  In Study BR1-128, all three readers successfully demonstrated statistically significant higher specificity for SonoVue-enhanced US versus unenhanced US.

  In Study BR1-130, readers 2 and 3 successfully demonstrated statistically significant higher specificity for SonoVue-enhanced US versus unenhanced US.

Conclusions from Studies BR1-128 and BR1-130

Results from BR1-130
Both readers 2 and 3 demonstrated superiority for both sensitivity and specificity in characterization of FLLs by SonoVue-enhanced ultrasound versus unenhanced ultrasound. Both readers satisfied the expected 20% superiority requirement for CE-US assessment of liver lesion nature (malignant/benign) compared to UE-US.

For reader 2 CE-US increased sensitivity to 76% compared to UE-US of 35%, and for reader 2 CE-US increased specificity to 83% compared to UE-US of 54%. For reader 3 CE-US increased sensitivity to 92% compared to UE-US of 16%, and for reader 2 CE-US increased specificity to 73% compared to UE-US of 22%.

Results from BR1-128
Only reader 2 demonstrated superiority for both sensitivity and specificity in characterization of FLLs by SonoVue-enhanced ultrasound versus unenhanced ultrasound. Reader 2 satisfied the expected 20% superiority requirement for CE-US assessment of liver lesion nature (malignant/benign) compared to UE-US.

For reader 2 CE-US increased sensitivity to 61% compared to UE-US of 41%,and for reader 2 CE-US increased specificity to 67% compared to UE-US of 7%.

Reduction in Indeterminate Diagnostic Reads
The superiority of SonoVue-enhanced ultrasonography versus UE-US for assessment of liver lesion nature (malignant/benign) was also apparent from the marked decrease in number of lesions with an indeterminate diagnosis (0-27 with CE-US versus 54-205 with UE-US across the 6 readers).

Final Diagnoses from the Truth Standard

The distribution of the final diagnoses by the truth standard for the ITD population in each study, as determined by the truth standards used, is shown in **Table 15**.

BR1-128: 124 of the subjects were diagnosed with malignant FLL; 116 of the subjects were diagnosed with benign FLL; HCC was the most frequent malignant lesion (68%) and hemangioma was the most frequent benign lesion (55%).

BR1-130: 119 of the subjects were diagnosed with malignant FLL; 140 of the subjects were diagnosed with benign FLL; HCC and metastases were equally common (both 40%) among the malignant lesions and hemangioma was the most frequent benign lesion (37%)

Study BR1-128 contained a higher proportion of malignant lesions and BR1-130 contained a higher proportion of benign lesions. Study BR1-128 contained a higher proportion of HCC lesions and hemangiomas. BR1-130 contained a higher proportion of metastatic lesions.

Table 15: Distribution of Final Diagnoses from Truth Standards – ITD Populations in BR1-128 and BR1-130

Characteristics	BR1-128 (N=240) n (%)	BR1-130 (N=259) n (%)
Malignant	**124 (51.7)**	**119 (45.9)**
Histology	45 (18.8)	46 (17.8)
Pathology	5 (2.1)	10 (3.9)
CT/MRI	74 (30.8)	63 (24.3)
Benign	**116 (48.3)**	**140 (54.1)**
Histology	10 (4.2)	24 (9.3)
Pathology	5 (2.1)	10 (3.9)
CT/MRI	101 (42.1)	106 (40.9)
Specific Lesion Types		
Malignant lesion characterization	**N=124**	**N=119**
Hepatocellular carcinoma (HCC)	84 (67.7)	47 (39.5)
Metastases	31 (25.0)	47 (39.5)
Cholangiocarcinoma	2 (1.6)	8 (6.7)
Other	7 (5.6)	8 (6.7)
Unable to determine	0	9 (7.6)
Benign lesion characterization	**N=116**	**N=140**
Hemangioma	64 (55.2)	52 (37.1)
Focal nodular hyperplasia (FNH)	25 (21.6)	38 (27.1)
Focal fatty sparing/change	8 (6.9)	4 (2.9)
Regenerating nodule	8 (6.9)	3 (2.1)
Simple cyst	5 (4.3)	8 (5.7)
Adenoma	1 (0.9)	8 (5.7)
Abscess	0	7 (5.0)
Other	4 (3.4)	13 (9.3)
Unable to determine	1 (0.9)	6 (4.3)

ITD, intent-to-diagnose; CT/MRI, computed tomography/magnetic resonance imaging.
Data source: Module 5, Section 5.3.5.2 Clinical Trial Reports BR1-128 and BR1-130

Analysis of Efficacy by Lesion Size [BRI-128]

Performance by the off-site readers in Study 128 were inconsistent in sensitivity (recognition of malignant FLLs) in all lesion size groups. All 3 readers in Study 128 performed successfully in recognizing significantly greater numbers of benign lesions in all lesion size groups (**Table 16**).

Table 16: Analysis of Efficacy by Lesion Size [BRI-128]

	Sensitivity N=16[a]			Specificity N=32[a]			Accuracy N=48[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Lesion Size ≤2 cm									
Reader 1	25.0	56.3	0.0956	25.0	71.9	0.0001	25.0	66.7	< 0.0001
Reader 2	25.0	37.5	0.3173	3.1	62.5	< 0.0001	10.4	54.2	< 0.0001
Reader 3	68.8	50.0	0.1797	59.4	81.3	0.0348	62.5	70.8	0.3173
On-site	*12.5*	*75.0*	*0.0016*	*34.4*	*90.6*	*< 0.0001*	*27.1*	*85.4*	*< 0.0001*
Lesion Size >2 cm to ≤4 cm									
	Sensitivity N=62[a]			Specificity N=57[a]			Accuracy N=119[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	54.8	62.9	0.3359	24.6	73.7	< 0.0001	40.3	68.1	< 0.0001
Reader 2	38.7	62.9	0.0053	7.0	68.4	< 0.0001	23.5	65.5	< 0.0001
Reader 3	61.3	48.4	0.1306	63.2	89.5	0.0006	62.2	68.1	0.3072
On-site	*35.5*	*85.5*	*< 0.0001*	*17.5*	*89.5*	*< 0.0001*	*26.9*	*87.4*	*< 0.0001*
Lesion Size >4 cm									
	Sensitivity N=46[a]			Specificity N=27[a]			Accuracy N=73[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	60.9	69.6	0.4328	22.2	66.7	0.0013	46.6	68.5	0.0114
Reader 2	50.0	65.2	0.1266	11.1	70.4	0.0003	35.6	67.1	0.0003
Reader 3	71.7	43.5	0.0093	48.1	92.6	0.0005	63.0	61.6	0.8694
On-site	*39.1*	*95.7*	*< 0.0001*	*25.9*	*92.6*	*< 0.0001*	*34.2*	*94.5*	*< 0.0001*

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
NOTE: The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
[b] McNemar's test.

Analysis of Efficacy by Lesion Size [BRI-130]

In Study 130, performance by off-site readers 2 and 3 were consistently superior in both sensitivity and specificity in all lesion size groups. Reader 1 performed successfully in recognizing significantly greater numbers of malignant lesions but not benign lesions in all lesion size groups (**Table 17**).

Table 17: Analysis of Efficacy by Lesion Size [BRI-130]

	Sensitivity N=16[a]			Specificity N=30[a]			Accuracy N=46[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Lesion Size ≤2 cm									
Reader 1	31.3	81.3	0.0047	66.7	76.7	0.3657	54.3	78.3	0.0116
Reader 2	6.3	56.3	0.0047	70.0	90.0	0.0339	47.8	78.3	0.0005
Reader 3	0.0	81.3	N/A	30.0	56.7	0.0209	19.6	65.2	< 0.0001
On-site	*18.8*	*81.3*	*0.0016*	*30.0*	*73.3*	*0.0003*	*26.1*	*76.1*	*< 0.0001*
Lesion Size >2 cm to ≤4 cm									
	Sensitivity N=50[a]			Specificity N=62[a]			Accuracy N=112[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	46.0	88.0	< 0.0001	67.7	67.7	1.000	58.0	76.8	0.0033
Reader 2	38.0	86.0	< 0.0001	61.3	87.1	0.0006	50.9	86.6	< 0.0001
Reader 3	14.0	90.0	< 0.0001	30.6	82.3	< 0.0001	23.2	85.7	< 0.0001
On-site	*42.0*	*94.0*	*< 0.0001*	*17.7*	*77.4*	*< 0.0001*	*28.6*	*84.8*	*< 0.0001*
Lesion Size >4 cm									
	Sensitivity N=53[a]			Specificity N=48[a]			Accuracy N=101[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Reader 1	56.6	86.8	0.0003	54.2	70.8	0.0881	55.4	79.2	0.0002
Reader 2	41.5	71.7	0.0006	35.4	72.9	0.0001	38.6	72.3	< 0.0001
Reader 3	22.6	96.2	< 0.0001	6.3	70.8	< 0.0001	14.9	84.2	< 0.0001
On-site	*45.3*	*90.6*	*< 0.0001*	*14.6*	*83.3*	*< 0.0001*	*30.7*	*87.1*	*< 0.0001*

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
NOTE: The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
[b] McNemar's test.

Demographic and Baseline Characteristics

The demographic and baseline characteristics for all subjects who received study agent (safety population) and for the subjects included in the ITD populations in the two studies are provided in **Table 18**. The majority of the subjects in each study were male and white. The mean age of the study participants was 56.1 years (range 18 to 88 years) in BR1-128 and 59.0 years (range 22 to 93 years) in BR1-130; mean weight was similar in the ITD population in the two studies (81.16 kg and 79.83kg, respectively).

In both studies, the demographic and baseline characteristics of the ITD population were similar to those in the efficacy phase and in the total populations.

Table 18: **Demographic and Baseline Characteristics of the Study Populations in BR1-128 and BR1-130**

Characteristic	BR1-128		BR1-130	
	Safety Population N = 337[a]	ITD Population n = 240[a]	Safety Population N = 340	ITD Population n = 259[a]
Sex, n (%)				
Male	180 (53.4)	123 (51.3)	182 (53.5)	136 (52.5)
Female	157 (46.6)	117 (48.8)	158 (46.5)	123 (47.5)
Age (years)				
Mean (SD)	56.1 (12.4)	55.0 (12.2)	57.2 (13.3)	56.9 (13.4)
Median	56.0	54.0	59.0	59.0
Range (min, max)	(18, 88)	(19, 88)	(22, 93)	(22, 93)
Age group, n (%)				
<18 years	0	0	0	0
≥18 to <65 years	254 (75.4)	190 (79.2)	238 (70.0)	185 (71.4)
≥65 years	83 (24.6)	50 (20.8)	102 (30.0)	74 (28.6)
Race, n (%)				
White	233 (69.1)	161 (67.1)	263 (77.4)	206 (79.5)
Black	45 (13.4)	32 (13.3)	35 (10.3)	22 (8.5)
Asian	17 (5.0)	15 (6.3)	19 (5.6)	12 (4.6)
Other	42 (12.5)	32 (13.3)	23 (6.8)	19 (7.3)
Weight (kg)				
Mean (SD)	82.55 (19.29)	81.16 (18.84)	78.70 (19.03)	79.83 (19.54)
Median	80.00	78.45	76.90	77.10
Range (min, max)	(44.40, 147.60)	(44.40, 147.20)	(41.80, 173.20)	44.40, 173.20)
Height (cm)				
Mean (SD)	169.4 (10.7)	169.1 (10.4)	169.50 (10.1)	169.6 (10.4)
Median	170.0	170.0	170.0	170.0
Range (min, max)	(137, 198)	(142, 198)	(137, 195)	(137, 195)

SD, standard deviation; min, minimum value; max, maximum value
[a] Denominator for percentage calculation.
Data source: Module 5, Section 5.3.5.2 Clinical Trial Reports BR1-128 and BR1-130

Study BR1-128: The majority of the 337 subjects who received study agent were male (180, 53%) and white (233, 69%). The mean age was 56 years (range 18 to 88 years), mean weight was 83 kg (range 44, 148 kg) and mean height was 169 cm (range 137 to 198 cm).

Study BR1-130: The majority of the 340 subjects who received study agent were male (182, 54%) and white (263,77%). The mean age was 57 years (range 22 to 93 years), mean weight was 79 kg (range 42, 173 kg) and mean height was 170 cm (range 137 to 195 cm).

In both studies, the demographic and baseline characteristics of the ITD population were similar to those in the efficacy phase and in the total populations.

Exposure

Exposure was similar in the two studies.
BR1-128: The mean volume (SD) of SonoVue administered was 2.6 mL (0.72) in the ITD

population and 2.7 mL (0.80) in the total population. Twenty-five (7%) patients received a second bolus, for technical reasons (failure of ultrasound machine, iv line or timer), suboptimal position of the transducer, patient movement, or atypical lesion location, depth, or size.

BR1-130: The mean volume (SD) of SonoVue was 2.6 mL (0.64) in both the ITD and total populations. Among all subjects who received SonoVue, 26 (8%) received a second SonoVue bolus injection (5 during training phase, 21 during efficacy phase). Reasons for technical failure of the first bolus included malfunction of ultrasound machine, wrong needle positioning, contrast medium extravasation and other reasons.

A medical review of the other reasons showed that a second injection was administered due to a difficult assessment of the target lesion because of a poor visualization of the lesion or a suboptimal position of the transducer (shadowed by rib), patient movement, failure of the IV line, and technical failure or incorrect use (e.g., video clip started too late).

Technical Adequacy of Contrast-enhanced Ultrasound Images

The adequacy of the images was evaluated. For each technically adequate image, the readers were to perform the target lesion assessment, including border definition, lesion shape, and pattern of enhancement.

For each technically adequate image, the readers were to perform the target lesion assessment, including border definition, lesion shape, lesion vascularity, echogenicity and pattern of enhancement. If the investigator determined that the images were inadequate, the investigator recorded the reason(s) for the inadequacy and the image assessment was not to be performed.

