CONFIDENTIAL

An Exploratory Study to Determine the Optimal Timing of BR55 Contrast Enhanced Ultrasound (CEUS) of the Ovaries in Pre-menopausal Women

BR55

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Protocol Title

An Exploratory Study to Determine the Optimal Timing of BR55 Contrast Enhanced Ultrasound (CEUS) of the Ovaries in Pre-menopausal Women.

Protocol No.

This study is being conducted under protocol number: BR55-109.
IND number: 114098

Objectives

The objectives of this exploratory study are:

- to determine the optimal phase of the menstrual cycle for performing BR55 CEUS of the target ovary in premenopausal women at high risk for ovarian cancer;
- to expand the safety profile of the BR55 in subjects with potential ovarian cancer.

Additional exploratory objectives are:

- to correlate the signal intensity (SI) within the target ovary as seen at BR55 CEUS with histopathology findings;
- to assess the correlation between SI within the target ovary as seen at BR55 CEUS and Vascular Endothelial Growth Factor Type 2 (VEGFR2) expression as determined by immunohistochemistry (IHC);
- to assess the correlation between SI within the target ovary as seen at BR55 CEUS and microvessel density (MVD).

Investigational Plan

This is an exploratory, single center, open label, prospective study of BR55 to determine the optimal phase of the menstrual cycle for performing BR55 CEUS of the target ovary in premenopausal women scheduled to undergo preventative surgery because of high familial/hereditary or genetic risk for ovarian cancer. This study will be conducted at Stanford University Medical Center.

Training Cases

The site will enroll up to 3 subjects as “training cases” to determine the optimal imaging technique with BR55 and a 2D intracavitary probe for this study population.

Subjects enrolled for training purposes will undergo all safety evaluations but not the efficacy assessments and will not be required to undergo oophorectomy. These subjects will receive a single CEUS examination with administration of BR55 at a dose of 0.03 mL/kg. Timing of the CEUS examination with respect to follicular phase of the menstrual cycle will not be considered.

Efficacy Cases

Subjects enrolled in the efficacy phase of the study will undergo two BR55 CEUS examinations: one examination during the early follicular phase (i.e. just after menstruation) and one examination during the late follicular phase (i.e. right before ovulation). The timing of the late follicular phase will be determined on the basis of the subject's clinical history with regard to the menstrual cycle and assessment of luteinizing hormone levels in the urine. SI within the target ovary will be quantitatively assessed for each of the two CEUS examinations with BR55 and compared for presence of significant differences.

All subjects will undergo preventative salpingo-oophorectomy within five days of the second BR55 CEUS (oophorectomy is not required for training cases); histopathology and IHC results will be compared with BR55 findings seen on the second CEUS examination.

Subjects will be randomized to the follicular phase for undergoing the first BR55 CEUS in the early or late follicular phase as follows:

Group A: BR55 CEUS will be performed in the early follicular phase first and in the late follicular phase thereafter

Group B: BR55 CEUS will be performed in the late follicular phase first and in the early phase thereafter

BR55 dose 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.

Study Duration

A subject's participation will begin at the time of signing the informed consent.

The BR55 CEUS exam will be completed within 60 minutes at maximum after BR55 administration (including 30 minutes of CEUS examination).

Safety monitoring will begin at the time of signing the Informed Consent Form and will continue through the follow-up evaluations at 24 hours after the first BR55 administration (first BR55 CEUS exam). Safety monitoring will continue from 24 h prior to the second BR55 administration through the follow-up evaluations at 24 h post second BR55 administration (second BR55 CEUS exam), if applicable.

Study Population

Subjects with a high risk of ovarian cancer scheduled to undergo preventative salpingo-oophorectomy within 5 days, but not before 24 hours, after the second BR55 CEUS examination will be prospectively enrolled in the study. It is planned to enroll about 50 female subjects meeting inclusion and exclusion criteria, in order to obtain 47 evaluable subjects with normal bilateral ovaries and no occult cancer.

