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BR55 Contrast Enhanced Ultrasound (CEUS) in Characterization of Ovarian Lesions

BR55

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Sponsor:
Bracco Diagnostics Inc.
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Protocol Title

BR55 Contrast Enhanced Ultrasound (CEUS) in Characterization of Ovarian Lesions.

Protocol No.

This study is being conducted under protocol number: BR55-108.
IND number: 114098.

Objectives

The objectives of this exploratory phase II study are:

- to determine the ability of BR55 CEUS to differentiate malignant from benign ovarian lesions in subjects with suspected ovarian cancer on the basis of a visual score (semi quantitative visual assessment of BR55 enhancement) using histopathology as the truth standard (TS);
- to expand the safety profile of the BR55 in subjects with suspected ovarian cancer.

Additional exploratory objectives of the study are:

- to correlate the signal intensity (SI) of the target lesion on BR55 CEUS with lesion type (malignant or benign) as determined by histopathology;
- to assess the relationship between the SI of the target lesion on BR55 CEUS and tumor size as determined by histopathology;
- to assess the correlation between *in vivo* SI of the target lesion on BR55 CEUS and *ex vivo* Vascular Endothelial Growth Factor Type 2 (VEGFR2) expression as determined by immunohistochemistry (IHC);
- to assess the correlation between *in vivo* SI of the target lesion on BR55 CEUS and *ex vivo* microvessel density (MVD) as determined by IHC.

Investigational Plan

This is an exploratory phase II, single center, open label, prospective study of BR55 CEUS for characterization of ovarian lesions in subjects with suspected ovarian cancer. This study will be conducted at Stanford University Medical Center.

A total of sixty (60) subjects scheduled to undergo salpingo-oophorectomy within 30 days of BR55 CEUS examination will be enrolled to obtain approximately 30 subjects with benign ovarian lesions and 30 with malignant ovarian lesions based on the TS.

BR55 will be evaluated in the study as follows:

The first 10 subjects enrolled in the study will receive BR55 at a dose of 0.03 mL/kg. Assuming these first 10 subjects will show technically adequate images, subsequent subjects enrolled in the

study will continue to receive 0.03 mL/kg dose of BR55; otherwise, subjects will be switched to a 0.05 mL/kg dose of BR55.

The final cancer diagnosis will be obtained for all subjects by histopathology.

Study Duration

A subject's participation will begin at the time of signing the informed consent through the 24 hour follow-up after the administration of BR55.

The procedure planned, namely the ovarian ultrasound examination with administration of BR55, will be completed within 60 minutes including up to 30 minutes of CEUS examination.

Safety monitoring will begin at the time of signing the Informed Consent and will continue for 24 hours after BR55 administration.

Study Population

It is planned to enroll a maximum of 60 female subjects scheduled to undergo salpingo-oophorectomy for suspected ovarian cancer within 30 days of the BR55 CEUS examination, but not before completion of the safety assessment at 24 hours post BR55 administration.

Inclusion Criteria

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

- Is at least 18 years of age;
- Has an ovarian lesion that is visible and assessable with trans-vaginal ultrasound;
- Is scheduled to undergo salpingo-oophorectomy for suspected ovarian cancer not earlier than 24 hours and not later than 30 days following BR55 administration;
- Provides written Informed Consent and is willing to comply with protocol requirements.

Exclusion Criteria

Exclude a subject from this study if the subject does not fulfill the inclusion criteria, or if any of the following conditions are observed:

- Is a pregnant or lactating female. Exclude the possibility of pregnancy:
  - by testing on site at the institution (serum Beta Human Chorionic Gonadotropin, βHCG) within 24 hours prior to the start of investigational product (IP) administration,
  - by surgical history (e.g., tubal ligation or hysterectomy),
  - by post-menopausal status with a minimum 1 year without menses;
- Has undergone prior systemic therapy for ovarian cancer;
- Has history of concurrent malignancy;

- Has history of any clinically unstable cardiac condition including class III/IV congestive heart failure;
- Has had any severe cardiac rhythm disorders within 7 days prior to enrolment;
- Has severe pulmonary hypertension (pulmonary artery pressure >90mmHg) or uncontrolled systemic hypertension and/or respiratory distress syndrome;
- Has open and/or non-healing wounds in the chest, abdomen and pelvis;
- Has other systemic vascular abnormalities associated with neovascularization, such as macular degeneration, that in the opinion of the investigator could significantly affect the ability to evaluate the effects of BR55;
- Is participating in a clinical trial or has participated in another trial with an investigational compound within the past 30 days prior to enrolment;
- Has previously been enrolled in and completed this study;
- Has any known allergy to one or more of the ingredients of the Investigational Product or to any other contrast media;
- Is determined by the Investigator that the subject is clinically unsuitable for the study;
- Has had major surgery, including laparoscopic surgery, within 3 months prior to enrolment;
- Has history of surgery to the ovaries or pelvic inflammatory disease.

