CONFIDENTIAL

1 Title Page

A Multicentre Study to Evaluate the Contribution of SonoVue® and CnTI Technology (Contrast Tuned Imaging) to a Correct Classification of Adnexal Masses as Benign or Malignant.

Name of Test Agent:	SonoVue® (sulfur hexafluoride microbubbles)
Protocol No.:	IGIT-008
Developmental Phase of Study:	III
Study Initiation Date (first subject enrolled):	01 February 2005
Study Completion Date (last subject completed):	29 December 2005
Clinical Trial Report Date:	Final August 2010
Sponsor:	Bracco Imaging S.p.A. Group Medical and Regulatory Affairs Via Folli 50 20134 Milan, Italy
Sponsor's Responsible Medical Officer:	Alberto Spinazzi, MD Bracco Group
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The study described in this report was performed in compliance with Good Clinical Practice (GCP).

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Title of Study: A Multicentre Study to Evaluate the Contribution of SonoVue® and CnTI Technology (Contrast Tuned Imaging) to a Correct Classification of Adnexal Masses as Benign or Malignant. (Protocol No. IGIT-008)

Investigators/Study Centers: This study was conducted in 8 investigational centers throughout Europe:

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Publication (reference, if any): Testa AC, Timmerman D, Van Delle V, Fruscella E, Van Holsbeke C, Savelli L, et al. Intravenous contrast ultrasound examination using contrast-tuned imaging (CnTI™) and the contrast medium SonoVue® for discrimination between benign and malignant adnexal masses with solid components. Ultrasound Obstet Gynecol. 2009;34:699-710.

Study Period: First subject enrolled: 01 February 2005 Last subject completed: 29 December 2005	**Phase of Development:** III

Objectives:

Primary Objective: The purpose of this study was to test prospectively the Contrast Tuned Imaging (CnTI) technology applied to an endovaginal probe after intravenous injection of the contrast agent SonoVue® in order to:

- define the benign and malignant pattern of microcirculation in adnexal masses, and
- determine if this technique could contribute to a correct classification (benign or malignant) of adnexal masses with complex ultrasound morphology.

The ultrasound diagnostic information obtained was to be correlated with pathology.

Secondary Objective: To obtain time-intensity curves on the microcirculation of adnexal masses with complex ultrasound morphology in order to define parameters which would help in characterizing the mass as benign or malignant.

The ultrasound diagnostic information obtained was to be correlated with pathology.

Study Design: This study was designed as a Phase III, multicenter, European, prospective, open-label, non-randomized study. Ten clinical centers were to enroll 160 women undergoing ultrasound examination of the pelvis, with at least one complex adnexal mass detected on baseline unenhanced ultrasound examination, to participate in the study. Each center was to enroll a minimum of 10 patients to a maximum of 30 according to a competitive enrollment. All the women enrolled in the study were to undergo SonoVue-enhanced ultrasonography.

Each patient was to be administered with 2 injections of 2.4 mL of SonoVue intravenously.

After the first injection of SonoVue was administered, the images relative to contrast enhancement were to be stored on the hard disk of the ultrasound equipment to evaluate off-line time intensity curves in the adnexal mass and in the normal tissue.

After the second injection of SonoVue was administered, a qualitative behavior of microcirculation within the adnexal mass was to be assessed in comparison to normal tissue microcirculation.

The primary endpoint of the study was, through the qualitative and quantitative assessment of haemodynamic behavior of SonoVue in the microcirculation of different adnexal masses and normal parenchyma, to characterize the adnexal mass as benign or malignant according to pathology.

Subject Population:

Number of Subjects Planned	160
Number of Subjects Enrolled	144*
Number of Subjects Randomized	144
Number of Subjects Dosed	144
Number of Subjects Evaluated for Efficacy	134
Number of Subjects Evaluated for Safety	144

* Enrollment was stopped after the sample size (141 evaluable subjects) was met.