Additionally, the site will enroll up to 3 subjects as "training cases" to determine the optimal imaging technique with BR55 and a 2D intracavitary probe for this study population. Subjects enrolled for training purposes will undergo all safety evaluations and will be part of the safety population but will be excluded from the efficacy analyses. Therefore, timing of the CEUS examination with respect to follicular phase of the menstrual cycle and oophorectomy are not requirements for subjects enrolled in the training phase of the study.

Inclusion Criteria

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

- Is a female subject of at least 18 years of age;
- Is premenopausal;
- Is scheduled to undergo preventative salpingo-oophorectomy for high risk of ovarian cancer not earlier than 24 hours and not later than 5 days following the second BR55 CEUS examination (with the exception of subjects enrolled in the training phase of the study);
- 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),
- 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 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]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[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.

Subjects enrolled in the training phase of the study will receive BR55 at a lowest dose of 0.03 mL/kg.

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 (that can be obtained within 10 days of BR55 administration) 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.

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.

To ensure optimal BR55 CEUS timing in the late follicular phase, i.e. just before ovulation, patient history with regard to the menstrual cycle will be assessed along with measurements of luteinizing hormone levels in the urine. This will not apply to subjects enrolled in the training

who will receive a single CEUS with BR55, independently from the phase of the menstrual cycle.

For each BR55 CEUS examination, 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 the Table B.

Table B: Study Schedule		**FIRST BR55 CEUS EXAMINATION**							
Events	**PRE-DOSE** *(before BR55 inj.)*					**BR55 inj.**	**POST-DOSE** *(after BR55 inj.)*		**POST-DOSE Follow-up**
	Within 10d	**Within 24h**	**Within 2h**	**-10 min**	**Immed. prior**		**+30 min**	**+1 hr**	**+24 h**
Written Informed Consent [a]	×								
Pregnancy test [b]		×							
Test for ovulation [c]		×							
Inclusion/Exclusion Criteria		×							
Concomitant medications [d]		×	⇒	⇒	⇒	⇒	⇒	⇒	×
Adverse Events Monitoring [e]	×	⇒	⇒	⇒	⇒	⇒	⇒	⇒	×
Medical History [f]		×							
Physical Examination		×							×
Laboratory Evaluations		×							×
Weight assessment			×						
Vital Signs [g]			×				×	×	×
Electrocardiogram			×				×	×	×
Pulse oximetry				×	⇒	⇒	×		
UEUS exam					×				
BR55 Administration						×			
BR55 CEUS [h]						×	×		

[a] Obtain prior to implementation of any study procedures, within 10 days prior to BR55 administration.

[b] For childbearing potential subjects perform a serum βHCG pregnancy test.

[c] To ensure optimal BR55 CEUS timing in the late follicular phase (i.e. just before ovulation) perform a luteinizing hormone urine test. To be performed only in subjects who will undergo the first BR55 CEUS during the late follicular phase of the menstrual cycle(not to be performed for the training subjects).

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

[e] Start monitoring from the time of signing Informed Consent and continue through the follow-up evaluations at 24 hours after the first BR55 administration. Only post-dose events will be tabulated as adverse events.

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

[g] Includes systolic and diastolic blood pressure and heart rate.

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

	SECOND BR55 CEUS EXAMINATION (not applicable for training cases)								
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	within 5 days
Pregnancy test [a]	×								
Test for ovulation [b]	×								
Concomitant medications [c]	×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Adverse Events Monitoring [d]	×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Physical Examination	×							×	
Laboratory Evaluations	×							×	
Weight assessment		×							
Vital Signs [e]		×				×	×	×	
Electrocardiogram		×				×	×	×	
Pulse oximetry			×	⇒	⇒	×			
UEUS exam				×					
BR55 Administration					×				
BR55 CEUS [f]					×	×			
Salpingo-oophorectomy [g]									×

[a] For childbearing potential subjects perform a serum βHCG pregnancy test.

[b] To ensure optimal BR55 CEUS timing in the late follicular phase (i.e. just before ovulation) perform a luteinizing hormone urine test. To be performed only in subjects who will undergo the second BR55 CEUS examination during the late follicular phase of the menstrual cycle (not to be performed for the training subjects).

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

[d] Resume safety monitoring 24 hours before the second BR55 administration and continue through the follow-up evaluations at 24 hours after the second BR55 administration. Only post-dose events will be tabulated as adverse events.

