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Name and Address of Company: Bracco Imaging S.p.A. Via Folli 50 I-20134 Milan Italy	**(For Bracco Regulatory Affairs Use Only) Study BR1-127** **Volume** **Page** **Item #:**	**(For National Authority Use only)**
Name of Finished Product: SonoVue®	**Item #:**	
Name of Active Ingredient: sulphur hexafluoride microbubbles	**Item #:**	

Title of Study: A Phase III study to compare SonoVue® guided prostate biopsy with systematic biopsy in the detection of prostate malignant lesions in patients with suspected prostate cancer (Protocol BR1-127)

Investigators/Study Centers: This study was conducted in 16 investigational centers across Europe by the following investigators:

[redacted]

Publication (reference, if any): None

Study Period: First patient enrolled: 02 February 2009 Last patient completed: 30 June 2010 Off-site assessment: 15 March 2011	**Phase of Development:** III

Objectives: *Primary Objective:* Determination of the potentiality of SonoVue to guide prostate biopsy increasing the detection rate of malignant lesions of 6% points in absolute terms compared to the detection rate of the conventional systematic biopsy on patients who were candidates for a bioptic procedure. The study population comprised both candidates for a first bioptic exam and candidates for a second bioptic procedure having had a previous negative result independently from the present study. *Secondary Objectives:* 1. Evaluation of the rate of patients negative to the systematic biopsy among the population that would not receive the targeted biopsy; 2. Assessment of the potentiality of SonoVue-guided biopsy to increase the percentage of positive bioptic cores compared to the percentage of positive cores obtained with systematic biopsy, intra-patient in the population of the patients that received both bioptic procedures; 3. Evaluation of the Gleason Score of bioptic samples and its relationship with the contrast-enhanced signal assessment scores.

Study Design: This was a Phase III multicenter, open-label, prospective study to assess the diagnostic accuracy of the use of SonoVue to guide prostate biopsies in comparison with the current practice of ultrasound-guided systematic biopsy. The overall study was to comprise 2 sequential parts: the first part was the technical optimization of the imaging procedure and the second part corresponded to the Main Part of the study. During the first part, up to 10 patients per center were to be enrolled for the technical optimization in order to assure an adequate preparation of all centers. This first part was to allow the Investigators to recognize SonoVue abnormal signal enhancement as a sign of a cancer lesion. Patients with a diagnosis of prostate cancer were to be enrolled in the first part and would have received one or two injections of SonoVue for lesions identification only. They were not to be submitted to the overall procedure planned in the Main Part of the study comprising additional SonoVue injections for guiding a targeted biopsy and were to be assessed for safety evaluation only. Afterwards, 452 patients (271 candidates for a first bioptic procedure and 181 for the second one) were to be enrolled with a competitive recruitment for the Main Part of the study and would have constituted the efficacy population. Two bioptic procedures were compared: 12 cores systematic biopsy versus SonoVue-guided biopsy with a number of cores variable from 2 up to 6. As a patient was admitted to a prostatic biopsy and met the inclusion criteria, he was to follow the 2 diagnostic procedures subsequently performed by 2 different investigators, during the same session in order to reduce patient discomfort. Each investigator was to be blinded to the other's judgment.

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Patient Population:	**Total**	**Optimization Part**	**Main Part**
Number of Patients Planned	602	150	452
Number of Patients Enrolled*****	282*	45*	237*
Number of Patients Dosed	273	45	228
Number of Patients Evaluated for Efficacy	206	0	206
Number of Patients Evaluated for Safety	273	45	228

***** *This study was terminated early due to a failure to meet the primary objective.*

Diagnosis and Main Criteria for Inclusion: For the Optimization part of the study, a patient was to be enrolled if the following inclusion criteria were met: male patient, ≥40 years old with a diagnosis of prostate cancer who provided written Informed Consent and was willing to comply with the protocol requirements. For the Main part, patients were included if they were men who were ≥40 years old with suspected prostate cancer and scheduled for their first biopsy (with a tPSA of <10 ng/mL), or who had already had one systematic bioptic procedure with negative results and currently under follow-up procedure due to a persistent indication, who provided written Informed Consent and were willing to comply with the protocol requirements.