Study BR1-128, the contrast-enhanced images were technically adequate for 98% to 99% of patients across the 3 readers. The reasons provided by the readers for inadequacy of the images were: (**Table 19**)

- "No liver tissue imaged or obvious inadequate anatomical coverage (target lesion partially visible)", stated by Reader 1 for one patient
- "Artifacts are present and hamper the assessment of the target lesion", stated by Readers 1 and 3, each for one patient
- "Other", stated by Readers 1 and 2, each for 4 patients and by Reader 3 for 2 patients

Study BR1-130, the contrast-enhanced images were technically adequate for 99% to 100% of patients across the 3 readers. The reasons provided by the readers for inadequacy of the images were

- "No liver tissue imaged or obvious inadequate anatomical coverage (target lesion partially visible)", stated by Reader 3 for one patient
- "Artifacts are present and hamper the assessment of the target lesion", stated by Reader 3 for 2 patients
- "Other", stated by Reader 1 for 3 patients and by Reader 3 for 2 patients

Table 19: Technical Adequacy of Off-site Ultrasound Assessments – ITD Population

Reader Assessment	BR1-128 N=240		BR1-130 N=259	
	Unenhanced n (%)	Contrast-enhanced n (%)	Unenhanced n (%)	Contrast-enhanced n (%)
Off-site Reader 1				
Technically Adequate	240 (100.0)	234 (97.5)	259 (100.0)	256 (98.8)
Technically Inadequate	0	6 (2.5)	0	3 (1.2)
-No liver tissue imaged or obvious inadequate anatomical coverage (target lesion partially visible)	0	1 (0.4)	0	0
-Artifacts are present and hamper the assessment of the target lesion	0	1 (0.4)	0	0
-Other	0	4 (1.7)	0	3 (1.2)
Off-site Reader 2				
Technically Adequate	239 (99.6)	236 (98.3)	259 (100.0)	259 (100.0)
Technically Inadequate	1 (0.4)	4 (1.7)	0	0
- No liver tissue imaged or obvious inadequate anatomical coverage (target lesion partially visible)	0	0	0	0
-Artifacts are present and hamper the assessment of the target lesion	0	0	0	0
-Other	1 (0.4)	4 (1.7)	0	0
Off-site Reader 3				
Technically Adequate	240 (100.0)	237 (98.8)	259 (100.0)	256 (98.8)
Technically Inadequate	0	3 (1.3)	0	3 (1.2)
-No liver tissue imaged or obvious inadequate anatomical coverage (target lesion partially visible)	0	0	0	1 (0.4)
-Artifacts are present and hamper the assessment of the target lesion	0	1 (0.4)	0	2 (0.8)
-Other	0	2 (0.8)	0	0

ITD, intent-to-diagnose
Data source: Module 5 Section 5.3.5.2 Study Reports of Uncontrolled Studies BR1-128 and BR1-130

Integrated Efficacy Analysis

Integrated Analysis of Studies BR1-128 and BR1-130

Integrated Efficacy Analysis

The diagnostic performance of the off-site ultrasound assessments are provided for the ITD population for studies BR1-128 and BR1-130 in **Table 20**. When the results are combined, the overall performance demonstrates superiority of CE-US compared to UE-US.

Table 20: **Diagnostic Performance of Off-site Ultrasound Assessment - Integrated Analysis of Studies BR1-128 and BR1-130**

Integrated Summary of Efficacy: N = 499									
	Sensitivity N=243 Malignant Lesions[a]			Specificity N=256 Benign Lesions[a]			Accuracy[a] N=499 Total Lesions[a]		
	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]	UE-US %	CE-US %	p-value[b]
Integrated Analysis[c]	43.6	72.4	<0.0001	34.0	80.5	<0.0001	38.7	76.6	<0.0001

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
[b] McNemar's test.
[c] The off-site evaluations of UE-US and CE-US images were performed by 3 off-site blinded readers in each study. For the purpose of the integration analysis, results from the 3 off-site readers in each study were collapsed into one result per patient following a majority rule.
Data source: Module 5, Section 5.3.5.3 Integrated Summary of Efficacy Tables, Table 2.1

Demographic and Baseline Characteristics: Integrated ITD Population

The demographics and baseline characteristics for the 499 patients in the ITD population in the two studies are summarized in **Table 21**. The majority of the ITD subjects were male (52%) and white (74%). The mean age was 56 years (range 19 to 93 years), mean weight was 80 kg (range 44 to 173 kg) and mean height was 169 cm (range 137 to 198 cm).

Table 21: **Demographic and Baseline Characteristics – ITD Population Integrated Analysis of Studies BR1-128 and BR1-130**

Characteristic	ITD Population N = 499[a]
Sex, n (%)	
Male	259 (51.9)
Female	240 (48.1)
Age (yr)	
N	499
Mean (yr) (SD)	56.0 (12.9)
Median (yr)	56.0
Range (yr) (minimum, maximum)	(19, 93)
Age group, n (%)	
≥18 to <65 years	375 (75.2)
≥65 years	124 (24.8)
Race, n (%)	
White	367 (73.5)
Non-white	132 (26.5)
Weight (kg)	
N	495
Mean (kg) (SD)	80.48 (19.20)
Median (kg)	78.00
Range (kg) (minimum, maximum)	(44.40, 173.20)
Height (cm)	
N	494
Mean (cm) (SD)	169.4 (10.4)
Median (cm)	170.00
Range (cm) (minimum, maximum)	(137, 198)

[a] Denominator for percentage calculation.
Data source: Module 5, Section 5.3.5.3 Integrated Summary of Efficacy Tables, Table 1

Integrated Efficacy Analysis by Age, Gender and Race Subgroups

The results for the integrated ITD population by subgroup are provided in **Table 22**. As for the subgroup analyses by age group and by race for the individual studies, the results by age group and by race in the ITD population integrated analysis must be considered in light of the smaller number of patients in the ≥65 yrs age group and the non-white race subgroup. Both sensitivity and specificity were significantly higher for CE-US than for UE-US across each all subgroup analyses.

Table 22: Diagnostic Performance of Off-site Ultrasound Assessment– ITD Population Integrated Analysis of Studies BR1-128 and BR1-130 by Subgroups

	Sensitivity (%)			Specificity (%)			Accuracy (%)		
By Age Group									
	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]
18-64 yrs, N = 375 (159M, 216B) [a]	45.9	67.9	0.0001	31.9	82.4	<0.0001	37.9	76.3	<0.0001
≥65 yrs, N = 124 (84M, 40B) [a]	39.3	81.0	<0.0001	45.0	70.0	0.0124	41.1	77.4	<0.0001
By Gender									
	Sensitivity (%)			Specificity (%)			Accuracy (%)		
	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]
Male, N = 259 (174M, 85B) [a]	43.7	70.7	<0.0001	41.2	76.5	<0.0001	42.9	72.6	<0.0001
Female, N = 240 (69M, 171B) [a]	43.5	76.8	<0.0001	30.4	82.5	<0.0001	34.2	80.8	<0.0001
By Race									
	Sensitivity (%)			Specificity (%)			Accuracy (%)		
	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]	UE-US	CE-US	p-value[b]
White, N = 367 (163M, 204B) [a]	42.3	77.3	<0.0001	34.8	79.4	<0.0001	38.1	78.5	<0.0001
Non-white, N =132 (80M, 52B) [a]	46.3	62.5	0.0326	30.8	84.6	<0.0001	40.2	71.2	<0.0001

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] M = Malignant lesions, B = Benign lesions
[b] McNemar's test.
[c] The off-site evaluations of UE-US and CE-US images were performed by 3 off-site blinded readers in each of the studies. For the integration analysis purpose, results from the 3 off-site readers within each study were collapsed into one result per patient following a majority rule.
Data Source: Module 5, Section 5.3.5.3 Integrated Summary of Efficacy Tables, Tables 2.2, 2.3, 2.4

Supportive Studies of SonoVue in the Characterization of Focal Liver Lesions

Prior to conducting the two pivotal studies (BR1-128 and BR1-130) for the FLL characterization program, other Bracco-sponsored studies have been conducted using SonoVue for either visualization or characterization of FLLs, including 2 Phase II/III studies (BR1-071 and BR1-072) carried out under the IND in the USA and summarized in **Table 23**. The image assessments in these studies were performed on site.

Table 23: Summary of SonoVue Bracco-Sponsored Studies in Characterization of FLLs: Conducted under the IND in the USA

Protocol No	No of Patients	Study Design	SonoVue Dose (Efficacy Population; N malignant/N benign)	Gold Standard	Sensitivity		Specificity		Accuracy	
					UE-US	CE-US	UE-US	CE-US	UE-US	CE-US
BR1-071	185 for efficacy	USA dose-finding, parallel groups, FLL characterization of indeterminate target lesion (onsite assessment)	0.6 mL (63 eff ; 25M/38B) 1.2 mL (63 eff; 26M/37B) 2.4 mL (59 eff; 21M/38B)	Final diagnosis Histology/ CT/MRI	48% 46% 43%	84% 85% 90%	32% 16% 24%	63% 76% 82%	38% 29% 31%	71% 79% 85%
BR1-072	207 for efficacy	USA dose-finding, parallel groups, FLL characterization of indeterminate target lesion (onsite assessment)	0.6 mL (63 eff; 29M/34B) 1.2 mL (73 eff; 30M/43B) 2.4 mL (71 eff; 35M/36B)	Final diagnosis Histology/ CT/MRI	41% 27% 43%	66% 83% 83%	21% 23% 25%	88% 91% 81%	30% 25% 34%	78% 88% 82%

FLL, focal liver lesion; UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; eff, efficacy; M, malignant; B, benign; CT, computed tomography; MRI, magnetic resonance imaging

Based on the results from these 2 Studies (BR1-071 and BR1-072), the 1.2 mL dose performed almost as well as the 2.4 mL dose.

Inter-reader Agreement

Inter-reader agreement among the off-site readers in diagnosing FLLs is presented in **Table 24** for each of the studies. CE-US reduced reader variability compared to UE-US and improved Kappa value in both studies.

BR1-128: The percentage of agreement among all 3 readers on the FLL diagnosis was 52% for CE-US, much higher than for UE-US (32%). The percentage of agreement among 2 out of 3 readers on the FLL diagnosis was 97% for UE-US and 92% for CE-US. The generalized Kappa value as a measure of agreement in diagnosis of the FLLs by CE-US (0.391) indicated moderate agreement among the 3 off-site readers. The higher generalized Kappa value for diagnosis from CE-US than from UE-US suggested that CE-US resulted in better agreement among the 3 readers in FLLs diagnosis than did UE-US. Study 128 had higher inter-reader variability.

BR1-130: The percentage for all 3 readers being in agreement on the diagnosis was 66% for CE-US, much higher than UE-US (28%). The percentage agreement for 2 of the 3 readers in agreement on the lesion diagnosis was 95% for UE-US and 99.6% for CE-US. The generalized Kappa value as a measure of agreement in diagnosis of the FLLs by CE-US (0.553) indicated

moderate to substantial agreement among the 3 off-site readers. The higher generalized Kappa value for diagnosis from CE-US than from UE-US suggested that CE-US resulted in better agreement among the 3 readers in FLLs diagnosis than did UE-US.

Table 24: **Inter-reader Agreement on Off-Site Ultrasound Diagnosis – ITD Population (Studies BR1-128 and BR1-130)**

Characteristics	BR1-128		BR1-130	
	UE-US N=240	CE-US N=240	UE-US N=259	CE-US N=259
% Agreement: All 3 off-site readers agree	32.1	51.7	28.2	66.0
% Agreement: 2 out of 3 off-site readers agree	97.1	91.7	94.6	99.6
Generalized Kappa value[a]	0.258	0.391	0.191	0.553

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to diagnose
[a] Measure of agreement in the diagnosis of 'Benign'/'Malignant'/'Indeterminate'/'Technically Inadequate'.
Data source: Module 5, Section 5.3.5.2 Clinical Trial Reports BR1-128 and BR1-130

Diagnostic Confidence: Off-site Ultrasound Assessment

BR1-128: Diagnostic confidence assessments from the three off-site readers in study BR1-128 are summarized in **Table 25**. The majority of scores for diagnostic confidence was "High" for CEUS for all 3 off-site readers, (62% for reader 1, 75% for reader 2, 61% for reader 3). On the other hand, the diagnostic confidence was generally much lower for UE-US evaluations, with percentage of high confidence ranging from 18% to 45% across the 3 readers.

Table 25: Diagnostic Confidence of Off-site Ultrasound Assessments – ITD Population (Study BR1-128)

Category	Off-site Reader 1 (N=240)		Off-site Reader 2 (N=240)		Off-site Reader 3 (N=240)	
	UE-US n (%)	CE-US n (%)	UE-US n (%)	CE-US n (%)	UE-US n (%)	CE-US n (%)
High	42 (17.5)	148 (61.7)	52 (21.7)	179 (74.6)	109 (45.4)	146 (60.8)
Low	70 (29.2)	59 (24.6)	27 (11.3)	32 (13.3)	77 (32.1)	85 (35.4)
Indeterminate	128 (53.3)	27 (11.3)	160 (66.7)	25 (10.4)	54 (22.5)	6 (2.5)
Technically Inadequate	0	6 (2.5)	1 (0.4)	4 (1.7)	0	3 (1.3)

For subjects with an FLL that had the assessment of indeterminate or technically inadequate, no confidence was provided by the reader.
UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to diagnose
Data source: Module 5, Section 5.3.5.2 Clinical Trial Report BR1-128

BR1-130: Diagnostic confidence assessment of the three off-site readers is summarized for study BR1-130 in **Table 26**. The diagnostic confidence was scored as "High" for CE-US in most cases by all 3 off-site readers; the percentage of lesions assigned a score of high diagnostic confidence by the reader ranged from 68% to 83%. On the other hand, the diagnostic confidence was generally much lower for UE-US evaluations, with percentage of high confidence ranging from 4% to 32%.

Table 26: **Diagnostic Confidence of Off-site Ultrasound Assessments – ITD Population (Study BR1-130)**

Category	Off-site Reader 1 N=259[a]		Off-site Reader 2 N=259[a]		Off-site Reader 3 N=259[a]	
	UE-US n (%)	CE-US n (%)	UE-US n (%)	CE-US n (%)	UE-US n (%)	CE-US n (%)
High	82 (31.7)	176 (68.0)	84 (32.4)	178 (68.7)	10 (3.9)	214 (82.6)
Low	104 (40.2)	80 (30.9)	63 (24.3)	79 (30.5)	44 (17.0)	42 (16.2)
Indeterminate	73 (28.2)	0	112 (43.2)	2 (0.8)	205 (79.2)	0
Technically inadequate	0	3 (1.2)	0	0	0	3 (1.2)

For subjects with a lesion that had the assessment of indeterminate or technically inadequate, no assessment of diagnostic confidence was provided by the readers.
UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to diagnose
[a] Denominator for percentage calculation.
Data source: Module 5, Section 5.3.5.2 Clinical Trial Report BR1-130

Efficacy Analysis by Demographic Subgroup
Results of the efficacy analysis have been summarized by demographic subgroups including age group (<65; ≥65), gender, and race group (white; non-white). Patients in the ≥65 years age group was a much smaller number as those <65 years of age were in the great majority of the total population (**Table 27** and **Table 28**).

BR1-128 (**Table 27**)
For the age group of patients between 18 and 64 years (N = 190), trends are very similar to those observed in the whole population. For the subjects with a final diagnosis of malignant FLL based on the truth standard (N=89), CEUS correctly diagnosed more subjects (i.e., more True Positive) than UE-US as determined by off-site readers 1 and 2. The sensitivity from CE-US was higher than that from UE-US for offsite readers 1 and 2, and the difference was statistically significant for off-site reader 2 in favor of CE-US. However, for off-site reader 3, sensitivity of UE-US was significantly higher than that of CE-US.

For subjects with benign FLL according to the truth standard (N=101), all 3 off-site readers were able to correctly diagnose more subjects (i.e. more True Negative subjects) from CE-US images than from UE-US images. In this age group, the difference in specificity between CE-US and UE-US in correctly diagnosing a lesion as benign was significant, in favor of CE-US, for each off-site readers.