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Diagnosis and Main Criteria for Inclusion: A total of 160 women, 18 years of age or older (pre- and postmenopausal patients, Hormone Replacement Therapy and contraceptive therapy users were to be included), with complex adnexal masses detected on baseline ultrasound examination who provided written informed consent were to participate in this study. These patients have should also had pelvic masses with presumed presence of solid tissue (vascularized or not at Power Doppler examination) within the mass (unilocular-solid, multilocular-solid or solid adnexal masses) and multilocular masses with a number of septae greater than 10. The largest diameter had to be less than 15 cm. In case of multiple masses only 1 mass was to be examined with SonoVue. The most complex one was to be chosen; in case of similar ultrasound morphology the largest one or the one most easily accessible for CnTI was to be selected. Patients should have undergone surgery within 3 months from the ultrasound examination.

Dose and Mode of Administration, Batch Number of Test Agent: SonoVue® was to be administered as an intravenous bolus injection as quickly as possible through a 20-gauge intravenous catheter placed in a large forearm vein. Each subject was to receive up to 2 quick intravenous boluses of SonoVue as follows:

1st injection: 2.4 mL of SonoVue®
2nd injection 2.4 mL of SonoVue®

The maximum total amount of contrast agent that could be administered during the study was up to 4.8 mL in 1 study visit.

Dose and Mode of Administration of Comparative Agent: None

Duration of Treatment: Total study duration of each subject: 1 day.

Evaluation Parameters:

Imaging Procedure: The examination was to be performed using a Technos MPX (ESAOTE SpA Via Siffredi 58 16153 Genova, Italy) with a dedicated intravaginal probe with CnTI technique.

Fundamental Mode Examination: Each patient was to be examined with a transvaginal ultrasound probe using the following imaging modalities:

The Investigator was to use fundamental modes to study morphology and vascularisation of the complex mass.

- B-mode examination: The morphological ultrasound characteristics of the mass were to be recorded in the CRF. A short representative clip was to be stored.
- Power Doppler examination: A short representative clip was to be stored and the characteristics of the vascularisation were to be recorded on the CRF. A JPEG image of the most vascularized section was to be stored.

CnTI and SonoVue-enhanced Ultrasound Examination: Using CnTI the Investigator was to evaluate the vascular pattern of the adnexal masses. A CnTI examination was to be performed on the most vascularized 'slice' of the pelvic mass as judged upon the power Doppler examination. A still image of the selected slice was to be stored on the hard disc of the ultrasound system. After selecting the most vascularized section of the pelvic mass, the preset "CnTI-Multicentric" was to be selected.

- The first dose of 2.4 mL of SonoVue was to be injected into the in-dwelling cannula in the antecubital vein. SonoVue was to be administered in bolus as quickly as possible. A 3-minute CnTI recording was to be started right after the completion of the injection. The recording was to be performed by using the predefined modality acquisition for the time-intensity analysis. It was mandatory that the examiner not move the transducer during the 3-minute recording. At the end of the recording the preset was to be changed back to the original one and a still image of the 'end image' was to be stored on the hard disc of the ultrasound system. The examination was to be excluded if the end image showed the examiner had moved the transducer during the examination.
- The second dose of 2.4 mL of SonoVue was to be performed to obtain a detailed qualitative documentation of the haemodynamic distribution of the contrast within the mass, using the "CnTI-Multicentric" preset. Three consecutive CnTI clips of 60 seconds each were to be recorded, the first clip starting right after the injection of SonoVue. The shortest time interval between the 3 clips was recommended.

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Evaluation Parameters (Continued):

Efficacy:

The primary endpoint of the study was, through the qualitative and quantitative assessment of haemodynamic behavior of SonoVue in the microcirculation of different adnexal masses and normal parenchyma, to characterize the adnexal mass as benign or malignant in comparison with pathology.