Dose and Mode of Administration, Batch Number of Test Agent: SonoVue was to be administered as multiple fast bolus injections. For the Optimization Part, a maximum of 1 SonoVue vial was to be administered as 2 bolus injections of 2.4 mL, for a total dose of 4.8 mL. Each bolus was to be followed by a 10-mL flush of saline. The entire procedure planned in the Main Part of the study was to require the use of a maximum of 2 SonoVue vials administered as 4 bolus injections of 2.4 mL, for a total dose of 9.6 mL. Each bolus was to be followed by a 10-mL flush of saline. The batch numbers of SonoVue used in this study were [redacted]).

Dose and Mode of Administration of Comparative Agent: Not Applicable.

Duration of Treatment: The complete study, either for the first optimization part or for the main part, was to last a maximum of 30 hours per patient. Safety monitoring was to begin at the time of signing the Informed Consent, and was to continue for 24 hours after the administration of SonoVue. At 24 hours postdose, a follow-up call was to be performed.

Evaluation Parameters:

Efficacy: The primary endpoint was the determination of the potentiality of SonoVue to guide prostate biopsy increasing the detection rate of malignant lesions of 6% points in absolute terms compared to the detection rate of the conventional systematic biopsy.

For assessing the type of detected lesions, a centralized histological evaluation of all bioptic samples was to be performed. The histology of bioptic core samples was to be used to compare the 2 methodologies in term of detection rate of malignant tumors. Moreover to support secondary objectives, the images and clips stored during the SonoVue procedure were to be analyzed by Bracco to correlate the contrast-enhanced signal assessment scores with the Gleason Score of the bioptic sample.

The qualitative signal enhancement scores were defined as follows (only 1 score could be selected): Very Low: the suspect area was barely distinguishable from the normal parenchyma; Low: the suspect area is relatively distinguishable from the normal parenchyma; Medium: the suspect area was sufficiently distinguishable from the normal parenchyma; High: the suspect area was well distinguishable from the normal parenchyma; Very High: the suspect area was perfectly distinguishable from the normal parenchyma.

A centralizing reading of both bioptic samples and prostatic sections was to be performed.

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Evaluation Parameters (cont'd):

Safety: Patients were to be monitored for any untoward medical occurrences from the time of signed Informed Consent through 24 hours post investigational product administration. An accurate medical history with special focus on the assessment of entity of cardiovascular risk and/or cardiovascular disease was to be obtained within 24 hours prior to investigational product administration in both parts of the study (Optimization Part and Main Part). All medications (prescription and over-the-counter) taken within 24 hours prior to investigational product administration were to be recorded. Vital signs (systolic and diastolic blood pressure and radial pulse) were to be collected at 1 hour prior to starting SonoVue administration, and at 10 minutes after the first SonoVue bolus administration for both the Optimization and Main parts of the study, and again at 10 minutes after SonoVue-guided biopsy in the Main Part of the study. A 12-lead electrocardiogram (ECG) was to be collected for all patients (in both the Optimization and Main parts of the study) within 1 hour prior to investigational product administration and again within 10 minutes after the end of the overall procedure. Blood samples were to be collected within 1 hour prior to the investigational product administration and at 2 hours after the overall procedure.

Statistical Methods: All data collected were to be presented in the listings. Descriptive statistics were to be provided for all variables in the summary tables. Continuous variables were to be summarized by using N, mean, standard deviation (SD), median and range. Categorical variables were to be summarised by using frequency distributions and percentages.

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, as well as for the actual dose received. The following baseline and demographic characteristics were to be summarised by descriptive statistics for all patients included in the safety population: age (year); sex; race; height (cm); weight (kg); TRUS results; and prostate volume.

For patients enrolled in the Main Part of the study, the following information related to the assessment of the eligibility for a prostatic biopsy was to be summarized for the safety population: Bioptic procedure history, and tPSA, free PSA, PSA ratio, PSA velocity values.