Discontinuation Criteria

Clearly document the reason for the subject's discontinuation on the Case Report Form (CRF). Discontinued subjects are not replaced. Discontinue a subject from the study if the subject:

- Withdraws consent;
- No longer meets the Inclusion Criteria;
- Experiences any of the Exclusion Criteria;
- Has an adverse event that, in the opinion of the Investigator, requires the subject's discontinuation.

Investigational Products

[redacted]

[redacted]

Administration

The calculated dose of BR55 will be administered by slow IV bolus injection through an angiocatheter (20 Gauge). Strict adherence to aseptic technique must be maintained and subjects will be observed throughout the procedure.

The first 10 subjects enrolled in the study will receive BR55 at a dose of 0.03 mL/kg. Assuming the first 10 subjects will show technically adequate images, subsequent subjects will receive the BR55 dose of 0.03 mL/kg; otherwise, subjects will be switched to a 0.05 mL/kg dose of BR55.

In each subject, the administration of BR55 will be followed by a 10 mL 0.9% saline flush.

Methodology

The Study Schedule is presented in Table B.

Participants will sign an Informed Consent Form prior to the conduct of any study procedures. Baseline evaluations will be conducted on all Study participants and include the collection of a complete medical history, gynecological history (including disease related medical history), a physical examination, weight measurement, vital signs, electrocardiogram (ECG), and collection of laboratory samples, plus a serum βHCG pregnancy test for women of child-bearing age and who have not had a hysterectomy. Post-menopausal women are defined as women having no menstrual period for at least one year. If there is any doubt the woman is post-menopausal, the test to rule-out pregnancy must be performed.

The unenhanced ultrasound (UEUS) and BR55 CEUS examinations will be performed with the same equipment and documented by images acquired according to instructions in the dedicated imaging manual.

Safety will be assessed through monitoring for Adverse Events, continuous monitoring of pulse oximetry from 10 minutes pre-dose through 30 minutes post dose, as well as measuring of vital signs, ECGs and collection of laboratory samples at the time points described in Table B.

Table B: Study Schedule

Events	PRE-DOSE *(before BR55 inj.)*				BR55 inj.	POST-DOSE *(after BR55 inj.)*		POST-DOSE FOLLOW-UP	
	Within 24h	Within 2h	-10 min	Immed. prior		+30 min	+1 hr	+24 h	30 days
Written Informed Consent [a]	×								
Pregnancy test [b]	×								
Inclusion/Exclusion Criteria	×								
Concomitant medications [c]	×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Adverse Events Monitoring [d]	×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Medical History [e]	×								
Physical Examination	×							×	
Laboratory Evaluations	×							×	
Weight assessment		×							
Vital Signs [f]		×				×	×	×	
Electrocardiogram		×				×	×	×	
Pulse oximetry			×	⇒	⇒	×			
UEUS exam				×					
BR55 Administration					×				
BR55 CEUS [g]					×	×			
Salpingo-oophorectomy [h]									×

[a] Obtain prior to implementation of any study procedure.

[b] A serum βHCG pregnancy test to be performed in subjects of childbearing potential and if post-menopausal status is doubtful (i.e. < 1 year without menses)

[c] Record all medications (prescription and over-the-counter) taken within 24 hours prior to BR55 administration up through 24 hours after BR55 administration

[d] Start monitoring from the time of signing Informed Consent up to 24 h post-dose. Only post-dose events will be tabulated as adverse events.

[e] Includes Demographics, Gynecological Medical History, disease related Medical History and General Medical History.

[f] Includes systolic and diastolic blood pressure and heart rate

[g] Imaging will be performed as per instructions in the imaging manual

[h] Salpingo-oophorectomy to be performed from 24 h up to 30 days post-dose.

Subject Evaluations

Medical History and Demographics

Obtain a complete medical history, gynecological history (including disease related medical history and scheduled date for salpingo-oophorectomy) and demographic information after the subject has signed the Informed Consent and within 24 hours prior to investigational product administration.

Pregnancy Test

Exclude the possibility of subject's pregnancy:

- by testing (serum βHCG) within 24 hours prior to the start of investigational product administration;
- by surgical history (e.g., tubal ligation or hysterectomy);
- by post-menopausal status with a minimum of 1 year without menses

Concomitant Medications

Record all medications (prescription and over-the-counter) taken within 24 hours prior up to 24 hours after BR55 administration and taken for treatment of an adverse event.