Ultrasound Baseline Characteristic of the Adnexal Mass: The following parameters were to be assessed during baseline ultrasound examination: Location and size of the lesion; monolateral or bilateral localization; presence of ascites and/or fluid (quantified in mm) in the pouch of Douglas; type of mass (unilocular-solid, multilocular, multilocular-solid, solid); color score (1=no vascularisation; 2=minimal vascularisation; 3=moderate vascularisation; 4=high vascularisation) based on power Doppler examination (no spectral Doppler measurements).

Characteristic of Vascularisation within the Adnexal Mass: The presence/absence of the vascularisation within the adnexal mass was to be assessed using both Power Doppler mode and SonoVue + CnTI technology.

Qualitative Assessment: Presence or absence of enhancement/microcirculation: within the solid tissue in case of unilocular-solid, multilocular- solid and solid masses; within septum/septae in case of multilocular masses; within the papilla in case of papillation.

Quantitative Assessment: The quantitative assessment was to be performed on the images obtained during the first injection of SonoVue. The following time intensity curve parameters in the area of neoangiogenesis and in the normal area were to be tested: Time to peak; Area under the curve; Peak enhancement value; Slope of the ascending curve; Time from peak to 50% of the peak.

Assessment After Fundamental Imaging (B-mode and Power Doppler): Subjective assessment (benign, malignant); Subjective probability of diagnosis; (1=benign; 2=probably benign 3=uncertain; 4 =probably malignant; 5 = malignant); Self Impression: diagnosis (i.e.=ovarian fibroma, endometrioma, mucinous ovarian carcinoma etc.).

Assessment After CnTI Imaging: Subjective assessment (benign in case of absence of microcirculation in the target area or malignant in case of presence of microcirculation in the target area and difference of quantitative parameters between normal and target area); Subjective probability of diagnosis (1=benign; 2=probably benign 3=uncertain; 4 =probably malignant; 5 = malignant).

Histopathology and Staging: Outcome (benign, primary invasive, borderline, metastatic invasive):

- Exact specific diagnosis
- Stage in case of malignancy (FIGO; Federation International de Gynecologie et Obstetrique)
- Degree of differentiation (well, moderate, poor)
- Date of surgery

Safety: The patients were to be monitored for adverse events up to 2 hours after the end of the administration of the ultrasound contrast agent.

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Statistical Methods:

Demographics and Baseline Characteristics: Summary tables were to be provided for the number of patients who had been screened, dosed and completed according to the protocol guidelines. The number of patients prematurely discontinued during the study and the reasons for their discontinuation were to be summarized. Demographic and baseline characteristics, including age, gender, race, height, weight and medical history were also to be presented using summary statistics.

Efficacy: **Primary Efficacy Analysis**

The primary endpoint of the study was to characterize the adnexal mass as benign or malignant, through the joint qualitative and quantitative assessment of haemodynamic behavior of SonoVue in the microcirculation of different adnexal masses and normal parenchyma in comparison with pathology.

The table below describes the relationship between baseline ultrasonography and/or CnTI and the reference standard according to the mass characterization.

Performance Characteristics - Truth Standard vs. Ultrasonography (Based On Definitive Diagnosis)

Truth Standard	**Baseline Ultrasonography/CNTI**			
	B	**I**	**M**	
B	*TN*		*[FP]*	B_{TS}
M	*[FN]*		*TP*	M_{TS}
	B_{US}	I_{US}	M_{US}	

The following definitions were to be used to calculate the primary endpoints:

- *True positive (TP)*: was defined as a patient with the target lesion characterized as malignant (M) on ultrasound examinations (baseline ultrasonography or CnTI) and the reference standard.
- *True negative (TN)*: was defined as a patient with the target lesion characterized as benign (B) on ultrasound examination (baseline ultrasonography or CnTI) and the reference standard.
- *Sensitivity* was defined as the number of true positives divided by number of patients with a target lesion characterized as malignant based on the reference standard.

$$Sensitivity = \frac{TP}{M_{RS}}$$

The hypotheses to be tested for each modes were as follows:

$$H_0: Sensitivity_{CNTI} = Sensitivity_{BaselineUltrasonography}$$

versus

$$H_a: Sensitivity_{CNTI} \neq Sensitivity_{BaselineUltrasonography}$$

The difference in sensitivity was to be tested using McNemar's 2-sided test.