Medical history and concomitant medications were to be summarized as well.

In addition, protocol violations that led to exclusion from the efficacy population were to be implemented. For the Main Part of the study, the following protocol violations were to be listed:

1) Not meeting any of the inclusion criteria #1 and 2;
2) Meeting any of the exclusion criteria #1 and 5;
3) No biopsy slides available for the central lab;
4) Incomplete systematic biopsy procedure, i.e. less than 10 biopsy cores available;
5) Not meeting the protocol requirement on SonoVue administration: maximum 2 SonoVue vials with no more than 4 injections in total;
6) Bolus injection not in the range of 2.2 mL to 2.6 mL;
7) SonoVue-guided biopsy performed when no suspected area identified during SonoVue-enhanced prostate scan;
8) SonoVue-guided biopsy performed with bioptic cores collected not in the range from 2 up to 6 bioptic cores in the suspected areas when abnormal contrast enhancement is observed during SonoVue-enhanced prostate scan;
9) Not meeting inclusion criterion #3;
10) Meeting any of the exclusion criteria #2, 3, 4, 7 and 8.

Patients with any of violations #1 to #8 were to be excluded from the efficacy analysis.

Descriptive statistics were to be presented to summarize the total volume of investigational product administered. Dosing for the contrast-enhanced ultrasound procedure was to be summarized and listed by patient.

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Statistical Methods (cont'd):

Efficacy: The efficacy analysis population was to include all patients who were enrolled in the Main Part of the protocol, dosed with the investigational product, SonoVue, and had related efficacy data available for both systematic and SonoVue-guided bioptic procedures from central histopathological evaluation following the data derivation process.

The primary endpoint was the determination of the potentiality of SonoVue to guide prostate biopsy increasing the detection rate of malignant lesions of 6% points in absolute terms compared to the detection rate of the conventional systematic biopsy.

For assessing the type of detected lesions, a centralized histological evaluation of all bioptic samples was to be performed. The histology of bioptic core samples was to be used to compare the 2 methodologies in term of detection rate of malignant tumors.

The cancer detection rate of SonoVue-guided targeted prostate biopsy was to be compared with the cancer detection rate of systematic biopsy using McNemar test based on central histopathological evaluation, with the final diagnosis from the central histopathological evaluation of all bioptic samples as the truth. The analysis was to be performed at the patient level.

Deriving Patient Level Diagnosis: The diagnosis of SonoVue-guided targeted biopsy and systematic biopsy from central histopathological evaluation was to be reported for each bioptic core assessed, with 5 categories: 0 = Benign, 1 = Inflammation, 2 = HGPIN, 3 = ASAP, and 4 = Adenocarcinoma.

For the purpose of the efficacy analyses, diagnosis of category 4 = Adenocarcinoma was to be considered as a positive outcome (cancer), while all other categories were to be regarded as a negative outcome (no cancer).

Based on the diagnosis of the cores, patient level diagnosis was to be derived as cancerous if at least one biopsy core was positive or had no cancer if all biopsy cores were negative.

Truth Standard Diagnosis: Final diagnosis provided by the central histopathological evaluation at the patient level based on all biopsy cores from both systematic and SonoVue-guided biopsies were to be the truth standard for the analysis.

Secondary Objectives: The following was done for each secondary objective:

- Secondary Objective 1: 95% Confidence Interval (C.I.) was to be provided for the proportion of patients negative to the systematic biopsy among the population that was not to receive the targeted biopsy.
- Secondary Objective 2: Difference in proportion of positive cores between SonoVue-guided targeted prostate biopsy and systematic biopsy was to be tested using the chi square test.
- Secondary Objective 3: Gleason score, type of signal enhancement and qualitative score of the signal enhancement were to be summarized descriptively; the relationship between Gleason score and qualitative score of the signal enhancement was to be evaluated by Spearman correlation coefficient.

