CONFIDENTIAL

TRANSABDOMINAL ULTRASOUND WITH BR55 FOR CHARACTERIZATION OF PANCREATIC LESIONS

BR55

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Protocol Title

Transabdominal Ultrasound with BR55 for Characterization of Pancreatic Lesions

Protocol No.

This study is being conducted under protocol number: BR55-110.
IND number: 114,098.

Objectives

The objectives of this exploratory study are:

- to assess the ability of BR55 contrast-enhanced ultrasound (CEUS) to characterize pancreas lesions using transabdominal ultrasound (US) in subjects with suspected pancreatic ductal adenocarcinoma (PDAC) 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 BR55 in subjects with suspected pancreatic 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 correlation between SI of the target lesion on BR55 CEUS and VEGFR2 expression as determined by IHC;
- to assess the correlation between SI of the target lesion on BR55 CEUS and microvessel density (MVD) as determined by IHC.

Investigational Plan

This is an exploratory, single center, open label, parallel-dose, and prospective study of BR55 CEUS for characterization of solid pancreatic lesions in subjects with suspected PDAC using transabdominal US. This study will be conducted in the US.

Approximately twenty-four (24) subjects with suspected PDAC and scheduled to undergo surgical resection or endoscopic ultrasound-guided fine needle biopsy (EUS-FNB) within 30 days (but not before 24 hours) after the transabdominal BR55 CEUS examination will be enrolled.

Subjects will be enrolled into 3 dose groups starting with the lowest dose group as follows:

- Dose group 0.03mL/kg
- Dose group 0.05mL/kg
- Dose group 0.08mL/kg

A maximum of 8 patients will be enrolled in each dose group according to the following methodology.

Enrollment into the next higher dose group will be based on the technical adequacy of the ultrasound acquisitions, as determined by a consensus medical review of images acquired in the first 4 subjects of the preceding dose group. Technical inadequacy of the ultrasound acquisitions will be determined in accordance with, but not limited to the following criteria:

- absent or insufficient contrast enhancement during the wash-in phase
- absent or insufficient contrast enhancement in subsequent image acquisitions (i.e. after the wash-in phase)
- lack of visualization of the target lesion in the post-contrast image acquisitions

Efficacy evaluations will be made on the US images that are acquired adequately. The Investigator will record the size, location and echogenicity of the lesion for UEUS. After BR55 is administered, the CEUS images will be evaluated for contrast enhancement using a semi-quantitative scale and a quantitative measurement of the SI in the target lesion vs. surrounding pancreatic tissue.

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

Safety will be evaluated in this study by performing physical exams, collecting concomitant medications and the monitoring of adverse events, vital signs, electrocardiograms, laboratory tests and pulse oximetry.

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 planned imaging procedures, namely the transabdominal unenhanced US and BR55 CEUS, 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

This is a single center study including one investigative site in the United States. It is planned to enroll approximately 24 subjects with solid pancreatic lesions and suspected PDAC who are scheduled to undergo surgical resection or endoscopic ultrasound-guided fine needle biopsy (EUS-FNB) 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 at least one solid pancreatic lesion;
- Is scheduled to undergo surgical resection or endoscopic ultrasound-guided fine needle biopsy (EUS-FNB) for suspected PDAC 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 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 1 year without menses;
- Has undergone prior systemic therapy for pancreatic 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 pancreatic surgery (e.g., cyst removal);
- Has acute pancreatic abnormalities (acute pancreatitis or trauma).

Investigational Products

The study IP is BR55.

[redacted]

Administration

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

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

Three different doses of BR55 will be evaluated: 0.03, 0.05 and 0.08 mL/kg. A total of eight (8) subjects will be enrolled sequentially into each dose group starting at 0.3 ml/kg.

A maximum of 8 patients will be enrolled in each dose group according to the following methodology.

Enrollment into the next higher dose group will occur either after a total of 8 subjects complete enrollment, or the first 4 subjects enrolled in the previous dose group have CEUS images that are not technically adequate based on consensus medical review by PI & Bracco. Reasons for technical inadequacy will be recorded on the CRF and may include the following:

- absent or insufficient contrast enhancement during the wash-in phase
- absent or insufficient contrast enhancement in subsequent image acquisitions (i.e. after the wash-in phase);
- lack of visualization of the target lesion in the post-contrast image acquisitions

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

Methodology

Study Schedule

The Study Schedule is presented in Table B.