Table 27: **Diagnostic Performance of Off-site Ultrasound Assessment by Age Group - ITD Population in BR1-128**

	Sensitivity N=89 Malignant Lesions[a]			Specificity N=101 Benign Lesions[a]			Accuracy N=190 Total Lesions[a]		
Age 18-64 years; N = 190									
	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value
Reader 1	52.8	57.3	0.5371	22.8	70.3	<0.0001	36.8	64.2	<0.0001
Reader 2	39.3	57.3	0.0114	5.9	69.3	<0.0001	21.6	63.7	<0.0001
Reader 3	69.7	41.6	0.0001	59.4	88.1	<0.0001	64.2	66.3	0.6547
Age ≥65 years; N = 50									
	Sensitivity N=35 Malignant Lesions[a]			Specificity N=15 Benign Lesions[a]			Accuracy N=50 Total Lesions[a]		
	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value
Reader 1	54.3	82.9	0.0253	33.3	80.0	0.0082	48.0	82.0	0.0011
Reader 2	45.7	68.6	0.0325	13.3	53.3	0.0143	36.0	64.0	0.0017
Reader 3	57.1	60.0	0.7963	53.3	86.7	0.0253	56.0	68.0	0.1797

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
Data source: Module 5, Section 5.3.5.3 BR1-128 Additional By Study Analysis Tables, Table 4.1

BR1-130 (**Table 28**)
For the age group of patients between 18 and 64 years (N = 185), trends are very similar to those observed in the whole population. For subjects with a final diagnosis of malignant FLL based on truth standard (N=70), CE-US correctly diagnosed more lesions (i.e., more True Positives) than UE-US as assessed by all 3 readers. The sensitivity from CE-US was significantly greater ($p < 0.05$) than that from UE-US for the 3 readers.

For the subjects with benign FLL according to the final diagnosis of truth standard (N=115), all 3 off-site readers were able to correctly diagnose more lesions (i.e. more True Negative lesions) from CE-US images than from UE-US images. Specificity from CE-US was higher than that from UE-US for all 3 readers, and the difference between CE-US and UE-US in correctly diagnosing a lesion as benign was significant for all 3 of the readers.

Table 28: Diagnostic Performance of Off-site Ultrasound Assessment by Age Group - ITD Population in BR1-130

	Sensitivity N=70 Malignant Lesions[a]			Specificity N=115 Benign Lesions[a]			Accuracy N=185 Total Lesions[a]		
Age 18-64 years; N = 185									
	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value
Reader 1	54.3	82.9	0.0003	64.3	75.7	0.0526	60.5	78.4	0.0001
Reader 2	34.3	74.3	<0.0001	50.4	86.1	<0.0001	44.3	81.6	<0.0001
Reader 3	15.7	91.4	<0.0001	23.5	76.5	<0.0001	20.5	82.2	<0.0001
Age ≥65 years; N = 74									
	Sensitivity N=49 Malignant Lesions[a]			Specificity N=25 Benign Lesions[a]			Accuracy N=74 Total Lesions[a]		
	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value	UE-US %	CE-US %	p-value
Reader 1	40.8	91.8	<0.0001	56.0	48.0	0.5271	45.9	77.0	0.0002
Reader 2	36.7	77.6	<0.0001	72.0	68.0	0.6547	48.6	74.3	0.0004
Reader 3	16.3	91.8	<0.0001	16.0	56.0	0.0039	16.2	79.7	<0.0001

UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; ITD, intent-to-diagnose.
The unit of analysis was the lesion; each subject had a single lesion that was to be characterized.
[a] Denominator for percentage calculation.
Data source: Module 5, Section 5.3.5.3 BR1-130 Additional By Study Analysis Tables, Table 4.1

Summary of the Relevant Literature for Efficacy

Several publications, including single center and multicenter trials, provide additional evidence for the diagnostic value of SonoVue-enhanced ultrasound in the characterization of FLLs in the population studied in the two Phase III studies. These studies specifically include assessment of incidental findings on routine unenhanced ultrasound of lesions or suspected lesions in subjects with chronic hepatitis or liver cirrhosis and of liver lesions in subjects with a history of malignancy.

A literature search was performed by the applicant to provide supportive evidence for the use of SonoVue in the characterization of liver lesions. The search was performed utilizing PubMed, a service of the US National Library of Medicine®. The search terms were (contrast-enhanced ultrasound OR SonoVue OR BR1 OR sulfur hexafluoride) AND (liver OR hepatic). The date range was 1993 to Sep 30, 2014. No limits were applied. A total of 1786 references were identified in the search results. All articles identified in the search were reviewed against the criteria listed below.

Eligibility Criteria

Publications that met all the following **inclusion criteria** were included in the literature summary or the use of SonoVue in the characterization of liver lesions:

1. Original publication of a clinical study in human subjects with prospective or retrospective enrollment;
2. SonoVue was used during liver ultrasound examinations;
3. Sufficient information for efficacy evaluation of the endpoints of sensitivity and specificity for SonoVue-enhanced ultrasound.

Publications that did not meet the inclusion criteria or met the following **exclusion criteria** were excluded from the SonoVue efficacy literature summary:

1. Report of contrast-enhanced ultrasound in non-human subjects (e.g. phantom, in vitro or animal studies);
2. Report from a Bracco-sponsored study;
3. SonoVue was used for post-interventional treatment, such as radiofrequency ablation;
4. Use of SonoVue in clinical indications other than characterization of liver lesions;
5. No appropriate truth standard in the study;
6. Subjects were <18 years of age;
7. Contrast agent other than SonoVue was used;
8. Used different ultrasound technique
9. Had assessment of abdominal organs/structures other than liver;
10. Fewer than 30 subjects who received SonoVue were evaluated;
11. Review articles, guidance/guideline articles, letters to the editor, or case report or commentary articles with no characterization of lesions provided
12. Reports from meta-analysis of study data.

Results

Based on the above inclusion and exclusion criteria, a total of 1740 of the 1786 publications were excluded from the SonoVue efficacy literature summary because they did not meet the criteria for inclusion. Of these:

- 160 publications are non-human studies;
- 3 publications are reports from Bracco-sponsored studies;
- 9 publications are about SonoVue use in post-interventional treatment, such as radiofrequency ablation;
- 468 publications report use of SonoVue in clinical indications other than characterization of liver lesions;
- 5 publications had no appropriately defined truth standard;
- 1 publication included subjects <18 years of age;
- 179 publications were studies in which a contrast agent other than SonoVue was used;
- 419 publications used a different ultrasound technique such as acoustic radiation force, intraoperative ultrasound, and/or no contrast agent was utilized;
- 84 publications had assessment of abdominal organs/structures other than liver;
- 6 publications evaluated fewer than 30 subjects who received SonoVue;
- 402 publications were review articles or guidance/guideline articles, letters to the editor, or case reports;
- 4 publications reported meta-analysis of studies of characterization of liver lesions.

Summary of Findings from the Literature

A total of 46/1786 publications met all inclusion and exclusion criteria and present the sensitivity, specificity and/or accuracy of SonoVue-enhanced ultrasound for the characterization of focal liver lesions determined from clinical studies reported in the peer-reviewed literature.

Results from the peer-reviewed literature confirm the high sensitivity and specificity of SonoVue-enhanced ultrasonography for characterization of FLLs. Three of the publications identified in the literature search reported the results of meta-analysis of studies of characterization of liver lesions. Findings from these 3 studies are summarized below.

Summary of Meta-analyses of SonoVue in the Characterization of FLLs: 3 Publications from the Literature

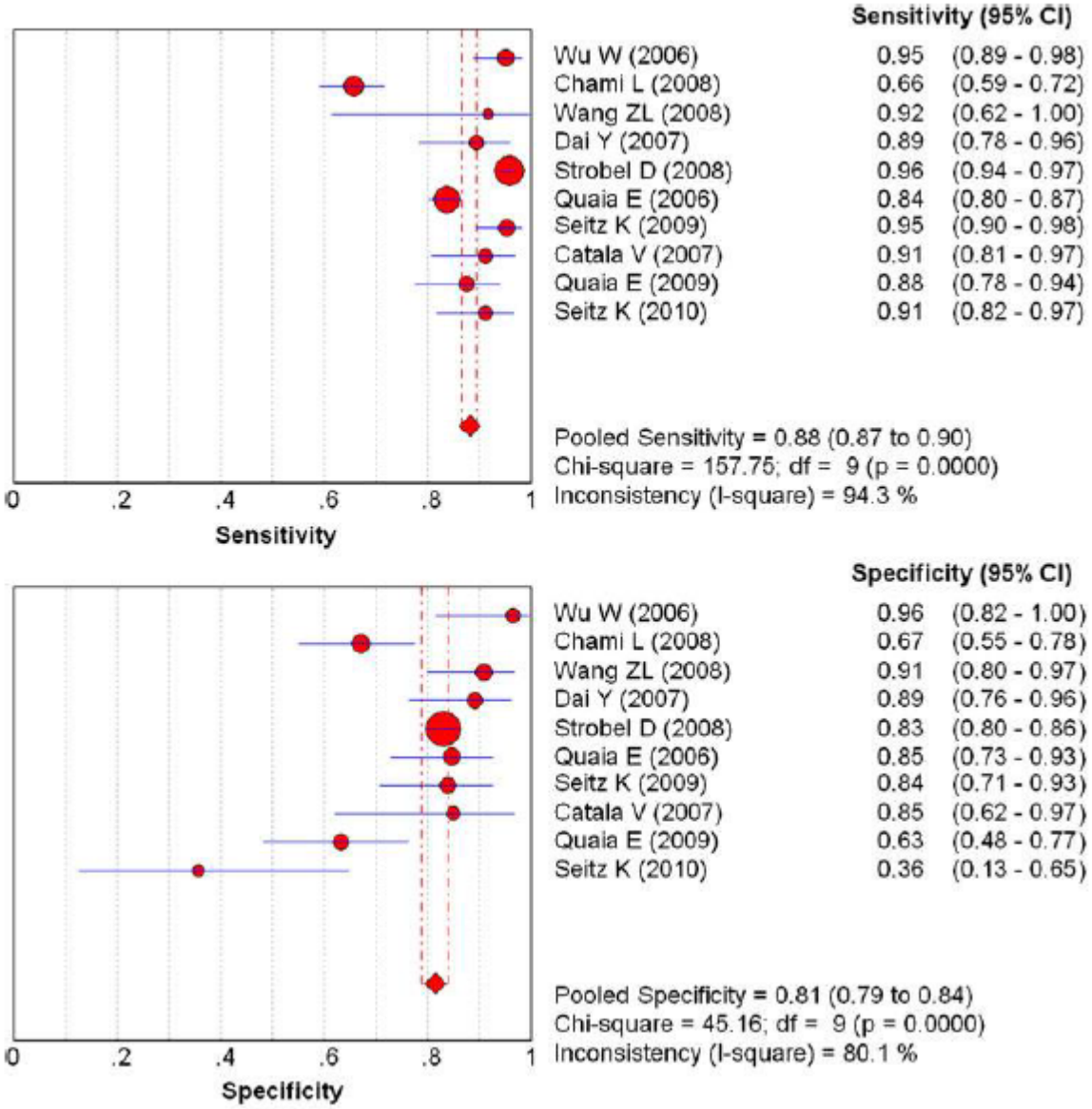
The meta-analysis by **Friedrich-Rust** on contrast-enhanced ultrasound for the differentiation of benign and malignant focal liver lesion included 35 papers with SonoVue use out of a total of 45 publications, with a total of 7231 focal liver lesions, 4221 of which were malignant. The pooled sensitivity was 0.93 (0.91, 0.95) and the pooled specificity was 0.90 (0.88, 0.93). This meta-analysis supported EFSUMB's recommendation that CE-US should be the first method of choice in the diagnostic work-up of focal liver lesions if B-mode and Doppler ultrasound were not conclusive.

The meta-analysis performed by **Niu** on contrast-enhanced ultrasonography for the diagnosis of small hepatocellular carcinoma included 15 publications, 12 of which presented data for SonoVue. The 12 papers included a total of 778 patients (878 lesions) and showed a pooled sensitivity of 0.84 (0.77, 0.90) and pooled specificity of 0.89 (0.81, 0.94) for SonoVue. These results provide support for the use of CE-US as a useful diagnostic tool based on sensitivity and specificity for the identification of small HCC.

A meta-analysis of the diagnosis value of focal liver lesions with SonoVue-enhanced ultrasound compared with CE-CT and CE-MRI was performed by **Guang**. It included 10 studies with SonoVue, 9 studies with CE-CT and 10 studies with CE-MRI; 2646 patients (2981 lesions) were included. **Figure 2** below provides the sensitivity and specificity with 95% CIs for the 10 studies and the pooled estimate for SonoVue-enhanced sonography. The pooled sensitivity was 0.88 (0.87, 0.90), and the pooled specificity was 0.81 (0.79, 0.84) for SonoVue-enhanced ultrasound. Among the 3 techniques, SonoVue-enhanced ultrasound had the highest specificity, and CE-CT had the highest sensitivity; however, there was no significant statistical difference between CE-CT or CE-MRI and SonoVue-enhanced ultrasound.

Conclusions from the Literature: Studies in Adults
Results from both single study reports and meta-analyses in the peer-reviewed literature confirm that based on sensitivity and specificity, SonoVue-enhanced ultrasonography is a useful diagnostic tool for characterization of FLLs in adults.

Figure 2. Sensitivity and Specificity of SonoVue-enhanced Sonography



5. Summary of Safety

Overview of Safety – Adults

This section of the overview summarizes safety data from studies in the full SonoVue safety evaluation plan and those that are most relevant to this population.

The safety data presented include:

- Analysis of pooled data from All Completed Studies (Healthy Volunteers and Patients);
- Analysis of pooled data from All Patient Studies;
- Subset analysis of pooled data from integrated Completed Liver Studies (23 studies: BBG-006, BR1-015, BR1-018, BR1-032, BR1-034, BR1-035, BR1-039, BR1-042, BR1-043, BR1-045, BR1-048, BR1-053, BR1-071, BR1-072, BR1-105, BR1-118, BR1-121, BR1-128, BR1-129, BR1-130, BRA-007, IGIT-002, IGIT-005)
- Analysis of safety data is based on subjects who received at least one dose of SonoVue.
- A brief overview of safety findings from post-marketing experience is also provided.
- A brief overview of safety findings from literature is also provided.
- Information about safety in special groups and situations is presented.

Overall Extent of Exposure
In all 77 clinical studies included in the integrated safety database, SonoVue was administered intravenously, either as a slow bolus injection and/or as a continuous infusion. Many studies employed crossover dosing, in which patients received multiple doses of SonoVue and/or control agents, usually on the same study day. The control agents included saline and Albunex®. At the time some of these studies were conducted, Albunex was the only contrast agent approved for use with ultrasound imaging. At present, Albunex is no longer marketed. All 77 studies were open-label or single-blind with respect to on-site assessments of safety.

SonoVue was provided by Bracco as a sterile, pyrogen-free, lyophilized powder, 25 mg or 50 mg, in a septum-sealed glass vial (10-mL or 20-mL capacity). The vials were to be stored at controlled room temperature (20-25°C). A white, milky suspension of sulfur hexafluoride (SF_6) lipid-type A microspheres [redacted (b) (4)]) was obtained by adding 5 mL or 10 mL (for the 25 and 50 mg vials, respectively) of 0.9% sodium chloride to the powder, using standard clinical aseptic techniques followed by hand agitation.

A 5 mg/mL concentration of SonoVue was used in all studies except study BR1-025, a Phase I dose escalation safety study in which a 15 mg/mL concentration was used. The increase in concentration in this study was achieved by reducing the amount of saline used in reconstitution of the 25 mg SonoVue vial from 5 mL to 1.7 mL. Study BR1-025 was the only study in the SonoVue clinical development program that used a concentration of 15 mg/mL. No further development of SonoVue as a 15 mg/mL concentration is planned.