Special Handling for SonoVue-Guided Biopsy: SonoVue-guided biopsy was not to be performed for a patient if no areas of abnormal contrast enhancement were seen from SonoVue-enhanced TRUS exam, while systematic biopsy was to be performed regardless if the patient had received SonoVue-guided biopsy or not. For a patient without SonoVue-guided biopsy due to there having been no suspected area identified from SonoVue-enhanced TRUS exam, the diagnosis of that patient was to be derived as no cancer for SonoVue-guided biopsy.

Qualitative Score of Signal Enhancement: For the purpose of analysis, to obtain qualitative score of signal enhancement for each location recorded on the SonoVue-guided biopsy CRF from the central histopathological evaluation, the linkage between the main CRF and the SonoVue-guided biopsy CRF was to be by location. However, if a patient had only one suspected area according to the main CRF, the qualitative score of signal enhancement of the suspected area was to be applied for all locations recorded on the biopsy CRF, therefore, the linkage between the main CRF and the biopsy CRF was to be by-patient.

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Statistical Methods (cont'd):

Safety: All patients who received investigational product were to be included in the safety population. Safety parameters were to be analysed on the safety population.

The safety data were to be summarized for all patients dosed, including the patients enrolled for the technical optimization. Summary tables, including change from pre-dose to postdose where applicable, were to be presented for the following safety endpoints:

- Adverse Events: monitoring from the time of signing informed consent form through 24 hours post investigational product administration;
- Postdose Vital Signs (systolic blood pressure, diastolic blood pressure, and radial pulse): measured within 10 minutes after first SonoVue bolus administration and within 10 minutes after SonoVue-guided biopsy;
- Postdose ECGs: measured within 10 minutes after the end of the overall procedure;
- Postdose Laboratory Evaluations: measured within 2 hours after the end of the overall procedure.

Interim Analysis: An interim analysis was performed based on the Amendment #3 to assess the sample size assumptions, i.e., difference in detection rate of cancer between SonoVue-guided prostate biopsy and conventional systematic biopsy. Based on the preliminary results of the interim analysis, the primary objective results were really not satisfactory and the pronounced trend observed should not have been modified even after data cleaning completion or even after achievement of the enrollment of the total number of patients planned originally. Potential study biases and protocol violations that could have been responsible for the above mentioned results of the interim analysis had been identified. Bracco therefore made the decision to terminate the study. All the analyses performed for the interim purpose were also part of the final analysis.

Summary and Conclusions:

A total of 282 patients were enrolled to participate in this study. All 45 patients enrolled for the Optimization Part were dosed and completed the study. Of the 237 patients enrolled in the Main Part, 9 did not receive SonoVue; therefore, 228 patients were included in the Safety Population, along with the 45 from the Optimization Part (total Safety Population: 273 patients). Patients enrolled in the Main Part of the protocol who received SonoVue, had efficacy data available for both systematic and SonoVue-guided bioptic procedures were included in the Efficacy Population (206/228, 90.3% of dosed patients). Among the 22 patients that were excluded from the Efficacy Population, 15 had protocol violations and 7 did not have both the systematic and SonoVue-guided bioptic procedures.

Demographics: Of the 273 male patients who participated in the study, 45 were enrolled and dosed in the Optimization Part and 228 were enrolled and dosed in the Main Part. The overall average age was 64.0 years, ranging from 43 to 82 years. The majority of the overall patients were White (93.8%; 256/273 patients). The mean height of the patients was 175.0 cm (range: 75 to 198 cm) and the mean weight was 81.2 kg (range: 43.0 to 166.0 kg). The mean prostate volume was 53.52 cc (range: 13.60 to 151.09 cc), obtained during the TRUS evaluation.

Protocol Violations: Protocol violations were recorded for 15 of the 228 patients in the Main Part of the study. The most commonly recorded violation was SonoVue-guided biopsy being performed with bioptic cores collected that were not in the range from 2 up to 6 cores in the suspected areas when abnormal contrast enhancement was observed during SonoVue enhancement. In addition, deviations from the protocol were noted for Patient [redacted] of the Optimization Part and Patients [redacted] and [redacted] of the Main Part as they received additional injections of SonoVue (more than the allotted 2 injections per examination); however, because the total volume did not exceed the total maximum allowed in this study (9.6 mL), they were not listed as protocol violators.