[e] Includes systolic and diastolic blood pressure and heart rate.

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

[g] Salpingo-oophorectomy to be performed from 24 h up to 5 days after the second BR55 CEUS exam (with the exception of subjects enrolled in the training phase of the study).

Subject Evaluations

Medical History and Demographics

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

Test for Ovulation

To ensure optimal BR55 CEUS timing in the late follicular phase, i.e. just before ovulation, patient history with regard to the menstrual cycle will be assessed along with measurements of luteinizing hormone levels in the urine (this test will not be performed in the subjects enrolled in the training phase).

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).

Concomitant Medications

For each BR55 administration, record all medications (prescription and over-the-counter) taken within 24 hours pre-dose and up to 24 hours post-dose in the Concomitant Medication section of the Case Report Form. Any medication taken for treatment of an adverse event that occurred after the subject signed the informed consent form should be recorded.

Safety Assessment

Adverse Events

Subjects will be monitored for any untoward medical occurrences from the time of signing of Informed Consent through 24 hours after the first administration of BR55 (first BR55 CEUS exam); monitoring will resume from 24 hours prior to the second BR55 injection and continue through the follow-up evaluation at 24 hours after the second administration of BR55 administration (second BR55 CEUS exam), if applicable. 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

For each BR55 administration, perform a physical examination within 24 hours pre-dose and at 24 hours post-dose.

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

Vital Signs

For each BR55 administration, collect the following vital signs within 2 hours pre-dose and approximately at 30 min, 1 hour and 24 hours post-dose:

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

Pulse Oximetry

Monitor pulse oximetry from approximately 10 minutes prior to BR55 administration and continue through 30 minutes after each BR55 administration. During the monitoring period, any worsening of blood oxygen saturation in pulse oximetry noted from pre-dose, including the timing in respect to BR55 administration, must be recorded on the CRF.

Laboratory Evaluations

For each BR55 administration, collect blood and urine samples within 24 hours pre-dose and at 24 hours post-dose for evaluation of the analytes listed in the protocol.

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

Electrocardiograms

For each BR55 administration, electrocardiograms (ECG) will be recorded within 2 hours pre-dose and at approximately 30 minutes, 1 hour, and 24 hours post-dose.

A local ECG laboratory will be utilized for analyzing and reporting ECG evaluations.

Imaging Procedures

Ultrasound equipment

The ultrasound examination will be performed in the Department of Radiology of Stanford University Hospital with a FDA-approved ultrasound equipment commercially available for clinical applications, with a CEUS dedicated platform and equipped with an endocavitary 2D probe for obstetrics and gynecology examinations.

A detailed description of the unenhanced ultrasound (UEUS) and BR55 contrast-enhanced ultrasound (CEUS) acquisition protocols will be provided in a dedicated Imaging Manual.

Imaging Procedure outline

Unenhanced Ultrasound

Each BR55 CEUS examination will be preceded by UEUS examination.

UEUS will be performed to ensure a comprehensive survey of the pelvic structures and organs and to identify the ovary with the corpus luteum, or any focal abnormality (if present in the absence of a corpus luteum). If neither the corpus luteum nor a focal abnormality is seen, then one of the two ovaries will be chosen as the target ovary by the investigator. The ovary chosen during this first examination will then also be used as the target ovary for the subsequent CEUS examination with BR55.

Contrast Enhanced Ultrasound

CEUS will be performed after administration of BR55 and upon activation of the low Mechanical Index (<0.2) contrast study preset. The image persistence needs to be set to OFF in order to facilitate the post-processing of acquired images with dedicated quantification software.

After BR55 administration, the contrast inflow within the target ovary will be observed for approximately 45 seconds using a low frame rate to minimize destruction of targeted bubbles. Subsequently, post-contrast images will be acquired using a 2D probe at predefined time intervals of 2 minutes, starting from 2 minutes after administration of BR55 and up to 30 minutes post BR55 administration, or until stationary enhancement is no longer visible, whichever occurs first.

