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Citation for published version (APA):

Smeenge, M., Tranquart, F., Mannaerts, C. K., de Reijke, T. M., van de Vijver, M. J., Laguna, M. P., Pochon, S., de la Rosette, J. J. M. C. H., & Wijkstra, H. (2017). First-in-human ultrasound molecular imaging with a VEGFR2-specific ultrasound molecular contrast agent (BR55) in prostate cancer a safety and feasibility pilot study. *Investigative Radiology*, 52(7), 419-427. <https://doi.org/10.1097/RLI.0000000000000362>

Document license:
TAVERNE

DOI:
[10.1097/RLI.0000000000000362](https://doi.org/10.1097/RLI.0000000000000362)

Document status and date:
Published: 01/07/2017

Document Version:
Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

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First-in-Human Ultrasound Molecular Imaging With a VEGFR2-Specific Ultrasound Molecular Contrast Agent (BR55) in Prostate Cancer

A Safety and Feasibility Pilot Study

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Objective: BR55, a vascular endothelial growth factor receptor 2 (VEGFR2)-specific ultrasound molecular contrast agent (MCA), has shown promising results in multiple preclinical models regarding cancer imaging. In this first-in-human, phase 0, exploratory study, we investigated the feasibility and safety of the MCA for the detection of prostate cancer (PCa) in men using clinical standard technology.

Materials and Methods: Imaging with the MCA was performed in 24 patients with biopsy-proven PCa scheduled for radical prostatectomy using a clinical ultrasound scanner at low acoustic power. Safety monitoring was done by physical examination, blood pressure and heart rate measurements, electrocardiogram, and blood sampling. As first-in-human study, MCA dosing and imaging protocol were necessarily fine-tuned along the enrollment to improve visualization. Imaging data were correlated with radical prostatectomy histopathology to analyze the detection rate of ultrasound molecular imaging with the MCA.

Results: Imaging with MCA doses of 0.03 and 0.05 mL/kg was adequate to obtain contrast enhancement images up to 30 minutes after administration. No serious adverse events or clinically meaningful changes in safety monitoring data were identified during or after administration. BR55 dosing and imaging were fine-tuned in the first 12 patients leading to 12 subsequent patients with an improved MCA dosing and imaging protocol. Twenty-three patients underwent radical prostatectomy. A total of 52 lesions were determined to be malignant by histopathology with 26 (50%) of them seen during BR55 imaging. In the 11 patients that were scanned with the improved protocol and underwent radical prostatectomy, a total of 28 malignant lesions were determined: 19 (68%) were seen during BR55 ultrasound molecular imaging, whereas 9 (32%) were not identified.

Conclusions: Ultrasound molecular imaging with BR55 is feasible with clinical standard technology and demonstrated a good safety profile. Detectable levels of the MCA can be reached in patients with PCa opening the way for further clinical trials.

Key Words: prostatic neoplasms, molecular imaging, ultrasonography, microbubbles, vascular endothelial growth factor receptor

(*Invest Radiol* 2017;52: 419–427)

The main diagnostic tools to diagnose prostate cancer (PCa) include serum prostate-specific antigen (PSA) measurement, digital rectal

examination, and transrectal ultrasound (TRUS)-guided systematic biopsy.¹ Unfortunately, this systematic untargeted biopsy approach results in sampling error with significant tumors being missed and undergraded.² In recent years, multiparametric magnetic resonance imaging (mpMRI) and mpMRI-targeted biopsies are increasingly used in clinical practice, and they have shown improvement of PCa detection following systematic TRUS-guided biopsies.³ However, still clinical significant tumors are missed using mpMRI alone. Therefore, following latest European Association of Urology guidelines, mpMRI cannot replace systematic TRUS biopsies and should be used in addition to systematic biopsies.¹ Consequently, there is an ongoing search for new reliable imaging technologies for adequate PCa detection.

Although conventional grayscale TRUS as imaging modality has a limited role in PCa detection at the moment, ultrasound-based imaging offers many advantages; it is widely available, far more portable than MRI, and less expensive in machine purchase and usage than MRI. In addition, TRUS has no confined working space, and biopsy-needle position can be monitored in real time, therefore allowing more core biopsies to be taken in less time. Transrectal ultrasound has excellent spatial and temporal resolution and allows real-time imaging without the use of ionizing radiation or kidney function impairing contrast. Therefore, targeted ultrasound-guided prostate biopsies would be preferable over targeted MRI-guided biopsies if both imaging modalities have similar PCa detection capacities.