All Completed Studies in Healthy Volunteers and Patients
For the 6918 subjects in the completed studies with exposure to SonoVue, the mean total volume administered was 9.76 mL (range: 0.2 to 161.3 mL) (**Table 29**). This includes subjects who received multiple bolus doses of SonoVue in crossover studies as well as those who received infusion dosing. Seventy-two percent (72%) of the subjects were in the group receiving cumulative doses ranging from greater than 1.0 mL to 10 mL in volume; 95% received cumulative doses ranging from greater than 1.0 mL to 50 mL in volume. Three additional subjects received SonoVue at an 'unknown' total volume.

Table 29: Extent of Exposure to SonoVue in All Completed Studies (Healthy Volunteers and Patients)

Total Volume SonoVue Administered[a]	
Number of subjects	6918[b]
Mean volume (mL) (SD)	9.76 (12.974)
Median volume (mL)	4.80
Range (minimum, maximum volumes) (mL)	0.2, 161.3
Cumulative Dose Categories	Number of patients (%)
≤1 mL	232 (3.4%)
>1 to 5 mL	3314 (47.9%)
>5 mL to 10 mL	1657 (24.0%)
>10 mL to 50 mL	1599 (23.1%)
>50 mL	116 (1.7%)

[a] Only exposure to SonoVue 5 mg/mL is summarized.

[b] Study BR1-129 (N=30) is excluded because the study was designed with multiple visits that were 2 to 8 weeks apart which is very different from all other studies included in the summary. Three additional subjects received SonoVue at an 'unknown' total volume. Study BR1-025 (N=33) is excluded because the study agent administered was of a different concentration (15 mg/mL).

For summary of exposure, undiluted volume was used. When diluted volume was administered, undiluted volume was computed for summary purposes.

Study BR1-006: 3/10 dilution was used for administration.

Study BR1-008: 3/10 dilution was used for administration to the renal arteries.

Study BR1-021: 1/2 dilution was used for infusion administration.

Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 2.1.

All Patient Studies

For the 6823 patients in the completed studies who had exposure to SonoVue, the mean total volume administered was 9.70 mL (range: 0.3 to 161.3 mL) (**Table 30**). This includes patients who received multiple bolus doses of SonoVue in crossover studies as well as those who received infusion dosing. Seventy-two percent (72%) of the patients were in the group receiving cumulative doses ranging from greater than 1.0 mL to 10 mL; 95% received cumulative doses ranging from greater than 1.0 mL to 50 mL. Three additional subjects received SonoVue at an 'unknown' total volume.

Table 30: Extent of Exposure to SonoVue in All Patient Studies

Total Volume SonoVue Administered (mL)	
Number of patients	6823
Mean volume (mL) (SD)	9.70 (12.978)
Median volume (mL)	4.80
Range (minimum, maximum volumes) (mL)	0.3, 161.3
Cumulative Dose Categories	
≤1 mL	224 (3.3%)
>1 to 5 mL	3302 (48.4%)
>5 mL to 10 mL	1631 (23.9%)
>10 mL to 50 mL	1551 (22.7%)
>50 mL	115 (1.7%)
Only exposure to SonoVue 5 mg/mL is summarized. Study BR1-129 (N=30) is excluded because the study was designed with multiple visits that were 2 to 8 weeks apart which is very different from all other studies included in the summary. Three additional subjects received SonoVue at an 'unknown' total volume. For summary exposure, undiluted volume was used. When diluted volume was administered, undiluted volume was computed for summary purposes. Study BR1-006: 3/10 dilution was used for administration. Study BR1-021: 1/2 dilution was used for infusion administration. Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 2.2.	

Completed Liver Studies

For the 2909 patients who received SonoVue in the Completed Liver Studies, the mean total volume administered was 6.02 mL (range: 0.6 to 72.0 mL) (**Table 31**). All except 6 patients received doses ranging from <1 mL to 50 mL.

Table 31: Extent of Exposure to Study Agent in Completed Liver Studies

Total Volume SonoVue Administered (mL)	
Number of patients	2909
Mean volume (mL) (SD)	6.02 (6.157)
Median volume (mL)	4.80
Range (minimum, maximum volumes) (mL)	0.6, 72.0
Cumulative Dose Categories	
≤1 mL	132 (4.5%)
>1 to 5 mL	1819 (62.5%)
>5 mL to 10 mL	548 (18.8%)
>10 mL to 50 mL	404 (13.9%)
>50 mL	6 (0.2%)
Study BR1-129 (N=30) is excluded because the study was designed with multiple visits that were 2 to 8 weeks apart, which is very different from all other studies included in the summary. Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 2.4.	

Demographic and Other Characteristics of Study Populations

All Completed Studies (Healthy Volunteers and Patients)

Disposition

A total of 7060 subjects were enrolled in the studies, with 71 subjects discontinuing prior to receiving SonoVue. Of the 6984 subjects who received SonoVue, 6598 (95%) completed the studies, while 386 (6%) discontinued prematurely (23 for adverse events, 4 were lost to followup, 48 for withdrawal of consent, 2 for protocol violations, 306 for other reasons, and 3 for no reason specified).

Demographic and Baseline Characteristics

The majority of the 6984 subjects who received SonoVue in the All Completed Studies were male (64%) and white (79%). The mean age was 59 years (range: 17 to 99 years), the mean weight was 76 kg (range: 35 to 210 kg), and the mean height was 169 cm (range: 118 to 204 cm).

All Patient Studies

Disposition

A total of 6932 patients were enrolled in the studies, with 71 patients discontinuing prior to receiving SonoVue. Of the 6856 patients who received SonoVue, 6473 (94%) completed the studies, while 383 (6%) discontinued prematurely (22 for adverse events, 4 were lost to follow up, 48 for withdrawal of consent, 2 for protocol violations, 304 for other reasons, and 3 for no specified reason).

Demographic and Baseline Characteristics

The majority of the 6856 patients who received SonoVue in the Completed Studies in Patients were male (63%) and white (78%). The mean age was 60 years (range: 17 to 99 years), the mean weight was 76 kg (range: 35 to 210 kg), and the mean height was 169 cm (range: 118 to 201 cm).

Completed Liver Studies

In the Completed Liver Studies, a total of 2984 patients were enrolled; 40 patients discontinued prior to receiving SonoVue (**Table 32**). Of the 2939 patients who received SonoVue, 2764 (94%) completed the studies and 175 (6%) discontinued prematurely (1 for adverse events, 1 was lost to follow-up, 35 for withdrawal of consent, 2 for protocol violations, 136 for other reasons).

Table 32: Disposition of Patients, Completed Liver Studies – Lumason (SonoVue)

Enrolled (signed informed consent)	2984
Discontinued Prior to Receiving Study Agent	40
Withdrawal of consent	3
Protocol violation	2
Other	35
Received Study Agent[a]	2939[b]
Completed Study	2764 (94.0%)
Prematurely Discontinued	175 (6.0%)
Adverse event	1 (<0.1%)
Lost to follow-up	1 (<0.1%)
Withdrawal of consent	35 (1.2%)
Protocol violation	2 (0.1%)
Other	136 (4.6%)
No reason specified	0

[a] Five (5) patients from study BBG-006 received commercial SonoVue and are excluded from this count and the integrated safety summary analyses.
[b] Percentages are based on the number of patients who received SonoVue.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 1.4.

As shown in **Table 33A and Table 33B**, the majority of the 2939 patients in the Completed Liver Studies were male (61%) and white (66%). The mean age was 57 years (range: 17 to 99 years), the mean weight was 73 kg (range: 39 to 173 kg), and the mean height was 169 cm (range: 135 to 198 cm).

Table 33A: Demographic and Baseline Characteristics, Completed Liver Studies - Lumason (SonoVue)

Enrolled (signed informed consent)	2984
Discontinued Prior to Receiving Study Agent	40
Withdrawal of consent	3
Protocol violation	2
Other	35
Received Study Agent[a]	2939[b]
Completed Study	2764 (94.0%)
Prematurely Discontinued	175 (6.0%)
Adverse event	1 (<0.1%)
Lost to follow-up	1 (<0.1%)
Withdrawal of consent	35 (1.2%)
Protocol violation	2 (0.1%)
Other	136 (4.6%)
No reason specified	0

[a] Five (5) patients from study BBG-006 received commercial SonoVue and are excluded from this count and the integrated safety summary analyses.
[b] Percentages are based on the number of patients who received SonoVue.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 1.4.

Table 33B: Demographic and Baseline Characteristics, Completed Liver Studies - Lumason (SonoVue)

Characteristic	*Liver sNDA Safety Update* N = 2939
Gender, n (%)	
Male	1782 (60.6)
Female	1157 (39.4)
Age Group	
<65 years, n (%)	1980 (67.4)
≥65 years, n (%)	958 (32.6)
Unknown, n (%)	1 (<0.1)
Age (years)	(N=2938)
Mean (SD)	57.0 (13.72)
Range (min, max)	17, 99
Race, n (%)	
White	1941 (66.0)
Black	110 (3.7)
Hispanic	69 (2.3)
Asian	803 (27.3)
Other	14 (0.5)
Unknown	2 (0.1)
Weight Group	
<75 kg, n (%)	1729 (58.8)
75 to 100 kg, n (%)	983 (33.4)
>100 kg, n (%)	158 (5.4)
Unknown, n (%)	69 (2.3)
Weight (kg)	(N=2870)
Mean (SD)	72.57 (15.746)
Range (min, max)	38.5, 173.2
Height (cm)	(N=2864)
Mean (SD)	168.55 (8.869)
Range (min, max)	135.0, 198.0
Location of Study, n (%)	
Europe	1393 (47.4)
North America	795 (27.1)
China	751 (25.6)
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 3.1.4.	

Adverse Events

Analysis of Adverse Events

Summaries were provided for:

- Adverse events commonly reported following administration of SonoVue for subjects in all Completed Studies, all Patient Studies and Completed Liver Studies.
- Deaths and other serious adverse events reported for all Completed Studies.

- Adverse events leading to study participation discontinuation for all Completed Studies

There is little to no change to the safety profile of SonoVue between the time of the filing of the last *Safety Update* to the EBD NDA and the submission of this *Liver sNDA*.

Common Adverse Events

All Completed Studies (Healthy Volunteers and Patients)

A summary of adverse events for All Completed Studies is presented in **Table 34**. Of the 6984 subjects who received SonoVue, 774 (11.1%) experienced 1227 adverse events; of these 774, 369 subjects (5.3%) had study-agent related adverse events. The majority of events were mild and resolved without sequelae. Only 11 subjects had nonserious adverse events that were considered severe in intensity and 2 of the 11 were considered to have agent-related events (1 patient experienced hypertension and chills and another experienced headache, both considered by the Investigator to be of 'unknown' relationship to study agent administration).

Twenty-three subjects (0.3%) discontinued study participation due to adverse events; 12 of the 23 had events considered to be related to the administration of SonoVue. Serious adverse events were reported for 36 subjects (0.5%); 5 of the 36 subjects had events that were considered to be related to study agent. For 2 of the 5 subjects, "unknown" relationship is recorded in the clinical trial database for the events, and for one of the 5, "probable" relationship is recorded; however subsequent information about these 3 cases suggests a possibility of no relationship to the investigational product. In addition to the 36 subjects, one subject experienced a non-serious adverse event during study participation that became serious when the patient was hospitalized due to worsening of symptoms outside of the protocol-defined reporting window (after the 24 hours post-dose monitoring period); the event was considered by the Investigator to be unrelated to the administration of SonoVue at both recordings.

Deaths: Eight (0.1%) of the 36 subjects with serious adverse events died during the study; 1 additional subject died 2 weeks after the protocol-defined adverse event reporting window was closed and is therefore not included in the integrated safety database as a death. None of the 9 deaths were considered related to study agent. One other subject suffered a myocardial infarction and died prior to receiving SonoVue.

Table 34: Summary of Adverse Events in All Completed Studies (Healthy Volunteers and Patients)

Category	No. (%) of Subjects (N=6984)	
	Total	Related[a]
No. (%) of subjects with at least 1 AE	774 (11.1)	369 (5.3)
No. (%) of subjects with at least 1 serious AE	36 (0.5)[c]	5 (0.1)[b]
No. (%) of subjects who discontinued due to AEs	23 (0.3)	12 (0.2)
No. (%) of deaths	8 (0.1)[d]	0
No of AEs[e]	1227	587
No. (%) of subjects with at least 1 non-serious AE by intensity:[f]	749 (10.7)	364 (5.2)
Mild AEs	596 (8.5)	315 (4.5)
Moderate AEs	142 (2.0)	47 (0.7)
Severe AEs	11 (0.2)	2 (<0.1)

AE/s = Adverse Event/s.
[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.
[b] One subject in Study BR1-066 experienced 3 related serious adverse events during the clinical trial, but it was reported after database lock by the investigator that these were not related to study drug administration.
[c] One subject in Study BR1-127 experienced a non-serious adverse event (prostatitis) during the study which became serious when the patient was hospitalized due to worsening of symptoms outside of the protocol-defined adverse event reporting window.
[d] One subject, who experienced 2 serious adverse events during the clinical trial, was reported to have died outside of the protocol-defined adverse event reporting window for Study BR1-071. One other death occurred in a subject who died of myocardial infarction before receiving SonoVue in Study BR1-020.
[e] Multiple occurrences of the same adverse event in a subject are counted individually.
[f] If a subject experienced more than 1 non-serious adverse event, the subject was counted only once at the maximum intensity.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 4.1.

The adverse events experienced most frequently (>0.5%) by the 6984 subjects in All Completed Studies are summarized in **Table 35**. The most frequently reported adverse event was headache (148 subjects, 2%), followed by nausea (69 subjects, 1%), chest pain (50 subjects, 1%), chest discomfort (32 subjects, 0.5%) and injection site pain (32 subjects, 0.5%). All other adverse events occurred at a frequency of <0.5%.

Table 35: Adverse Events by System Organ Class Reported in ≥0.5% of Subjects in All Completed Studies (Healthy Volunteers and Patients)

MedDRA System Organ Class Preferred Term	Number (%) of Subjects (N=6984)	
	Total	Related[a]
No. (%) of subjects with at least 1 adverse event	774 (11.1)	369 (5.3)
Gastrointestinal Disorders		
Nausea	69 (1.0)	37 (0.5)
General Disorders/Administration Site Conditions		
Chest discomfort	32 (0.5)	18 (0.3)
Chest pain	50 (0.7)	12 (0.2)
Injection site pain	32 (0.5)	25 (0.4)
Nervous System Disorders		
Headache	148 (2.1)	71 (1.0)

[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.
Data source: Module 5, Section 5.3.5.3, Liver sNDA Safety Update Tables 4.6.1 and 4.6.2.

All Patient Studies

A summary of adverse events for All Patient Studies is presented in **Table 36**. Of the 6856 patients who received SonoVue, 737 (11%) experienced 1171 adverse events. The majority of events were mild and resolved without sequelae. Only 10 patients had non-serious adverse events that were considered severe in intensity; 1 of the 10 experienced hypertension and chills and another experienced headache, both considered by the Investigator to be of 'unknown' relationship to study agent administration.

Study-agent related adverse events were reported by 340 patients (5%). Since no serious adverse events or deaths occurred in the Healthy Volunteer Studies, the summary of serious adverse events and deaths in the All Patients Studies is the same as that described for All Completed Studies (Healthy Volunteers and Patients).

Twenty-two patients (0.3%) discontinued due to adverse events; 11 of the 22 patients had events considered to be related to the administration of SonoVue.