To determine the optimal imaging technique for this study population, up to 3 subjects will be enrolled as “training cases”. The technical aspects in the training phase of the study will focus on the CEUS examination using the wash-in phase on a predefined plane of the target ovary followed by slow sweep acquisitions over the entire ovary (starting from one pole to the other or one site of the ovary to the other) for at least 2 sec per plane. After the images are acquired on the training subjects, a consensus review will be performed to determine the optimal imaging technique to be used for the remainder of the study.

The Imaging Manual will be modified accordingly to describe the nuances in imaging technique.

DICOM images will be transferred to Bracco Imaging Core Laboratory.

Efficacy Evaluations

For CEUS exams, a quantitative assessment of BR55 enhancement will be performed at each acquisition time point by drawing a Region of Interest (ROI) within the following predefined areas in the target ovary, as applicable:

- corpus luteum, or focal abnormality (if present)
- area of focal enhancement (if present)
- normal tissue.

If more than one area of focal enhancement will be identified, the largest one will be considered for measuring of the SI.

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

Statistical Methods

Any major statistical methods deviated from the original statistical plan in this protocol will either be justified and approved in a protocol amendment or described in the separate statistical analysis plan, and then reported in the clinical trial report.
In general, summary statistics (mean, median, standard deviation, minimum, and maximum) will be provided for continuous variables, and the number and percentage of each category will be provided for categorical data.

All the data will be presented in the subject data listings.

Subject Disposition and Demographic and Baseline Characteristics

Subject completion status will be tabulated with number and percentages of subjects dosed and completed according to the protocol guidelines, and the reasons for any premature discontinuation will be presented.

Summary tables will be provided for demographic and baseline characteristics, including age, weight, height, gender, and race for each dose, as applicable.

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. Training subjects will not be included in the efficacy analysis.

Extent of Exposure

Descriptive statistics will be presented to summarise the volume of investigational medicinal product 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 Investigational Medicinal Product (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. Training subjects will not be included in the efficacy analysis. 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.

Comparison of signal intensity (SI) on BR55 CEUS between two phases of the menstrual cycle

The objective of this study is to determine the optimal phase of the menstrual cycle for performing BR55 CEUS of the target ovary in premenopausal women at high risk for ovarian cancer.

The analysis will be performed on subjects determined by pathology to be negative for cancer. The descriptive summary of the SI will be presented for early follicular phase and late follicular phase of the menstrual cycle. A scatter plot will be graphed to present the distributions of the paired observations for the early follicular phase SI versus the late follicular phase SI. The null hypothesis that early and late follicular phase imaging time points yield equal SI difference will be tested:
H_0: $SI_{early} = SI_{late}$
H_a: $SI_{early} \neq SI_{late}$

If the data at each time point can be normalized, a paired t- test will be performed. Otherwise the Wilcoxon signed-rank test will be used.

Correlation between SI at BR55 CEUS within the target ovary and histopathology findings

Descriptive statistics will be presented for SI on BR55 CEUS by the histopathology findings.

Correlation between SI within the target ovary on BR55 CEUS and VEGFR2 expression as determined by immunohistochemistry (IHC)

Descriptive statistics will be presented for the relationship between SI within the target ovary on BR55 CEUS and VEGFR2 expression level, as measured by the angiogenesis score. If data are

available, Spearman correlation coefficient may be estimated to explore the correlation between SI within the target ovary on BR55 CEUS and VEGFR2 expression level.

Correlation between SI within the target ovary on BR55 CEUS and microvessel density (MVD*)*

Descriptive statistics will be presented to explore the relationship between the SI within the target ovary on BR55 CEUS and MVD. If data are available, Spearman correlation coefficient may be estimated to explore the correlation between SI within the target ovary on BR55 CEUS and MVD.

Sample Size

Since this is the first-in-women clinical trial to determine the optimal phase of the menstrual cycle for performing BR55 CEUS of the target ovary in premenopausal women at high risk for ovarian cancer, no data exist from which to estimate variability or anticipate effect size, nor are there data to indicate a clinically relevant difference. Hence, this is an exploratory study. The sample size determination is not based on the statistical considerations and assumptions. About 50 female subjects meeting inclusion and exclusion criteria will be prospectively enrolled in order to obtain 47 evaluable subjects with normal bilateral ovaries and no occult cancer.

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.