In addition to providing point estimates, the corresponding 95% two-sided confidence intervals for the individual sensitivities were to be calculated. Cross-tabulation of masses characterization was also to be presented.

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Statistical Methods (Cont'd):

Efficacy (Cont'd): **Secondary Efficacy Analysis**

Secondary Performance Characteristics

Diagnostic Confidence

The diagnostic confidence was to be summarized for CnTI and Baseline Ultrasonography.

Other Performance Characteristics

In addition to the above defined performance characteristics, the accuracy and the negative and positive predictive values with their associated 95% two-sided confidence intervals were also to be calculated as follows:

- *Specificity* was defined as the number of true negatives divided by the number of patients with a target lesion characterized as benign based on the reference standard.

$$Specificity = \frac{TN}{B_{RS}}$$

- *Accuracy* was defined as the number of true negatives plus true positives divided by the total number of patients in the efficacy population.

$$Accuracy = \frac{TN + TP}{B_{RS} + M_{RS}}$$

- *Negative Predictive Value (NPV)*: was defined as the number of true negatives divided by the number of patients with an adnexal mass characterized with baseline ultrasonography as benign.

$$NPV = \frac{TN}{B_{US}}$$

- *Positive Predictive Value (PPV)*: was defined as the number of true positives divided by the number of patients with an adnexal mass characterized with baseline ultrasonography as malignant.

$$PPV = \frac{TP}{M_{US}}$$

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Statistical Methods (Cont'd):

Efficacy (Cont'd):

Furthermore, all performance characteristics were to be calculated based on definitions of true negatives and true positives, using the combined diagnosis.

- If the diagnosis was malignant and the diagnostic confidence was high then the combined diagnosis was malignant (M)
- If the diagnosis was malignant and the diagnostic confidence was low then the combined diagnosis was probably malignant (m)
- If the diagnosis was indeterminate and the diagnostic confidence was either high or low then the combined diagnosis was malignant (I)
- If the diagnosis was benign and the diagnostic confidence was low then the combined diagnosis was probably benign (b)
- If the diagnosis was benign and the diagnostic confidence was high then the combined diagnosis was benign (B)

  - *True positive (TP)*: a patient with the target lesion characterized as malignant (M) or probably malignant (m) based on both CnTI or baseline ultrasonography and the reference standard.
  - *True negative (TN)*: was defined as a patient with the target lesion characterized as benign (B) or probably benign (b) based on both CnTI or baseline ultrasonography and the reference standard.

Performance Characteristics - Truth Standard vs. Ultrasonography (Based On Definitive and Probable Diagnosis Combined)

Truth Standard	**Ultrasonography**					
	B	**b**	**I**	**m**	**M**	
B	*TN*		*[FP]*			B_{RS}
M	*[FN]*			*TP*		M_{RS}
	B_{US}		I_{US}	M_{US}		

Other Secondary Variables:

Ultrasound baseline characteristics of the adnexal mass, including the presence/absence of vascularization, qualitative and quantitative assessment, were to be presented in summary tables.

Histopathology and staging assessments were to be described.

Safety:

The safety data were to be summarized and presented for all patients dosed.

Adverse events were to be summarized by COSTART (Coding Symbols Thesaurus and Adverse Reaction Terminology) body system and preferred term, by intensity and by causal relationship to study agent.

Physical examination results and concomitant medication data were to be provided in data listings.

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Summary and Conclusions:

Demographics: A total of 144 female patients were enrolled and dosed in this study, of which only 126 completed. Of the 18 patients who discontinued prematurely, 2 did not meet entry criteria, 13 discontinued for 'other' reasons and 3 gave no reason.