Table B: Study Schedule

Events	PRE-DOSE					BR55 inj.	POST-DOSE			
	Within 10d	Within 24h	Within 2h	-10 min	Immed. prior		+30 min	+1 h	+24 h	Up to 30 days
Written Informed Consent [a]	×	⇒								
Inclusion/Exclusion Criteria		×								
Demographics		×								
Medical History [b]		×								
Pregnancy test [c]		×								
Concomitant Medications [d]		×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Adverse Events Monitoring [e]		×	⇒	⇒	⇒	⇒	⇒	⇒	×	
Physical Examination		×							×	
Vital Signs [f]			×				×	×	×	
Pulse Oximetry				×	⇒	⇒	×			
Laboratory Evaluations		×							×	
Weight assessment			×							
Electrocardiogram			×				×	×	×	
UEUS exam					×					
BR55 Administration						×				
BR55 CEUS [g]						×	×			
Surgical resection or EUS-FNB for suspected PDAC [h]										×

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

[b] Includes disease related and general Medical History.

[c] A serum βHCG pregnancy test to be performed in subjects of childbearing potential.

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

[e] 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.

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

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

[h] Surgical resection or EUS-FNB for suspected PDAC to be performed from 24 h up to 30 days post-dose.

Written Informed Consent

Obtain written Informed Consent from the subject prior to the implementation of study procedures required by the protocol. Give to the subject a copy of the signed and dated written informed consent form including any other written information regarding the study. Document Informed Consent process in detail in the source record at the site.

Subject Evaluations

Demographics

Obtain demographic information on each subject (including; height, age, sex and race) and record on the Case Report Form.

Medical History

Obtain a complete medical history including disease related medical history and scheduled date for surgery for resection or endoscopic ultrasound-guided fine needle biopsy (EUS-FNB) of the suspected PDAC after the subject has signed the Informed Consent and within 24 hours prior to IP administration. Record the subject's medical history on the Medical History section of the Case Report Form.

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 to IP administration in the Concomitant Medication section of the Case Report Form. Additionally, record newly prescribed pharmacological treatments in this section up through 24 hours post IP administration.

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 at 24 hours after BR55 administration.

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

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 from 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 at 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

Acquire a 12-Lead ECG within 2 hours prior to and at approximately 30 min, 1 hour and 24 hours after BR55 administration. Attach a photocopy of the ECG report to the appropriate page of the Case Report Form.

ECG examinations will be performed locally and the ECG reports will be evaluated by a local cardiologist.

Imaging Procedures

Ultrasound Equipment

The transabdominal UEUS and CEUS examinations will be performed with FDA-approved ultrasound equipment, commercially available for clinical applications. The BR55 CEUS evaluation of the pancreas should be performed with a contrast dedicated platform and equipped with a multi-frequency curved array transducer used for conventional transabdominal US examinations.

For standardization purposes, the same ultrasound system and transducer will be used for all subjects enrolled at the investigational site. Additionally, any technical interventions (including software release updates and periodic system maintenance) will be evaluated by the Sponsor in order to make sure the image quality is not affected.

A study dedicated preset will be installed on the selected ultrasound machine.

Ultrasound Image Acquisition

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

The BR55 CEUS examination of the pancreas must be preceded by a standardized transabdominal UEUS examination of the pancreas to identify the focal target lesion. Oral fluid may be administered as needed to reduce shadowing gas in the stomach. Optimally, it should be possible to view the entire lesion within the field of view of the ultrasound transducer. UEUS will also serve to identify the optimal plane for imaging the target lesion after BR55 administration.

For the subsequent BR55 CEUS, to prevent microbubble destruction, the procedure should be performed in contrast mode using a low Mechanical Index.

Contrast inflow into the target lesion will be observed in the optimal imaging plane for approximately 60 seconds and using a low frame rate. Post-contrast images will be acquired at 2 minute time intervals, starting at 2 minutes and continue up to 30 minutes post BR55 administration, or until stationary enhancement in the lesion is no longer visible, whichever occurs first.