Table 36: Summary of Adverse Events in All Patient Studies

Category	No. (%) of Subjects (N=6856)	
	Total	Related[a]
No. (%) of patients with at least 1 AE	737 (10.7)	340 (5.0)
No. (%) of patients with at least 1 serious AE	36 (0.5)[c]	5 (0.1)[b]
No. (%) of patients who discontinued due to AEs	22 (0.3)	11 (0.2)
No. (%) of deaths	8 (0.1)[d]	0
No of AEs[e]	1171	545
No. (%) of patients with at least 1 non-serious AE by intensity:[f]	712 (10.4)	335 (4.9)
Mild AEs	563 (8.2)	288 (4.2)
Moderate AEs	139 (2.0)	45 (0.7)
Severe AEs	10 (0.1)	2 (<0.1)

AE/s = Adverse Event/s.

[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.

[b] Patient No. 1403 of Study BR1-066, experienced 3 related serious adverse events during the clinical trial but was reported post database lock by investigator to be not related.

[c] One additional patient, Patient No. 0610 of Study BR1-127, experienced a non-serious adverse event (prostatitis) during the study which became serious when the patient was hospitalized due to worsening of symptoms outside of the protocol-defined adverse event reporting window.

[d] One additional patient, who experienced 2 serious adverse events during the clinical trial, was reported to have died outside of the protocol-defined adverse event reporting window for Study BR1-071. One other death occurred in a patient who died of myocardial infarction before receiving SonoVue in Study BR1-020.

[e] Multiple occurrences of the same adverse event in a patient are counted individually.

[f] If a patient experienced more than 1 non-serious adverse event, the patient was counted only once at the maximum intensity.

Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 5.1.1.

The adverse events experienced most frequently (>0.5%) by the 6856 patients in All Patient Studies are summarized in **Table 37**. The most frequently reported adverse event was headache (141 patients, 2%), followed by nausea (68 patients, 1%), chest pain (50 patients, 1%), and chest discomfort (31 patients, 0.5%). All other adverse events occurred at a frequency of <0.5%.

Table 37: Adverse Events by System Organ Class Reported in ≥0.5% of Subjects in All Patient Studies

MedDRA System Organ Class Preferred Term	Number (%) of Subjects (N=6984)	
	Total	Related[a]
No. (%) of patients with at least 1 adverse event	737 (10.7)	340 (5.0)
Gastrointestinal Disorders		
Nausea	68 (1.0)	37 (0.5)
General Disorders/Administration Site Conditions		
Chest discomfort	31 (0.5)	17 (0.2)
Chest pain	50 (0.7)	12 (0.2)
Nervous System Disorders		
Headache	141 (2.1)	65 (0.9)

[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.
Data source: Module 5, Section 5.3.5.3, Liver sNDA Safety Update Tables 5.2.1 and 5.2.2.

Similar incidences of adverse events were reported among patient subgroups by gender, age, and cumulative normalized dose (**Table 38**). The numbers of patients reporting at least one adverse event were comparable among the cumulative normalized dose groups.

Table 38: Summary of Adverse Events by Subgroup, All Patient Studies, SonoVue

Subgroup/Category	Number of Patients in the Studies	Number (%) of Patents With at Least 1 Adverse Event	
		Total	Related[a]
All Patients	6856	737 (10.7)	340 (5.0)
Gender			
Male	4343	441 (10.2)	201 (4.6)
Female	2512	296 (11.8)	139 (5.5)
Age Group			
<65 yrs	4182	461 (11.0)	226 (5.4)
≥65 yrs	2672	276 (10.3)	114 (4.3)
Race			
White	5370	590 (11.0)	284 (5.3)
Black	269	55 (20.4)	17 (6.3)
Hispanic	89	19 (21.3)	9 (10.1)
Asian	1085	64 (5.9)	24 (2.2)
Other	40	9 (22.5)	6 (15.0)
Weight Group			
<75 kg	3492	308 (8.8)	140 (4.0)
75-100 kg	2789	333 (11.9)	163 (5.8)
≥100 kg	463	96 (20.7)	37 (8.0)
Location of Study			
Europe	4452	405 (9.1)	195 (4.4)
North America	1501	297 (19.8)	130 (8.7)
China	903	35 (3.9)	15 (1.7)
Cumulative Normalized Dose			
≤0.02 mL/kg	396	50 (12.6)	19 (4.8)
>0.02 to ≤0.04 mL/kg	1063	115 (10.8)	44 (4.1)
>0.04 to ≤0.06 mL/kg	1003	132 (13.2)	73 (7.3)
>0.06 to ≤0.08 mL/kg	1001	96 (9.6)	53 (5.3)
>0.08 to ≤0.10 mL/kg	643	80 (12.4)	47 (7.3)
>0.010 to ≤ 0.12 mL/kg	532	57 (10.7)	25 (4.7)
>0.12 mL/kg	2073	206 (9.9)	78 (3.8)

[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Tables 5.1.1 to 5.1.6, and 5.3.

Completed Liver Studies

A summary of the adverse events reported in the Completed Liver Studies is provided in **Table 39**. Of the 2939 patients who received SonoVue in the Completed Liver Studies, 180 patients (6%) experienced 282 adverse events; the events were reported as study agent-related for 74 patients (3%).

Fifteen patients (0.5%) experienced serious adverse events; none were considered related to administration of study agent. Three patients (0.1%) died while participating in a clinical trial and 1 additional patient died as a result of his underlying disease 2 weeks after completing a clinical trial (the occurrence of death was reported outside of the protocol-defined adverse event reporting window); all 4 deaths were considered unrelated to study agent administration. All adverse events were mild or moderate in intensity, with the exception of 3 events reported to be of severe intensity.

No patient in the Completed Liver Studies discontinued participation as a result of an adverse event.

Table 39: Summary of Adverse Events, Completed Liver Studies

	(N=2939)	
Category	**Total**	**Related[a]**
No. (%) of patients with at least 1 AE	180 (6.1)	74 (2.5)
No. (%) of patients with at least 1 serious AE	15 (0.5)	0
No. (%) of patients who discontinued due to AEs	0	0
No. (%) of deaths	3 (0.1)[b]	0
No of AEs[c]	282	116
No. (%) of patients with at least 1 non-serious AE by intensity[d]	172 (5.9)	74 (2.5)
Mild AEs	137 (4.7)	66 (2.2)
Moderate AEs	32 (1.1)	7 (0.2)
Severe AEs	3 (0.1)	1 (<0.1)

AE/s = adverse event/s.
[a] Includes definite, probable, possible, doubtful, unknown, and missing relationships.
[b] One additional patient (0816 in Study BR1-071), experienced serious adverse events (worsening of nausea and vomiting) during the study and died 2 weeks after completing the study.
[c] Multiple occurrences of the same adverse event in a patient are counted individually.
[d] If a patient experienced more than 1 non-serious adverse event, the patient was counted only once at the maximum intensity.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Table 9.1.

Adverse events by MedDRA System Organ Class and Preferred Term reported in >0.5% of the patients in the Completed Liver Studies are provided in **Table 40**. The only adverse events occurring in >0.5% were headache (26 patients, 1%), nausea (24 patients, 1%), abdominal pain (20 patients, 1%), and dysgeusia (14 patients, 0.5%). All other events occurred in <0.5% of the patients. The only study agent-related adverse event reported in >0.5% of the patients was headache (17 patients, 0.6%).

Table 40: Adverse Events by System Organ Class Reported in >0.5% of the Patients, Completed Liver Studies

MedDRA System Organ Class / Preferred Term	Number (%) of Patients (N=2939)	
	Total	Related[a]
No. (%) of patients with at least 1 adverse event	180 (6.1)	74 (2.5)
Gastrointestinal Disorders		
Abdominal pain	20 (0.7)	2 (0.1)
Nausea	24 (0.8)	9 (0.3)
Nervous System Disorders		
Dysgeusia	14 (0.5)	13 (0.4)
Headache	26 (0.9)	17 (0.6)

[a] Includes definite, probable, possible, doubtful, unknown, and missing relationship.
Table data derived from Module 5, Section 5.3.5.3, Liver sNDA Safety Update Tables 9.2.1 and 9.2.2.

Deaths and Other Serious Adverse Events

Deaths

No difference is observed between deaths reported for the completed and ongoing studies in the Integrated Summary of Safety (ISS) provided in the Original NDA and those reported in this sNDA submission. No deaths were reported in Healthy Volunteer Studies. Eight (0.1%) of the 36 subjects with serious adverse events in the All Completed Studies in the Integrated SonoVue Safety Database had a fatal outcome during a clinical study. One additional subject died 2 weeks after the protocol-defined adverse event reporting window was closed and is therefore, not included in the integrated safety database as a death. All 9 deaths were considered to be unrelated to study agent.

Deaths occurred in both cardiac and non-cardiac studies. Among the 8 deaths:

- –1 subject had procedural complications during percutaneous coronary interventions following a well-tolerated echocardiographic exam with SonoVue;
- –1 subject had procedural complications during coronary angioplasty performed after the completion of stress echocardiography with SonoVue;
- –1 subject died 3 days after SonoVue administration and shortly after undergoing right hepatectomy;
- –5 subjects died 10 to 26 days after exposure to SonoVue. In none of these 5 cases did the death follow any reaction or complication related to the administration of SonoVue.

One other subject suffered a myocardial infarction and died prior to receiving SonoVue.

Five deaths occurred in Study BR1-128 and 4 in Study BR1-130; none were related to SonoVue.

Serious Adverse Events

No notable difference was observed between the incidence of serious adverse events reported for the completed and ongoing studies in the ISS provided in the Original NDA and those reported in this sNDA submission (0.4% versus 0.5%, respectively).

No serious adverse events were reported in Healthy Volunteer Studies. Of the 6984 subjects who received SonoVue in All Completed Studies, 36 (0.5%) had serious adverse events. Of these 36 subjects, 31 reported events that were considered unrelated to SonoVue administration. Four of the 5 cases potentially related to the investigational product occurred in patients with cardiovascular diseases treated within cardiac studies.

Completed Liver Studies
In the completed liver studies, 15 patients (0.5%) experienced serious adverse events; none was considered related to administration of study agent. Three patients (0.1%) died while participating in a clinical trial, and 1 patient died as a result of his underlying disease 2 weeks after completing a clinical trial (the occurrence of death was reported outside of the protocol defined adverse event reporting window); all the deaths were considered unrelated to study agent administration.

Adverse Events Leading to Discontinuations

All Completed Studies (Healthy Volunteers and Patients)
Twenty-three (0.3%; 1 Healthy Volunteer, 22 Patients) of the 6984 subjects who received SonoVue discontinued due to adverse events in the All Completed Studies. Of these 23 subjects, 11 subjects reported events that were considered unrelated to SonoVue administration. The most commonly reported study agent-related adverse events resulting in discontinuation were hypotension reported by 4 subjects and nausea reported by 3 subjects (<0.1% each). All other study agent-related adverse events resulting in discontinuation each occurred in 1 subject.

Completed Liver Studies
No patient discontinued as a result of an adverse event in the Completed Liver Studies.

Clinical Laboratory Evaluations

All Patient Studies
Clinical laboratory evaluations were summarized only for All Patient Studies. A small number of patients had increases and/or decreases in hematology, serum chemistry, and/or urinalysis values that met the criteria for substantial changes from baseline. The incidence of specific marked abnormalities for hematology, serum chemistry, and urinalysis was low, reported in <5%, <3% and <1.%, respectively, of the subjects in All Patients Studies.

Vital Signs and Other Observations Related to Safety

All Patient Studies
Vital signs and other observations related to safety were summarized only for All Patient Studies. For All Patient Completed Studies, vital sign and oxygen saturation values that met the sponsor defined criteria for changes from baseline of potential clinical importance were summarized. For each vital sign parameter (systolic blood pressure, diastolic blood pressure and heart rate), the percentage of patients with increases from baseline of potential clinical

importance was similar to the percentage with decreases from baseline of potential clinical importance. For vital signs and oxygen saturation, changes from baseline that met the criteria for potential clinical importance were relatively low for the patients who received SonoVue.

Electrocardiograms

All Patient Studies

Electrocardiogram evaluations were summarized only for All Patient Studies. For All Patient Studies, a summary of clinically significant changes from baseline in ECG parameters (heart rate, QT interval and QTc intervals using Bazett and Fridericia corrections) were displayed by time points. Overall, the post dose changes in ECG parameters include small increases and decreases from baseline in the majority of patients across all time points. Most changes in heart rate were ≤10 bpm. Most changes in QT and QTc values were ≤30 msec; changes >60 msec were few and occurred at sporadic time points.

Safety in Special Groups and Situations

Intrinsic Factors

In the All Patient Studies for patients in Completed Microvasculature Studies (including the 23 Liver Studies), similar incidences of adverse events were reported among patient subgroups by gender, age, and race.

Extrinsic Factors

There is no apparent trend in the incidence of adverse events associated with the dose of SonoVue administered. Similar incidences of adverse events were reported among subgroups by cumulative normalized dose and cumulative volume groups.

Drug Interactions

No specific interaction studies have been performed in humans. In preclinical studies, SonoVue did not interact with the action of aspirin in vitro, or with the action of antihypertensive drugs (captopril, propranolol, or nifedipine), heparin, isosorbide dinitrate, or digoxin in rats in vivo. There was no apparent relationship with respect to occurrence of adverse events in the clinical studies for patients receiving various categories of the most common concomitant medications.

Use in Pregnancy and Lactation

Reproduction studies have been performed in rats and rabbits at daily doses up to at least 17 times and 35 times the human exposure, respectively, based upon body surface area, and have revealed no evidence of impaired fertility or harm to the fetus due to SonoVue.

Because animal reproduction studies are not always predictive of human response, and there are no adequate and well-controlled studies in pregnant women, SonoVue should not be used during pregnancy unless the physician determines the benefit of the use of SonoVue-enhanced procedure exceeds the risk to the fetus, infant and/or mother.

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when SonoVue is administered to a nursing woman.

Pediatric
Based on the 31 March, 2015 teleconference with FDA, the Sponsor is seeking an indication for use of Lumason in characterization of FLLs in pediatric patients.

Overdose
Since there have been no cases of overdose reported to date, neither signs nor symptoms of overdose have been identified.

In a Phase I study, doses up to 52 mL of SonoVue (i.e., approximately 11 times the highest recommended dose, 4.8 mL, in humans) were administered to healthy volunteers without any serious adverse events. In SonoVue clinical trials, cumulative doses of up to 161 mL have been administered to patients, including those who received multiple bolus doses in crossover studies and those who received infusion dosing regimen. No dose-related trend was observed in the safety of SonoVue.

In the event of overdose, treatment is directed toward the support of all vital functions and prompt institution of symptomatic therapy.

Drug Abuse
SonoVue is only administered intravenously 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
SonoVue is given as a single administration and has no pharmacologic effect. A rebound effect is not expected. No rebound was observed after stopping SonoVue use.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Ability
No impairment of driving or psychometric performance was observed following SonoVue administration.

Postmarketing Data

Exposure
SonoVue is currently approved for intravenous use in 39 countries throughout the world and is marketed in 26 countries, indicated for use with echocardiography to provide opacification of cardiac chambers and enhance left ventricular EBD, Doppler of macrovasculature, and Doppler of microvasculature. SonoVue has recently been approved by the FDA under the trade name Lumason™ (sulfur hexafluoride lipid-type A microspheres) for use in patients undergoing transthoracic echocardiography with suboptimal images to improve visualization of EBD and left ventricular opacification.