Safety Assessment

Adverse Events

Monitor subjects for any untoward medical occurrences from the time of signing of Informed Consent through 24 hours post BR55 administration. Record all untoward medical events in the Adverse Event section of the CRF as specified in the protocol. Only post-dose untoward medical occurrences will be tabulated as adverse events.

All serious adverse events that occur during the study monitoring period are required to be collected regardless of the relationship to BR55 on the Serious Adverse Event Report (SAER) Form.

In addition, the investigator should report any serious adverse events that occur after the monitoring period that he/she believes may be related to the BR55 on the SAER Form.

Physical Examination

Perform a physical examination within 24 hours prior to and 24 hours after BR55 administration.

Weight measurements will be collected within 2 hours prior to BR55 administration for dose calculation.

Vital Signs

Collect the following vital signs within 2 hours pre-dose and approximately at 30 min, 1 hour and 24 hours after BR55 administration:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Heart rate (beats/minute)

Pulse oximetry will be performed starting at 10 minutes prior to BR55 administration and continuing through 30 minutes after BR55 administration. During the monitoring period, any pathological change in pulse oximetry noted form pre-dose, including the timing in respect to BR55 administration, must be recorded on the CRF.

Laboratory Evaluations

Collect blood and urine samples within 24 hours prior and 24 hours post BR55 administration for evaluation of the analytes listed in the protocol.

A local laboratory will be utilized for analyzing and reporting laboratory results.

Electrocardiograms

Electrocardiograms (ECG) will be recorded within 2 hours prior to BR55 administration, and at approximately 30 minutes, 1 hour, and 24 hours after IMP administration. A local ECG laboratory will be utilized for analyzing and reporting ECG evaluations.

Imaging Procedures

Ultrasound equipment

The ultrasound examination will be performed with an FDA-approved ultrasound equipment, commercially available for clinical applications, with contrast dedicated platform and equipped with an endocavitary probe for obstetrics and gynecology examinations.

A detailed description of the unenhanced ultrasound and BR55 CEUS acquisition protocols will be provided in a dedicated Imaging Manual.

Imaging procedure outline

A standardized UEUS, as used in clinical imaging, will be conducted in all subjects to ensure a comprehensive survey of pelvic structures and organs and to identify the target ovarian lesion (as defined in the imaging manual). In case of complex cystic ovarian lesions, the target lesion is defined as the solid component of the lesion itself. Optimally, it should be possible to view the entire lesion within the field of view of the ultrasound probe. UEUS will also serve to identify the optimal plane for imaging the target lesion after BR55 administration.

After BR55 administration, the contrast inflow into the target lesion will be observed on the optimal imaging plane for approximately 45 seconds using a low frame rate to minimize

destruction of targeted bubbles. Subsequently, post-contrast images will be acquired at predefined time intervals (outlined in the dedicated imaging manual) up to 30 minutes post BR55 administration or until stationary enhancement in the lesion is still no longer visible, whichever occurs first.

DICOM images will be transferred to the Bracco Imaging Core Laboratory.

Efficacy Evaluations

Visual Assessment of BR55 Enhancement

BR55 contrast-enhanced images will be assessed by the investigator using the following semi quantitative scale:

1. No enhancement: No focal targeted, stationary imaging signal is detectable
2. Weak enhancement: Weak focal targeted imaging signal is detectable and considered as possibly stationary
3. Strong enhancement: Well defined and strong focal targeted imaging signal is detectable and considered as definitely.

Quantitative Assessment of BR55 Enhancement

A quantitative assessment of contrast enhancement with BR55 will be performed by drawing a Region of Interest (ROI) within the target lesion to measure SI.

Pathology and immunohistochemistry measurements for Ovary lesions

Histopathology measurements and immunohistochemistry analysis will be described in a separate dedicated manual.

Statistical Methods

Analysis Population

Safety analysis population will consist of all subjects who are dosed.

Efficacy analysis population will include the data from all subjects who are dosed and have efficacy data available.

Extent of Exposure

Descriptive statistics will be presented to summarise the volume of investigational medicinal product (IMP) administered. Dosing of the BR55 will be listed.

Safety Analysis

All patients receiving any dosage of investigational product will be included in the safety population on which safety analyses will be carried out. Summary tables, including change from pre-dose to post-dose where applicable, will be presented for the following safety endpoints:

- Adverse Events
- Clinical Laboratory Evaluations
- Vital Signs
- ECG abnormalities

All adverse events will be coded using applicable dictionaries and summarised by body system organ class and preferred term, by intensity and by causal relationship to the IMP. Only those occurrences which occur from the start of IP/IMP administration through the follow-up period defined in the protocol will be tabulated in the Clinical Trial Report as "adverse events". Concomitant medications will be coded according to therapeutic area using the WHO drug reference list. Concomitant medications recorded between signing of informed consent and follow-up will be presented in data listings for all subjects dosed.