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

Efficacy Evaluations

Unenhanced Ultrasound (UEUS) Assessment

The investigator will draw the area corresponding to the target lesion on the pancreas map in the Case Report Form, indicating the location of the lesion (head, neck, body or tail of the pancreas), the size in centimeters, and the echogenicity (i.e., hypoechoic, isoechoic, or hyperechoic).

Contrast Enhanced (CEUS) Ultrasound Assessment

The investigator should indicate in the Case Report Form whether or not contrast enhancement was seen in the target lesion during the 60 second wash-in phase. Also the investigator should record the degree of specific (e.g., BR55-VEGFR2 binding) enhancement seen according to the 3-point semi-quantitative visual scale outlined below.

Visual Assessment of BR55 Enhancement of the Target Lesion

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 stationary.

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 and measuring the SI within that ROI. The SI fold-change will be calculated as the ratio of the SI in the target lesion ROI to the SI in the surrounding tissue ROI. Further details on these measurements will be provided in the Imaging Manual.

Diagnosis Confirmation

Pathology and Immunohistochemistry Measurements for Pancreatic Lesions

Standard protocols for collecting tissue samples from pancreas subjects undergoing surgery will be used as part of Standard of Care. Tissue sections from the suspected pancreatic lesion and normal tissue will be collected, macro dissected free of stroma and necrosis and processed using established standard clinical protocols. These samples will be used to correlate BR55 CEUS imaging signal with molecular markers of angiogenesis. For patients who will not undergo surgery after BR55 CEUS but will receive EUS-FNB, core tissue sample will be collected and used for correlation of CEUS imaging signal with IHC.

On the formalin fixed collected samples, the target lesion ROI or the core tissue sample will be assessed using standard histopathology and IHC analysis methods, including staining for the expression of VEGFR2 and CD31.

Histopathology measurements and IHC analysis will be described in detail in a separate dedicated Pathology Manual and performed on-site.

A copy of the pathology report will be collected to confirm the subject's diagnosis.

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 summarize the volume of IP administered.

Safety Analysis

All patients receiving any dosage of IP 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 MedDRA and summarized by system organ class and preferred term, by intensity and by causal relationship to the IP. Only those occurrences which occur from the start of IP administration through the follow-up period defined in the protocol will be tabulated in the Clinical Study 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

<u>General</u>

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.

<u>Characterization of Pancreatic Lesions – by BR55 CEUS Visual Score Using Histopathology as the Truth Standard (TS)</u>

The objective of this study is to determine the ability of BR55 CEUS to characterize pancreatic lesions in subjects with suspected PDAC on the basis of a visual score (semi quantitative visual assessment of BR55 enhancement) using histopathology as the TS.

CEUS images of the pancreas will be assessed by the investigator as 3-point scale for contrast enhancement of BR55 (none, weak enhancement, and strong enhancement), while TS assessment result will be classified as malignant if cancer is found or as benign if no cancer is found. Frequency tables will be provided for BR55 enhancement scores by TS assessment results (malignant and benign).

Signal Intensity (SI) of the Target Lesion on B55 CEUS vs. TS

SI will be defined as the quantitative assessment of BR55 enhancement of the target lesion on BR55 CEUS.

Summary statistics will be provided for SI by TS (malignant and benign). If data are available, a t-test or non-parametric test will be performed to test for a difference in mean SI on BR55 CEUS between the malignant and benign lesions per TS.

Furthermore, SI on BR55 CEUS will be categorized as no signal, low signal, and high signal, cross tabulation of the 3 SI categories versus TS will be presented.

In addition, exploratory analyses, such as ROC analysis of SI on BR55 CEUS in predicting presence of a malignant lesion as determined by TS and logistic regression will be performed if data is available and deemed as needed for this exploratory study.

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

To investigate relationship between SI of the target lesion on BR55 CEUS and VEGFR2 expression level, the Spearman correlation coefficient between the SI and VEGFR2 will be estimated.

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

To investigate the relationship between SI of the target lesion on BR55 CEUS and MVD, The Spearman correlation coefficient between SI and MVD will be estimated.

Sample Size

This is an exploratory study. The sample size determination is not based on the statistical considerations and assumptions. Approximately 24 subjects will be prospectively enrolled into three groups to receive BR55 at 0.03 mg/kg, 0.05 mg/kg and 0.08 mg/kg dose levels.

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.