The majority of the dosed patients were white with a mean age of 50 years old (range: 17 to 85 years). Medical history was recorded for 37 patients; the most frequently reported abnormality was hypertension, followed by thyroid disorders. Twenty-three patients reported having some type of allergy; penicillin was the most frequently reported. Sixteen patients were found to have an abnormality upon physical examination; no worsenings were reported during the study. Concomitant medications were recorded for 69 patients most frequently taken for hypertension, followed by thyroid disorders.

Exposure to Investigational Product and/or Comparator Product: All 144 patients received at least one 2.4-mL bolus injection of SonoVue. Among them, 142 patients received a second bolus injection, 2 patients received a third bolus injection, and 1 patient received a fourth bolus injection (the fourth injection was of 4.8 mL of SonoVue).

Efficacy: Of the 144 dosed patients in this study, 10 were excluded from the efficacy analysis due to inclusion criteria violations. An additional 43 patients were excluded for reasons such as technical difficulties (related to SonoVue administration or to acquisition of image) and no surgery reported within 3 months of this study. The remaining 91 patients had histology; 2 of these patients were subsequently excluded because of the presence of multilocular lesions. Therefore, 89 patients with solid tumors were included in the qualitative analysis. Of these 89, 27 (30%) were invasively malignant at pathological examination and 10 (11%) were borderline malignant. Thirteen patients were excluded from the quantitative analysis due to the inability to detect perfusion in the solid components of the tumors following the administration of SonoVue, and 4 others were excluded because the clip was missing for 1 patient and 3 patients had hyperechoic solid tissue that prevented quantification of the perfusion parameters, leaving 72 patients with detectable perfusion in the solid components after SonoVue injection to be included in the quantitative analysis. Of these 72, 26 (36%) were invasively malignant and 9 (13%) were borderline malignant.

In the selected tumor population comprising only of tumors with solid components, many ovarian masses were considered difficult to characterize as benign or malignant when using pattern recognition. In this selected tumor population, the results of SonoVue-enhanced CnTI differed between benign and malignant tumors. However, there was substantial overlap between the benign and malignant tumors, and in particular between the benign and borderline tumors. Even though the contrast variables seemed to have a better diagnostic performance with regard to discrimination between benign and malignant masses than the other ultrasound variables, their performance was not clearly superior, and it was not superior to that of pattern recognition.

The values for peak contrast signal intensity and area under the contrast signal intensity curve in malignant tumors were significantly higher than those in borderline tumors and benign tumors, while those for the benign and borderline tumors were similar. The area under the receiver-operating characteristics (ROC) curve of the best contrast variable with regard to diagnosing borderline or invasive malignancy (0.84) was larger than that of the best gray-scale (0.75) and power Doppler ultrasound variable (0.79) but smaller than that of pattern recognition (0.93).

The qualitative contrast analysis did not perform any better than the qualitative power Doppler analysis: the sensitivity with regard to malignancy of detectable flow in papillary projections, septa or solid parts after contrast injection was only slightly superior to that of detectable flow at power Doppler examination, but the false positive rate was much higher.

Safety: Of the 144 dosed patients with SonoVue, only 1 (Pt No [redacted]) reported a non-serious adverse event (headache) of mild intensity. The Investigator considered the event to be possibly related to the administration of SonoVue as the onset of headache occurred 30 minutes postdose. The patient was reported to have recovered 2 days later. No other adverse event occurred. No patient died or discontinued the study due to an adverse event.

Conclusions: Findings on the contrast-enhanced ultrasound examination differed between benign and malignant tumors but there was a substantial overlap in contrast findings between benign and borderline tumors. Although SonoVue-enhanced ultrasound did not prove to be superior over unenhanced ultrasound, it easily differentiated invasive malignancies from other tumors.

As only 1 adverse event was reported during this study, SonoVue has proven to still be a safe and well-tolerated product.

Date of Report: August 2010