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Summary and Conclusions (cont'd):

Exposure to Investigational Product and/or Comparator Product: The overall total volume of SonoVue administered per patient was 7.18 mL (range: 2.4 to 12.0 mL); the mean volume of SonoVue injected per injection was 2.4 mL. The overall mean number of injections per patient was 3 (range: 1 to 5 injections).

Efficacy: *Primary Objective:* Of the 228 patients in the Main Part of this study, 22 were excluded from the Efficacy Population. Among the 206 patients included in the Efficacy Analysis Population, 75 patients were diagnosed with cancer per the final diagnosis from the central histopathological evaluation. Of those 75 diseased patients, 4 patients were diagnosed as having no cancer according to the systematic biopsy evaluation as was correctly diagnosed by the SonoVue-guided targeted biopsy evaluation. The cancer detection rates of SonoVue-guided biopsy and systematic biopsy from the central histopathological evaluation were 45.3% (34/75 patients) and 94.7% (71/75 patients), respectively, with a difference of -49.3% in the detection rate of cancer (SonoVue-guided – systematic biopsy) which was deemed as significant ($p<0.0001$, McNemar's test). *Secondary Objectives:* Of the 206 patients in the Efficacy Population, 80 patients did not have a SonoVue-guided biopsy performed as no suspected area was identified from the initial scan; among them, 60 patients (75%) showed negative at systematic biopsy. For the remaining 126 patients with SonoVue-guided biopsy performed, 51 patients (40%) were positively diagnosed with cancer by systematic biopsy. There were 426 bioptic cores taken during SonoVue-guided biopsy for those 126 patients who had SonoVue-guided biopsy performed; the percentage of cancer-positive cores was 22.3% (95/426). For the 206 efficacy patients who had systematic biopsy performed, there were 2461 bioptic cores taken in total; the percentage of cancer-positive cores was 10.9% (269/2461). The percentage of cancer-positive cores from SonoVue-guided biopsy is significantly higher than that from systematic biopsy ($p<0.0001$, chi-square test).

Safety: Of the 273 patients dosed in this study, 20 (7.3%) reported 24 adverse events, 11 of which the Investigator could not rule out an association with the administration of SonoVue. The majority of the events reported were mild in intensity; no event was reported as severe in intensity. A local injection site reaction (injection site hypersensitivity) was reported for 1 patient, which was considered by the Investigator to be probably related to the administration of SonoVue. The most frequently reported adverse event was urinary retention (3/273; 1.1%), followed by proctalgia and haematuria (2/273; 0.7% each), none of which are unusual for this study population. A serious adverse event (acute urinary retention) was reported for 1 patient, which was considered by the Investigator to be unrelated to the administration of SonoVue. No patient died or otherwise discontinued the study due to an adverse event. However, 1 patient reported a worsening of event (prostatitis) the day after he completed the study. This report occurred outside of the protocol-defined reporting period, and therefore, is not presented in the study database. The patient was hospitalized the following day, therefore upgrading this event to serious. The event was considered to be mild in intensity and unrelated to the administration of SonoVue.

Overall, no clinically meaningful trends were noted for vital signs, ECG or laboratory parameters following the administration of SonoVue.

Conclusions: This multicenter study was aimed at comparing SonoVue-target biopsy to systematic biopsy in patients who were candidates for a prostate biopsy procedure. A population of 452 patients was to be enrolled in the Main Part of the study. However, the study was terminated after enrollment of 282 patients (45 in the Optimization part and 237 in the Main Part), because of potential study biases and protocol violations identified during the interim analysis performed on the basis of Amendment #3.

The administration of SonoVue within the dose range of 2.4 to 12.0 mL in patients undergoing prostate TRUS resulted to be safe and well tolerated. No serious adverse events with a suggested causal relationship to the investigational product were reported. No death occurred and no patient discontinued as a result of an adverse event. No clinically meaningful changes from baseline were observed for vital signs, ECGs, and laboratory evaluations. No previously unknown safety risks for SonoVue were detected.

Date of Report: 08 March 2012