Efficacy Analysis

General

Efficacy analysis will include the data from all evaluable subjects who are dosed and have efficacy data available. The results will be presented for each dose group, if applicable. Except as noted, the statistical tests will be two-sided at the 0.05 level of significance with 95% confidence limit.

Differentiation between malignant and benign ovarian lesions - visual score vs. histopathology as the (TS)

The objective of this study is to determine the ability of BR55 CEUS to differentiate between malignant and benign ovarian lesions in subjects with suspected ovarian cancer on the basis of a visual score (semi quantitative visual assessment of BR55 enhancement) using histopathology as the TS.
Contrast-enhanced US images of the ovaries will be assessed by the investigator as 3-point scale for contrast enhancement of BR55. Frequency tables will be provided for enhancement scores (none, weak enhancement, and strong enhancement) by histology assessment results (malignant and benign). The primary analysis will be performed based on the assumption that benign lesions will not show VEGFR2 expression and therefore will not present stationary enhancement at BR55 CEUS.
The primary variables for the characterization are sensitivity and specificity in differentiation of target ovary lesions. The following parameters will be calculated and the 2-sided 95% confidence intervals for the sensitivity and specificity will be estimated.

Characterization of BR55 CEUS in Ovary Cancer

BR55 CEUS	Truth Standard		
	Negative	**Positive**	**Total**
Negative	TN	FN	**N_{CEUS}**
Positive	FP	TP*	**P_{CEUS}**
Total	**N_{truth}**	**P_{truth}**	**TOTAL**
TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative. * TP is exact match of malignant lesion between BR55 CEUS and TS.			

$$Sensitivity = \frac{TP}{TP + FN}, \quad Specificity = \frac{TN}{TN + FP}, \quad Accuracy = \frac{TP + TN}{TP + FN + TN + FP},$$

B55 CEUS Differentiates between Malignant and Benign Ovarian Lesions - Ultrasound Signal Intensity (SI) vs. TS

The purpose of the analyses is to explore the threshold of SI (quantitative assessment of BR55 enhancement) on BR55 CEUS for the characterization of ovarian lesions.
Hypothesis: SI on BR55 CEUS is significantly higher for ovarian malignant versus benign lesions.
SI on BR55 CEUS will be defined as that of the target lesion of the subject; while the TS will be classified as malignant lesion if cancer is found or as benign lesion if no cancer is found. The empirical distribution of SI on BR55 CEUS will be assessed in each group (malignant and benign lesion) and, if necessary, apply a transformation to approximate normality. A t-test will be performed to test for a difference in mean signal intensity on BR55 CEUS between the malignant and benign groups. In addition, a Receiver Operating Characteristic Curve (ROC) analysis will be performed of SI on BR55 CEUS in predicting presence of a malignant lesion as determined by TS. This will be performed per subject as defined above.
Additionally, SI on BR55 CEUS will be categorized as no signal, low signal, and high signal and will be analyzed versus the TS results. Cross tabulation of the 3 SI categories versus TS will be presented and a logistic regression will be performed.

Relationship between the SI of the target lesion on BR55 CEUS and the tumor size as determined by TS

To explore if SI at BR55 CEUS is related to tumor size, SI of the target lesion will be plotted against tumor diameter as measured by TS, where tumor diameter is set to 0 for subjects without cancer. The plots will be analyzed qualitatively to determine detection limits of the technology.

Correlation between in vivo SI of the target lesion on BR55 CEUS and ex vivo VEGFR2 expression as determined by IHC

Hypothesis: There is a significant relationship between *in vivo* SI of the target lesion on BR55 CEUS and *ex vivo* VEGFR2 expression level, as measured by the angiogenesis score.
A two-sample t-test will be performed to test for a relationship between angiogenesis score and *in vivo* SI of the target lesion on BR55 CEUS.

Correlation between SI of the target lesion on BR55 CEUS vs. microvessel density (MVD)

Hypothesis: SI of the target lesion on BR55 CEUS significantly correlates with *ex vivo* MVD. The correlation coefficient between the subject's target lesion SI and MVD will be estimated and the null hypothesis that correlation coefficient is zero will be tested.

Sample Size

This is an exploratory study. The sample size determination is not based on the statistical considerations and assumptions. About 60 female subjects (approximately 30 subjects with a malignant lesion and 30 with a benign lesion) will be prospectively enrolled.

Data Handling

All data collected will be entered into the database and displayed in the subject data listings. In general, no imputation algorithms will be used to estimate missing safety and efficacy data, and tables will display counts of missing values.

Interim Analyses

No interim analysis is planned.