SonoVue should be administered using a 5-mL single vial per investigation (doses: 2.0 mL for endocardial border detection or 2.4 mL for Doppler of macrovasculature and Doppler of

microvasculature, repeated once if necessary). An estimate of patient exposure is thus calculated on the basis of the number of single dose vials sold from April 1, 2001 to September 30, 2014. Denominators are estimated from sales statistics, with each unit sold representing a patient exposed to the agent.

During market use (April 1, 2001 through September 30, 2014), an estimated (b) (4) patients have been exposed to SonoVue.

Adverse Events

Reporting rates (RR) of spontaneously reported adverse events are patient-based (case-based) and are expressed as percentages, calculated by dividing the number of patients (cases) who experienced one or more adverse events by the number of patients estimated to have been exposed during a reporting period, then multiplying by 100. The adverse events could have been serious (RR for serious adverse events) or non-serious (RR for non-serious adverse events). Any patient who reported at least one adverse event is counted as a case. If a patient reported both serious and non-serious events, the patient is counted as a serious case and not a non-serious case. Each case is assessed as related ("probably", "possibly", "unknown") causality or unrelated causality ("unrelated") by both the Reporter and the Sponsor. The assessment of "related" is assigned only if both Reporter and Sponsor assess the event as related.

A total of 968 cases (RR: (b) (4)%) which were not considered to be "unrelated" to the administration of SonoVue by both Reporter and Sponsor assessments have been spontaneously reported during market use. Among the 968 cases, a total of 388 cases were classified as serious (RR: (b) (4)%) and 580 as non-serious (RR: (b) (4)%). As of September 30, 2014, a total of 14 cases (b) (4)%) with fatal outcome have been received by the Sponsor during post-marketing use of SonoVue (since the launch of the product in 2001). The association of the deaths with SonoVue administration could not be ruled out in 9 of the 14 cases; there was no relation to SonoVue reported for the remaining 5 cases.

In addition to these 14 patients, 5 other patients experienced serious adverse events after the administration of SonoVue and subsequently died. Among these 5 patients: 1 patient experienced a serious adverse event of anaphylactoid shock with recovered / resolved outcome, considered to be related to the administration of SonoVue, and subsequently died almost 7 weeks later due to underlying cardiac disease; 1 other patient experienced an allergic reaction considered to be related to the administration of SonoVue with an unknown outcome, and subsequently died 3 days later following multi-organ failure (unrelated to the administration of SonoVue); 1 patient experienced serious adverse events of bleeding in the left carotid artery and acute respiratory insufficiency 12 hours after the administration of SonoVue, both events considered unrelated; 1 patient experienced serious adverse events of hypersensitivity (considered related to SonoVue administration) and pulmonary embolism (considered unrelated to SonoVue administration) from which the outcome was considered to be unknown, however, the patient died 3 days later due to multi-organ failure; 1 patient experienced a serious adverse event of acute renal failure, considered to be unrelated to SonoVue administration, from which he did not recover with death occurring 6 days later.

The overall RR of allergic-like reactions during SonoVue market use based on the Reporter's diagnosis, is 0.01% (1 in 10,000). Allergic-like reactions included the terms anaphylactic reaction/shock, anaphylactoid reaction/shock and hypersensitivity.

The total number of cases of serious adverse events has been low and generally stable across the last 13.5 years of market use of SonoVue. The RR for serious cases per year through 30 September 2014 remained low (ranging from (b) (4)%) and stable across the last 13.5 years of Pharmacovigilance surveillance.

In the period between the data lock point of this Safety Update (30 September, 2014) and 31 March 2015, approximately (b) (4) patients were exposed to SonoVue, worldwide. In this period, 66 new post-marketing ADR reports were received from worldwide sources (RR= (b) (4)%). Of the 66 ADR reports received, 32 ((b) (4)%) were serious (of which 1 case was fatal) and 34 were non-serious ((b) (4)%). For the case with fatal outcome, relationship was considered as unlikely

The reported reactions included 26 hypersensitivity/ anaphylactoid reactions (RR= (b) (4) of which 8 were non-serious (RR (b) (4)) and 18 were assessed as serious (RR= (b) (4)).
In summary, data from post-marketing surveillance database show:

- the adverse reactions reported for SonoVue were, in general, non-serious, transient, and resolved spontaneously without residual effects;
- serious allergy-like reactions may unpredictably and rarely occur after SonoVue administration, and the reporting rate is low;
- no significant change in the safety profile of SonoVue has been demonstrated since the original NDA submission in December 2011 for the now approved EBD indication.

Detailed summaries of postmarketing safety data were provided.
Narratives of deaths reported during postmarketing surveillance were available.

Safety Data Reported in the Literature

For the *Original NDA ISS* and each subsequent safety update report, a literature search was performed to provide supportive evidence of the safety of intravenous SonoVue administration during echocardiography and non-cardiac ultrasound studies. The same search criteria ((SonoVue OR Sulfur hexafluoride OR BR1) AND (echocardiography OR ultrasound); search was limited to "human"; no other limits applied) were used for each search. A total of 816 references were identified in the cumulative PubMed search result, among which 98 publications met the inclusion criteria. Of the 98 relevant publications, 22 reported 12,426 patients undergoing echocardiography examination for cardiac indications (*Cardiac Population*) and 76 reported 31,369 patients undergoing ultrasound examinations for non-cardiac indications (*Noncardiac Population*), including 32 reporting 29,848 patients undergoing SonoVue-enhanced imaging of the liver (*Liver Population*). A tabular representation of the results of these searches is provided in **Table 41**.

Table 41: Results of Literature Safety Searches

Safety Document Name	Data Cut-off Date for Inclusion of Published Articles	Results of Literature Searches			
		Number of Articles Retrieved	Number of Relevant Articles	Cardiac Population	Non-cardiac Population (Liver Population)
Original NDA ISS	September 30, 2011	763	87	20	67 (29)
4-Month Safety Update	January 31, 2012	10	8	0	8 (3)
12-Month Safety Update	December 31, 2012	27	3	2	1
Safety Update	September 30, 2013	9	0	0	0
Liver sNDA Safety Update	September 30, 2014	7	0	0	0
NOTE: A copy of each relevant article was provided within the submission of the respective safety document.					

The literature search performed for this *Liver sNDA Safety Update* did not yield any new publication reporting safety information regarding the use of SonoVue. A summary of the relevant literature among the Cardiac and Non-cardiac Populations yielded in previous searches was provided in the *Original NDA ISS* and the *12-Month Safety Update* (included those reported in the *4-Month Safety Update*).

A total of 29,848 patients underwent contrast-enhanced ultrasound imaging of the liver reported in 32 studies. The reported incidence of adverse events after SonoVue administration in these studies was low (30 events in 29,848 patients, or 0.10%). A large retrospective survey of 23,188 patients undergoing abdominal ultrasound with SonoVue (the large majority, 21,346, were from liver clinical studies) reported 29 of the 30 events, including 27 non-serious (23 mild, 3 moderate and 1 severe) and 2 serious events. Non-serious events included itching, mild dizziness, moderate hypertension, headache, warm sensation, nausea and vomiting. Serious events included one anaphylactoid reaction and another (clouding of consciousness, dorsolumbar pain, severe hypotension and cutaneous rash) in a patient with suspected renal artery in-stent restenosis. The role of SonoVue in this event could not be assessed.

6. Overview of Efficacy and Safety of SonoVue in the Pediatric Population

There are no Bracco-sponsored studies in the pediatric population. FDA requested a meeting with the sponsor by teleconference on 31 March, 2015. FDA indicated interest in considering all available pediatric data (prior pediatric clinical experience for Lumason, including retrospective studies, published literature and clinical practice guidelines) as part of the upcoming sNDA submission. Therefore, the Sponsor has included available information about intravenous use of Lumason in the pediatric population, with specific reference to liver lesion characterization, in this sNDA.

Guidelines

Use of CE-US in pediatric patients is acknowledged by the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) in the guidelines and recommendations on the

clinical practice of contrast enhanced ultrasound released in 2011. One major advantage of CEUS in pediatric applications, compared to alternative imaging modalities, is the absence of exposure to ionizing radiation which is a concern in pediatric patients.

Literature Search

To investigate efficacy and safety related to the use of SonoVue in the pediatric population, a literature search was performed by the sponsor utilizing PubMed, a service of the US National Library of
Medicine® and the Excerpta Medica dataBASE (EMBASE®), provided by Elsevier. The search terms were (SonoVue or sulfur hexafluoride or BR1) AND (focal liver lesion or FLL). A second literature search was performed utilizing PubMed and the terms (SonoVue or sulfur hexafluoride or BR1) AND (intravenous or IV). For both searches, the date range was 2001 to March, 2014 and the following filter for age range was applied "child: birth to 18 years".

Five additional papers about the intravenous administration of SonoVue in pediatric patients were identified through the Google Scholar browser. In total, 64 references were identified. After exclusion of duplicate papers (n=14), the search results comprised 50 references. All 50 publications underwent medical review to identify those that reported efficacy data for FLL characterization and/or safety information after intravenous administration of SonoVue in pediatric patients.

Eligibility Criteria

Publications that met all the following inclusion criteria were included in the SonoVue efficacy and safety summaries for the pediatric population:

- Original publication of a clinical study in pediatric patients (birth to 18 years) with prospective or retrospective enrollment;
- SonoVue was administered intravenously during a non-cardiac ultrasound examination; and
- Information on safety (e.g. adverse events, side effects, complications) after intravenous SonoVue administration was reported (safety summaries only);
- Information on efficacy of SonoVue–enhanced ultrasonography for FLL characterization (efficacy summaries only) Publications that did not meet the inclusion criteria or met the following exclusion criteria were excluded from the SonoVue safety and efficacy summary for the pediatric population:
- Study was performed in non-human subjects (e.g. phantom, in vitro or animal studies);
- SonoVue was not administered or was not administered intravenously;
- Results were from Bracco-sponsored clinical trials; or
- Publications were other than study reports, such as review articles, author correspondence, editorials, letter-to-editor, case reports or conference or scientific meeting abstracts that have no or insufficient data of study population, study methodology and results or if there is a lack of completeness in the reports.

Results

Among the 50 unique references identified during the literature searches, 38 papers reported data for SonoVue use in adults, and 17 of the 38 included at least one patient <18 years of age (age

range, 12-17 years) in the study population; however, no separate or specific information was reported for the patients <18 years and, therefore, these papers are not included in the efficacy or safety summaries.

Among the 50 unique references, 12 reported on the use of SonoVue in a population <18 years of age; this included 8 papers in which SonoVue was administered intravenously, 3 papers in which a route of administration other than IV was used, and 1 paper in which IV and intravesicle administration of SonoVue were reported.

A total of 6 references met all selection criteria. Of these 6 publications, one publication, Jacob et al, reports efficacy of SonoVue in the characterization of FLLs in the pediatric population; safety information is also presented in the paper. The findings from this study are provided. Additional clinical experience with SonoVue in the characterization of FLLs in the pediatric population, published in abstract format is also provided.

Safety information for intravascular administration of SonoVue from the 6 publications is also provided.

Pediatric Patient Usage of SonoVue Contrast-Enhanced US

Ultrasonography (US) may be a useful screening study in a child with a suspected liver mass, as there is no ionizing radiation and no need for sedation. If there are multiple liver masses, the main diagnostic consideration is metastatic disease and the primary tumor should be sought. Color Doppler evaluation may suggest a vascular nature of the tumor, as with infantile hemangio-endothelioma or FNH. Ultrasound is also useful for distinguishing solid tissue from liquid (e.g., flowing blood or the contents of abscesses or cysts).

If a FLL cannot be properly characterized at US, further imaging with computed tomography (CT) or magnetic resonance (MR), or biopsy and pathologic assessment of the lesion, are usually performed to obtain a final diagnosis. Use of ultrasound contrast in children with FLLs that are indeterminate at plain ultrasonography has been reported in a survey carried out in the Europe among members of the European Society of Pediatric Radiology to investigate the use of SonoVue (brand name in Europe) showed that, out of 146 respondents, 88 did not perform contrast-enhanced US in children.

Forty-five centers reported 5,079 examinations with SonoVue in children (mean age: 2.9 years; range: birth-18 years). The majority (4,131, 81%, 29 centers) were intravesical applications for detection of vesico-ureteral reflux. The minority (948, 19%, 30 centers) were intravenous (IV) applications. As for IV use, the mean patient age was 5 years (range: newborn to 18 years, gender distribution: 1 male to 2 females). The most frequently targeted organ was indeed the liver, less frequently the spleen, rarely the kidney and pancreas, with a few additional target organs such as the ovary or vessels. The indications were mostly oncological, traumatic or inflammatory conditions, either lesion detection, as in subacute or moderate trauma, or lesion characterization (e.g. abscess vs. neoplasm), or some dedicated rare queries (such as transplant and vascular examinations).

To provide evidence supporting the use of SonoVue for the characterization of focal liver lesions in children, the Sponsor has evaluated the efficacy data available in the peer-reviewed literature (**Table 42**). Although the approved product name in the United States is Lumason, within this document, the contrast agent is referred to as SonoVue when presenting summary efficacy data.

Table 42: Clinical Efficacy Experiences in Pediatric Patients (Literature)

Study	Patients Number /Ages	Dose	Indication	Efficacy	Safety
Jacob et al 2013	44 Median age 11 4 – 18yrs	1.2 or 2.4 mL	FLL	44 - benign	No adverse reactions
Franke 2013	35 5-7 years	2.4 mL	FLL	21 – benign 11- malignant	No side effects
Gronthues et al 2006	6 7 days – 12 years	0.5 to 2.0 mL	Hepatoblastoma	6- malignant	-
Franke et al 2013	10 0.5 – 17 years	not provided	FLL	Benign and malignant	No side effects

Efficacy in the Pediatric Population (Literature)

1. Jacob et al [2013]

The usefulness of SonoVue-enhanced ultrasound in characterizing indeterminate focal liver lesions (FLLs) was demonstrated by Jacob et al in a study conducted at the Pediatric Liver Unit of King's College Hospital in London. The prospective study was conducted to evaluate the diagnostic performance of Lumason enhanced ultrasound in the characterization of grey-scale sonographic indeterminate FLLs in pediatric practice.

Over a 5-year period (September 2007 to August 2012), all children referred for a contrast-enhanced ultrasound assessment following an inconclusive evaluation of a FLL on plain greyscale sonography were included in this study. An indeterminate FLL was defined as a lesion not characterized (as benign or malignant) on grey scale sonography, precluding appropriate clinical management and requiring further imaging or histology.

Indeterminate FLL evaluation included those children with established background chronic liver disease as well as incidental lesions on a background normal liver. Any underlying liver disease was established in patients by conventional non-focal liver biopsy and histology, clinical features, biochemical blood tests or imaging findings.

The grey-scale lesion appearances (either iso-, low- or high-echogenicity in comparison to adjacent liver), location (within a specific liver segment) and size (in two dimensions, with the

largest measurement representing the maximum lesion size) were documented. Once designated as an indeterminate FLL on grey-scale sonography by experienced radiologists, a SonoVue enhanced examination was performed by experienced operators using commercially available equipment and contrast-specific techniques at a low mechanical index (0.10 or lower). This use of a lower mechanical index than the usually utilized 2-10 MHz frequencies, may have labeling implications. (The original publication states "A CEUS examination was performed employing a low mechanical index (MI) technique." The exact MI was not provided.)

Lumason was injected as a bolus at the dose of 1.2 or 2.4 mL followed by 10mL of normal saline flush via an arm vein cannula previously sited by an experienced pediatrician. A three-phase (arterial, portal, and late) examination of the FLL was performed with imaging for at least 3 minutes following administration of Lumason. Images were recorded on cine loop, and transferred to a Picture Archiving System.

SonoVue-enhanced images were jointly evaluated by consensus by three experienced readers prior to any other imaging or histology. The pattern of enhancement throughout the three phases was observed, with attention paid to presence or absence of early increased enhancement, and to the detection of any late phase washout of contrast. A diagnosis was then made.

All patients then underwent either a CT or MR examination of the liver using standard imaging protocols and approved contrast agents. If the FLL was still indeterminate, patients underwent image guided-biopsy of the FLL and histopathology exam of the lesion. The results of the CT (consensus read by experienced radiologist), MR (consensus read by experienced radiologist), or pathology exams were used as standard of truth. In patients with background chronic liver disease, who underwent non-lesion biopsy, any evidence of liver steatosis was used to indicate focal fatty change and evidence of cirrhosis to suggest a regenerative nodule was the FLL observed on the SonoVue-enhanced images. This finding had to be confirmed on subsequent follow-up ultrasonography (6 months or longer) demonstrating no change.

Finally, a consensus read of the cine loop recordings of all the SonoVue-enhanced exams were evaluated in random order and with the patient details obscured, and the final diagnosis recorded. The final diagnosis of this blinded read was matched with the final diagnosis of the first unblinded read and any differences in interpretation noted.

Study Results

Forty-four children (female = 21, male = 23, median age 11.5yrs, range 4 – 18yrs) were included in the study. The predominant reason for referral was the presence of a FLL in a child with known chronic liver disease (n = 30) followed up with ultrasonography, a new FLL (n = 3) following treatment for a non-hepatic malignancy, and incidental finding of a FLL in children with no underlying chronic liver disorder or known primary malignancy (n = 11).
All 44 patients underwent a successful SonoVue-enhanced examination with no adverse reactions to the product identified in the first 30 minutes after the examination, and none recorded in the clinical notes attributed to the administration of the contrast agents.

Standard of truth for FLL characterization in the 44 patient studies was:

- Only CT and/or MR imaging (n = 33 patient studies);
- Histology following lesional/excisional biopsy or liver transplantation (n = 8);
- Follow-up (6-month or longer) with plain ultrasonography (n = 3).

The background liver was subject to biopsy in 14 patients showing liver steatosis (n = 9) or cirrhosis (n = 5). CT and/or MR imaging was available for 34 patients (14 CT, 10 MRI, 10 both CT and MRI). In 15 patient studies, CT and/or MRI and SonoVue-enhanced findings were compared to histology (in one patient for FLL characterization, in 14 patients for diffuse liver disease that could explain focal manifestations at SonoVue-enhanced ultrasonography or other imaging).

The median FLL size was 30 mm (range 12 – 79 mm), of which:

- 20 showed lower echogenicity compared to normal liver parenchyma (hypoechoic lesions)
- 15 showed similar echogenicity (isoechoic lesions)
- 9 were hyperechoic.

Based on the blinded assessment of US images, 43 lesions were benign and 1 lesion was malignant. FLLs were identified as:

- Regenerative nodule (n=15)
- Focal fatty sparing (n=11)
- Focal nodular hyperplasia (n=7)
- Focal fatty infiltration (n=6)
- Hepatocellular adenoma (n=2)
- Abscess (n=1).
- Uncertainty (FNH or adenoma) (n=1)
- Malignant lesion (n=1) (false-positive)

Based on the final diagnosis, specificity was 98% (43 lesions were correctly diagnosed as benign), with a 95% CI: 86-100%; the negative predictive value was 100%. One single lesion was misdiagnosed as malignant by all imaging modalities (CE-US, CT/MRI). Sensitivity could not be calculated in this study.

In 20/22 cases (91%), the SonoVue-enhanced diagnosis (for FLL or diffuse liver disease) concurred with histology. In 13 cases, where all the imaging modalities concurred, histology demonstrated a different type of lesion (hepatic adenoma in 1 case, while all the imaging modalities suspected a malignant lesion; regenerative nodule in liver cirrhosis on imaging, different type of benign lesion at histology, lesion type not reported in the article).

The SonoVue-enhanced diagnosis agreed with that of the reference imaging procedure (either CT, MR or both) in 29/34 (85 %) of the cases. In the 5 cases where there was disagreement:

- In 4 cases, SonoVue -enhanced sonography identified 4 focal lesions thought to be the effects of fatty change (focal fatty infiltration fatty sparing or focal on diffuse liver steatosis), not seen on either CT or MR imaging; all 4 lesions were still present and unchanged on follow-up sonography;
- In 1 case, the FLL was thought to be a regenerative nodule in liver cirrhosis, not seen on CT or MR imaging, still present and unaltered on follow-up sonography.

No adverse reactions to SonoVue were identified in the first 30 minutes after the examination, and none recorded in the clinical notes attributed to the administration of the contrast agent.

Conclusions and Limitations

In agreement with previously reported adult data, the continuing iso- or hyper-echoic nature of the FLL in the late portal-venous phase imaging allowed accurate interpretation indicating a benign abnormality in over 90% of patient studies. Compared to CT and/or MRI, in 5 cases (11% of this patient population), the grey-scale sonographic examination identified a FLL, which was not identified on the reference imaging (n = 5), and these FLLs, focal manifestations of diffuse liver disease, were properly characterized as benign lesions by the SonoVue-enhanced exam. Of these, particular importance should be given to FLLs in the context of liver steatosis when considering the obesity epidemic apparent in the pediatric population.

No immediate-type adverse reaction, of any type, was observed following the administration of the agent, suggesting a positive risk-benefit profile of the agent for this use in pediatric patients. The main limitation of this study is the small number of patients. It should be noted, however, that all patients with an inconclusive exam have been enrolled in this study, and that it took 5 years to gather data from these 44 children. This reflects the frequency in which a FLL that is indeterminate on grey-scale sonography is encountered in clinical practice. Also, the study did not include any true malignant FLL, which again relates to the very low incidence of liver cancer in pediatric patients, and the fact that this study was carried out at a tertiary referral treatment center and children with malignancies presented with disease already characterized by CT, MRI or liver biopsy.

2. Three Additional Clinical Experiences in Pediatric Patients

In addition to the study by Jacob et al, 3 other studies utilizing intravenous SonoVue in pediatric patients with FLLs, published in an abstract format, were identified.

In **one study (1)**, the accuracy and safety of CE-US in pediatric patients with FLLs were assessed.

Thirty-five (35) consecutive pediatric patients (22 males; age range: 5-17 years) with FLL detected at UE-US were investigated; 20 of the 35 patients had a known neoplastic disease and 15 had an incidental liver lesion. All 35 patients underwent CE-US with intravenous administration of 2.4 mL of SonoVue. The diagnostic accuracy of CEUS against findings on CT, MRI or histopathology was assessed.

Overall, 11 *malignant lesions* included:

- 2 hepatocellular carcinoma [HCC]
- 2 non-Hodgkin lymphoma [LNH]
- 6 metastases,

24 lesions were *benign*

- 13 angiomas,
- 4 focal steatosis,
- 3 liver abscesses,
- 4 focal nodular hyperplasia [IFN]).

SonoVue-enhanced ultrasound showed 91.5% accuracy (32/35 liver lesions). No side effects were observed in any patient.

In **one study (2)**, the clinical value of SonoVue-enhanced ultrasound was assessed in 6 children with hepatoblastoma (age range 7 days to 12 years). Patients underwent CE-US with intravenous administration of 0.5-2 mL of SonoVue.

In **one study (3)**, 10 pediatric patients (age range 0.5 to 17 years; median 11.5 years) with FLLs including focal nodular hyperplasia, bilioma, hemangioma, adenoma, focal fatty changes, lymphoma (1) and HCC (1). The SonoVue dose was not provided.

Pharmacokinetics in Pediatric Patients (excerpted from the sponsor)

Lumason has a well-established pharmacokinetic profile that has been studied and characterized in clinical trials in adults with healthy and impaired lungs.

The gas phase in the Lumason vial is an innocuous gas, sulfur hexafluoride (SF_6). The total amount of SF_6 administered in a clinical dose is extremely small (2 mL dose contains 16 µL of SF_6 microspheres). Most or all of the SF_6 from a Lumason dose rapidly dissolves in the blood and subsequently eliminates by the lungs. The cumulative recovery of SF_6 in expired air averaged 86% to 94% of the administered dose in healthy subjects. Furthermore, the recovery of SF_6 in expired air in subjects with impaired lungs averaged 102%. This finding indicates that the patients eliminate all of the SF_6 from Lumason via their lungs rather than an alternate elimination route, despite the impairment of lung function. In addition, a published study by Morel et al had shown that Lumason rapidly removed from the blood by the pulmonary route with 40% to 50% of the injected dose eliminated within the first minute after administration and 80% to 90% eliminated by 11 minutes after administration.

The pharmacokinetics of Lumason have been previously tested in adults through the analysis of SF_6 from expired air and blood samples taken sequentially over approximately 60-120 minutes post dose. The method for collection of exhaled air is technically challenging and also dependent on a high degree of patient compliance. The technique involves collection of expired air into plastic bags via a respiratory mask and the use of a pulmonary monitoring system (e.g., Spirobank). Collection of expired air continues up to 120 min postdose. Apparently, such a level of compliance cannot be expected from children unless they are appropriately sedated during the whole procedure.

In addition, conducting a comprehensive pharmacokinetic study in healthy children administered Lumason would be not feasible since the children enrolled in such a study would not gain any benefit from exposure to Lumason while in order to detect the small quantities of SF_6 in expired air, doses 10 times higher than the proposed efficacious Lumason dose may be needed. Bracco would anticipate overwhelming ethic obstacles in obtaining approvals from Institutional Review Boards (IRB) and Ethics Committees (EC), considering unfavorable risk-benefit ratio for thistype of study.

SonoVue Safety in the Pediatric Population (Literature)

Among the 6 original publications with safety information reported after intravenous administration of SonoVue), 5 were reports from single center studies (3 prospectively conducted) and one reported the results from a large multicenter retrospective survey on the safety of SonoVue in 5,079 examinations, 948 of which were after intravenous administration of the contrast agent. The total number of pediatric patients included in the 6 publications is 1259; the age range in the publications is 2 months up to 18 years. Clinical indications for the use of SonoVue in the single center studies were post-operative follow-up of pediatric liver transplant recipients (n=1 paper), assessment of abdominal solid organ injuries in hemodynamically stable patients after blunt abdominal trauma (n=2 papers), characterization of FLLs (n=1 paper); one study enrolled oncologic pediatric patients including 39 children in whom CE-US was focused on the liver. Clinical indications reported in the retrospective survey included oncological, traumatic or inflammatory conditions (either lesion detection, e.g., in subacute or moderate trauma, or lesion characterization, e.g., abscess vs tumor), and some dedicated rare queries, such as transplant and vascular examinations.

A summary of the 6 original publications is provided in **Table 43**. Overall in these 6 publications, 7 adverse events were reported in 6 patients, including one serious adverse reaction of hypersensitivity reported in an 11-year-old female. All events were reported in 2 of the 6 publications; for the remaining 4 studies, the publications stated that no adverse events were observed after SonoVue administration.

In addition to the 6 original peer-reviewed publications, 3 other studies about the intravenous use of SonoVue in pediatric patients, published in an abstract format, included safety information. In one study abstract, 35 consecutive pediatric patients (22 males; age range: 5-17 years) with FLL detected at UE-US underwent CE-US with intravenous administration of 2.4 mL of SonoVue to assess the diagnostic accuracy of CE-US against findings on CT, MRI or histopathology. No side effects were observed in any of the study patients.

In a second study abstract, no side effects were reported in 10 pediatric patients (age range 0.5 to 16.9 years; median 11.5 years) with FLLs including focal nodular hyperplasia, bilioma, hemangioma, adenoma, focal fatty changes, lymphoma and HCC.

One abstract reported on 129 SonoVue-enhanced ultrasound examinations performed in 106 children (51 females, 55 males) at a single site, over a five-year period; patients' mean age was 8.9 years (range 0.2 – 17.9 years). In 1 out of 129 examinations a possible allergic side-effect occurred in a 16 year old female.

Table 43: SonoVue Safety Information in Pediatric Patients (Literature)

I.V. SonoVue Safety Information in Pediatric Patients (Literature)				
Author Year Published Journal	**SonoVue Dose Objective**	**Patient Population**	**Methods**	**Safety Results**
Bonini G, et al. 2007 J Ultrasound	IV Bolus 0.5 mL maximum (up to 3 injections)	40 patients range 2 months-10 yrs	CE-US to R/O post-transplant vascular/biliary complications	No adverse reactions
Piskunowicz M, et al. 2015 US in Medicine & Biology	IV Bolus 0.1 -1.8 mL	137 children (167 exams) (39 liver exams) Mean age: 10 yrs (range 0-18 yrs)	Multiple body organs examined	One adverse event of severe anaphylactic shock reported in 11-year-old girl. Event was potentially life-threatening and considered to be related to the administration of SonoVue (0.6 mL). After supportive Rx all symptoms had resolved by 2 Hrs
Riccabona M 2012 Pediatric Radiology	i.v. administration Dose: not reported	948 examinations from 30 centers Mean age: 5 yrs range newborn – 18 yrs	Targeted organs by frequency: - Liver: most frequent - Kidney, spleen, pancreas: rare	5 patients reported 6 side effects, all mild - 1 strange taste and skin reaction - 1 skin reaction - 2 unusual taste - 1 hyperventilation
Valentino M, et al. 2008 Radiology	i.v. administration bolus 2.4 mL, 2 doses	27 patients Mean age: 9 yrs range 4 – 13 yrs	CE-US abdomen	No adverse events were observed
Menichini G, et al. 2015 Radiologia Medica	i.v. bolus 1.2 mL; 2 injections	73 patients Mean age: 9 years Range 0-16 years	CE-US abdomen history minor abdominal trauma,	No adverse effects were observed
Jacob J, et al. 2013 Ultraschall in der Medizin	i.v. bolus 1.2-2.4 mL	44 patients median 11.5 yrs range 4-18 yrs;	referred for CE-US with indeterminate FLL evaluation on UEUS	No adverse reactions

Tables 42 and 43 collectively provide safety data following I.V. administrations of Lumason to >900 pediatric patients (ages 2 months to 18 years) to characterize FLLs.

Postmarketing Surveillance
SonoVue is currently approved for intravenous use in adults in 39 countries throughout the world outside the USA and is marketed in 26 countries. SonoVue has recently been approved by the FDA under the trade name Lumason™ (sulfur hexafluoride lipid-type A microspheres). SonoVue is not approved for use in pediatric population in any country.

During market use of SonoVue from April 1, 2001 through September 30, 2014, Bracco received sporadic reports pertaining to the pediatric population, for which SonoVue was used off-label. Most of the pediatric reports in the Bracco pharmacovigilance database were derived from the published literature.

In addition to the literature-based cases, Bracco has received 8 spontaneous cases of adverse events after intravenous administration of SonoVue in pediatric patients. These included 5 cases of serious adverse reactions, 3 of which were hypersensitivity, and 3 cases of non-serious adverse reactions.

There are no changes in the reported serious cases since the last safety update, and all narratives for these cases have been submitted. Three additional cases are present in the Pharmacovigilance Database that were entered as "off-label use" with no adverse effect reported.

As reported for adults, the type, frequency and severity of adverse events observed following intravenous administration of SonoVue in pediatric patients are similar to that observed in adults.

Benefits and Risks Conclusions
Characterization of focal liver lesions (FLLs) is a common problem in everyday clinical practice, since liver can be the site of both malignant and benign lesions in patients with a history of cancer or in patients with chronic liver disease as well as in patients who are asymptomatic.

Adults

In the 2 confirmatory studies BR1-128 and BR1-130, the administration of SonoVue to 499 patients with at least 1 focal liver lesion requiring work-up for characterization improved the performance of ultrasonography:

- Sensitivity (characterization of lesions as malignant) and specificity (characterization of lesions as benign) increased significantly with SonoVue-enhanced ultrasound, as compared to unenhanced ultrasound in one of the 3 blinded readers in Study 128 and in two of the 3 blinded readers in Study 130.
  US; the increase in sensitivity was >10% (marginally significant) for the 5th reader;
- Marked decrease in the number of lesions with an indeterminate diagnosis was observed after administration of SonoVue (0-27 with CE-US versus 54-205 with UE-US across the 6 readers).

In 2 other multicenter clinical trials performed in the USA under the IND (n=392 efficacy patients), SonoVue-enhanced ultrasonography showed higher sensitivity and specificity (66-90% and 63-91%, respectively) than unenhanced ultrasonography (27-48% and 16-32%, respectively) for characterization of FLLs (**Table 44**).

Table 44: Summary of SonoVue Bracco-Sponsored Studies in Characterization of FLLs: Conducted under the IND in the USA

Protocol No	No of Patients	Study Design	SonoVue Dose (Efficacy Population; N malignant/N benign)	Gold Standard	Sensitivity		Specificity		Accuracy	
					UE-US	CE-US	UE-US	CE-US	UE-US	CE-US
BR1-071	185 for efficacy	USA dose-finding, parallel groups, FLL characterization of indeterminate target lesion (onsite assessment)	0.6 mL (63 eff ; 25M/38B) 1.2 mL (63 eff; 26M/37B) 2.4 mL (59 eff; 21M/38B)	Final diagnosis Histology/ CT/MRI	48% 46% 43%	84% 85% 90%	32% 16% 24%	63% 76% 82%	38% 29% 31%	71% 79% 85%
BR1-072	207 for efficacy	USA dose-finding, parallel groups, FLL characterization of indeterminate target lesion (onsite assessment)	0.6 mL (63 eff; 29M/34B) 1.2 mL (73 eff; 30M/43B) 2.4 mL (71 eff; 35M/36B)	Final diagnosis Histology/ CT/MRI	41% 27% 43%	66% 83% 83%	21% 23% 25%	88% 91% 81%	30% 25% 34%	78% 88% 82%

FLL, focal liver lesion; UE-US, unenhanced ultrasound; CE-US, contrast-enhanced ultrasound; eff, efficacy; M, malignant; B, benign; CT, computed tomography; MRI, magnetic resonance imaging

Results from the 46 peer-reviewed papers identified in the extensive literature search confirm the high sensitivity and specificity of SonoVue-enhanced ultrasonography for characterization of FLLs.

The safety profile of SonoVue in humans has been extensively documented in both clinical trial settings and during post-marketing surveillance world-wide. Assessment of safety in 6984 subjects who received SonoVue in clinical trials (6856 adult patients and 128 healthy volunteers in 77 studies) allows the conclusion that SonoVue is well tolerated in the adult population and has a safety profile that is comparable to that of other US contrast agents:

- the overall incidence of adverse events was relatively low (11.1% overall, 5.3% study agent-related) in subjects receiving SonoVue;
- most adverse events were mild and resolved spontaneously within a short time without sequelae;
- serious adverse events occurred in 0.5% of subjects and only 0.1% were considered to be of some relationship to the administration of SonoVue (relationship assessed as probable, possible, or unlikely);
- no investigational product-related deaths were reported within Bracco-sponsored trials. The favorable safety profile of SonoVue is confirmed in the subpopulation of patients who received the contrast agent in a Bracco-sponsored liver study (2909 patients in 23 liver studies):

- adverse events were reported by 6.1% of patients (2.5% patients reported study agent related events);
- most adverse events were mild and resolved spontaneously within a short time without sequelae;
- serious adverse events occurred in 0.5% of patients; none of the events was considered to be related to the administration of SonoVue;
- no investigational product-related deaths were reported and no patient discontinued study participation due to an adverse event. Experience from post-marketing surveillance of the estimated 3,286,840 patients exposed to SonoVue from April 1, 2001 through 30 September 2014 during the market use of this product, shows that
- the reporting rate of serious adverse events after administration of SonoVue is low;
- the observed pattern of serious AE cases possibly related to the administration of SonoVue is similar to that reported for anaphylactic or anaphylactoid reactions to other intravascular imaging agents;
- serious hypersensitivity reactions are observed in fewer than 1 in 10,000 exposures Overall, no significant changes to the safety profile of SonoVue have been observed between the filing of the Original NDA and subsequent Safety Updates and submission of this Liver sNDA.

Pediatric Patients

The incidence of FLLs in pediatric patients is lower than in adults; however, a number of benign and malignant liver lesions can be observed during follow-up studies for known neoplastic conditions or chronic liver disease that require characterization and monitoring. Furthermore, with the rise in the prevalence of obesity among children, there is an increased need for proper characterization of hepatosteatosis-related changes (i.e. focal fatty infiltration and fatty sparing) and their differentiation from solid liver masses.

Overall, primary liver tumors are rare in children, with approximately 100 to 150 new cases of primary liver tumors diagnosed in the United States annually. Malignant liver tumors account for 1.1% of all pediatric malignancies, with hepatoblastoma comprising over two-thirds of liver malignancies in children and hepatocellular carcinoma accounting for most of the remaining cases. Most patients with hepatoblastoma are younger than 4 years of age at diagnosis, while hepatocellular carcinoma occurs primarily after 10 years of age. The most common benign primary tumor is a congenital form of hepatic hemangioma (or infantile hemangioendothelioma), followed by mesenchymal hamartoma, especially in toddlers, and focal nodular hyperplasia (FNH). Hepatic adenoma is almost exclusively a disease of older children; primary hepatic teratoma is exceedingly rare

Ultrasonography is commonly performed in children with a suspected liver mass, because of the lack of exposure to ionizing radiation and because the examination can be performed without the need for sedation. Color Doppler evaluation may suggest a vascular nature of the tumor, as with infantile hemangioendothelioma or FNH. Ultrasonography is also the best method for distinguishing solid tissue from liquid (e.g., flowing blood or the contents of abscesses or cysts). However, if the hepatic lesion cannot be properly characterized at unenhanced ultrasound, further imaging with CT or MRI, or biopsy and pathologic assessment of the lesion, are needed

to obtain a final diagnosis.

Contrast-enhanced ultrasound has the potential to improve the diagnostic performance of ultrasonography in characterizing FLLs in pediatric patients by providing high resolution images of tissue vascularization and by showing specific enhancement patterns of liver lesions during all vascular phases, from the early arterial phase to the late phase. The avoidance of ionizing radiation is a clear benefit of ultrasound imaging in all patients and is of even greater significance in diagnostic examinations in children and adolescents.

In one study, 44 pediatric patients with an indeterminate FLL (23 males, 21 females, age range: 4-18 years, median 11.5 years) were evaluated after intravenous bolus administration of 1.2-2.4 mL of SonoVue. The objective was to correlate the findings of contrast-enhanced ultrasound with the findings on CT, MRI or histology. Specificity of SonoVue-enhanced ultrasound was 98% (43/44). In agreement with previously reported adult data, the continuing iso- or hyperechoic nature of the FLL in the late portal-venous phase imaging allowed accurate interpretation indicating a benign abnormality in over 90% of the patient studies.

Safety information about the intravenous use of SonoVue in pediatric patients, as retrieved from the published literature and the Bracco Drug Safety database are summarized below:

- 6 original publications were identified in the literature search with safety information reported after intravenous administration of SonoVue for a total number of 1259 patients (age range: 2 months up to 18 years). Overall in these 6 publications, 7 adverse events were reported in 6 patients, including one serious adverse reaction of hypersensitivity reported in an 11-year-old female.
- In addition to the 6 original peer-reviewed publications, 3 other studies about the intravenous use of SonoVue in pediatric patients, published in an abstract format, included safety information. Among the total of 174 patients included in the studies, a possible allergic side-effect occurred in a 16-year-old female.
- In addition to the literature-based cases, Bracco has received 8 spontaneous cases of adverse events after intravenous administration of SonoVue in pediatric patients. These included 5 cases of serious adverse reactions, 3 of which were hypersensitivity

The types, frequency and severity of adverse events observed following intravenous administration of SonoVue in pediatric patients are similar to those observed in adults. Overall, the benefit-to-risk ratio for SonoVue is high in both adult and pediatric patients, indicating an advantage to patients undergoing ultrasonography for characterization of focal liver lesions.

DPMH Consultation

Currently there is limited Lumason-enhanced US data available on dosing among the pediatric population. Bracco has a currently outstanding PMR to evaluate the echocardiography dosing of Lumason among patients 9-17 years of age for characterization of left ventricular endocardial

border. [redacted]

Therefore, DPMH questioned the adequacy of justification for a proposed pediatric dose based on available data and recommended one or more pediatric clinical studies to determine pediatric dose and obtain more safety data.

Bracco's Recommendations Regarding Proposed Pediatric Dosing

Pediatric FLL Dosing Considerations

Blood volume is proportional to body weight (all ages)
Adult FLL dose = 2.4 mL = 0.034 mL/kg in 70 kg person
Adult EBD dose = 2.0 mL = 0.03 mL/kg in 70 kg person
[redacted]

Bracco's Proposed FLL Pediatric Dosing

[redacted]

0.03 mL/kg for [redacted] liver imaging

APPENDIX I

To aid in the assessment of ultrasound images of FLLs, the readers were provided with detailed charts to help in distinguishing malignant and benign FLL patterns for both UE-US and CE-US images. These following charts were provided:

UE-US Benign FLLs Patterns

Lesion Type	Gray Scale Findings	Color/Power Doppler Findings
Hemangioma	Hyperechoic, homogenous, solid lesion with sharp margins	No color Doppler flow
Focal Nodular Hyperplasia (FNH)	No classic appearance. Lesion may be hyperechoic, hypoechoic or isoechoic with or without a central scar.	In addition to peripheral flow, intralesional (spoke-wheel pattern) flow may be seen.
Focal Fatty Sparing	Area of liver parenchyma of relatively low echogenicity with lack of mass effect	
Focal Fatty Change (FFC)	FFC appears as a patchy hyperechoic or hypoechoic focal areas in the liver.	
Regenerating Nodule	No classic appearance. Lesion may be hyperechoic, hypoechoic, isoechoic or of mixed echogenicity in cirrhotic liver.	Not Available
Simple Cyst	Anechoic lesion, with a pencil thin rim which demonstrates posterior acoustic enhancement	No color Doppler flow
Adenoma	No classic appearance. Lesion may be hyperechoic, hypoechoic, isoechoic or of mixed echogenicity.	Intralesional/peripheral flow may or may not be seen
Abscess	Hypoechogen, but it could be hyperechoic or of mixed echogenicity.	

UE-US Malignant FLLs Patterns

Lesion Type	Gray Scale Findings	Color/Power Doppler Findings
HCC	No classic appearance. Lesion may be hyperechoic, hypoechoic, isoechoic or of mixed echogenicity. Lesions smaller than 3 cm tend to be hypoechoic.	Intralesional and peripheral flow on color Doppler sonography
Hypovascular Metastases	Target pattern, hypoechoic, hyperechoic, mixed echogenicity, calcified	No intralesional or peripheral flow; may see vessel displacement from lesion
Hypervascular Metastases	Target pattern, hypoechoic, hyperechoic, mixed echogenicity, calcified	No intralesional or peripheral flow; may see vessel displacement from lesion
Cystic Metastases	Target pattern, hypoechoic, hyperechoic, mixed echogenicity, calcified and cystic	No intralesional or peripheral flow; may see vessel displacement from lesion
Cholangiocarcinoma	Predominately isoechoic but may be hyperechoic, hypoechoic or mixed echogenicity. Look for: a) ductal dilatation b) displacement of vascular structures c) ductal irregularity	May see portal vein encasement or occlusion

CE-US Benign FLLs Patterns

Lesion Type	Features	Arterial Phase	Portal Phase	Late Phase
Hemangioma	typical	peripheral-nodular enhancement, no central E	partial/complete centripetal filling	complete enhancement
	additional	small lesion: complete, rapid centripetal enhancement		non-enhancing areas
		rim enhancement		
FNH	typical	hyper-enhancing, complete, early	hyper-enhancing	iso-/hyper-enhancing
	additional	spoke-wheel arteries, centrifugal filling	hypo-enhancing central scar	Hypo-enhancing central scar
		feeding artery		
Focal Fatty Sparing	typical	iso-enhancing	iso-enhancing	iso-enhancing
Focal Fatty Change	typical	iso-enhancing	iso-enhancing	iso-enhancing
Regenerating Nodule	typical	iso-enhancing	iso-enhancing	iso-enhancing
	other	hypo-enhancing		
Simple Cyst	typical	non-enhancing	non-enhancing	non-enhancing
Adenoma	typical	hyper-enhancing, complete	iso-enhancing	iso or hypo
	additional	non-enhancing areas	hyper-enhancing	
			non-enhancing areas	non-enhancing areas
Abscess	typical	rim enhancement, no central enhancement	hyper-/iso-enhancing rim, no central enhancement	hypo-enhancing rim, no central enhancement
	additional	enhanced septa	hypo-enhancing rim	
		hyper-enhanced liver segment	enhanced septa	
			hyper-enhanced liver segment	

CE-US Malignant FLLs Patterns

Lesion Type	Features	Arterial Phase	Portal Phase	Late Phase
HCC	typical (in cirrhosis)	hyper-enhancing, complete	iso-enhancing	hypo/iso-enhancing
		non-enhancing areas	non-enhancing areas	
	additional	basket pattern/chaotic vessels		
		enhancing tumor thrombus in PV and/or HV		
	atypical	non-enhancing lesion	non-enhancing lesion	non-enhancing lesion
	HCC in non cirrhotic liver	hyper-enhancing	hypo-/non enhancing	hypo-/non enhancing
Hypovascular Mets	typical	rim enhancement	hypo-enhancing	hypo-/non enhancing
	additional	complete enhancement	non-enhancing areas	
		non-enhancing areas		
Hypervascular Mets	typical	hyper-enhancing, complete	hypo-enhancing	hypo-/non enhancing
	additional	chaotic vessels		
Cystic Metastasis	typical	hyper-enhancing nodular/rim component	hypo-enhancing	hypo-enhancing
Cholangiocarcinoma	typical	rim enhancement	hypo-/non enhancing	hypo-/non enhancing
	additional	non-enhancing		

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/s/

SCHELDON KRESS
02/29/2016

ALEXANDER GOROVETS
02/29/2016