Various ultrasound modalities are in development striving to increase cancer detection. Dynamic contrast-enhanced ultrasound (DCE-US) has shown promising results for PCa detection using different analysis methods, such as quantification techniques as dispersion imaging based on angiogenesis visualization.⁴

Angiogenesis, the formation and recruitment of new blood vessels from preexisting vessels and the host surrounding tissue, is known to play a central role in tumor growth up to clinically significant disease in many cancers, including PCa.^{5–7}

Several opposing factors challenge neovascularization perfusion imaging in cancerous tissue. Low resistance results from a lack of vaso-motor control and an increase in arteriovenous shunts, but this can be counterbalanced by an interstitial pressure increase resulting from extravascular leakage and by the small diameter and high degree of tortuosity of neovascularization.⁸ As a result, many perfusion measurements in DCE-MRI have difficulties producing reliable diagnostic results, and different analytic approaches may be needed.^{9–11}

A promising development in DCE-US is molecular imaging using molecularly targeted contrast microbubbles, which has emerged in recent years as a new promising ultrasound imaging technique.^{12,13} Molecularly targeted contrast agents can visualize disease processes at the molecular level by accumulating at tissue sites overexpressing certain molecular markers.¹³ These microbubbles, consisting of an insoluble gas stabilized by a shell, remain within the vasculature due to their size and are therefore suited for detection of disease processes characterized by alterations in vascularity and microvascular flow patterns such as angiogenesis.¹⁴

Received for publication September 27, 2016; and accepted for publication, after revision, January 3, 2017.

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ISSN: 0020-9996/17/5207-0419

DOI: 10.1097/RLI.0000000000000362

FIGURE 1. Schematic drawing of an attached BR55 microbubble. The bubble consists of a gas mixture of N₂ and C₄F₁₀ stabilized by a phospholipid shell. The shell is functionalized with a heterodimer peptide specific for VEGFR2. VEGFR2 indicates vascular endothelial growth factor receptor 2; PEG, polyethylene glycol.

The expression and interaction with the vascular endothelial growth factor receptor 2 (VEGFR2) plays a key role in the signaling cascade responsible for endothelial proliferation and migration, hence contributing to tumor angiogenesis and progression.^{8,15,16} In PCa, VEGFR2 is overexpressed compared with benign hyperplasia or normal gland tissue.^{8,17,18} VEGFR2 therefore represents an ideal target for angiogenesis imaging. VEGFR2-specific microbubbles, which are restricted to the vascular compartment, might specifically localize in the neovasculature of prostate tumors.¹⁸ Until now, molecular ultrasound contrast agents used proteins such as streptavidin or antibodies to bind to the designated receptor. Because of potentially immunogenic effects, use of these contrast agents is prohibited in humans. BR55, however, is a specific microbubble ultrasound contrast agent designed

to bind to the VEGFR2 receptor (Fig. 1) without the use of streptavidin or antibodies and allows the in vivo clinical use in humans.^{19–21} BR55 has been evaluated for several applications in preclinical in vitro and in vivo studies.^{21–27} Tardy et al²⁰ investigated its use in a rat human prostate tumor model, demonstrating exquisite binding of these targeted microbubbles to VEGFR2 receptors. Molecular ultrasound imaging using BR55, hereafter named as molecular contrast agent (MCA), resulted in a strong enhancement of the lesions by highlighting the sites of active angiogenesis and as a result could be valuable for PCa detection and biopsy guidance in men. In this proof-of-concept, phase 0 study, we investigated the feasibility and safety of the MCA in patients with biopsy confirmed PCa scheduled for radical prostatectomy. We report on the results with

TABLE 1. Main Inclusion and Exclusion Criteria

Main inclusion criteria	<ul style="list-style-type: none">• Male patient, age ≥40 y• Has a histology-proven focal PCa lesion• Scheduled for prostatectomy not earlier than 3 d and at the latest 15 d after BR55 administration• Provides written informed consent and is willing to comply with protocol requirements
Main exclusion criteria	<ul style="list-style-type: none">• Has a body weight greater than 95 kg (this weight limitation is required to maintain the active component of the drug under 100 µg) according to the indication of the EMA guideline M3 for this type of study• Has documented acute prostatitis or urinary tract infections• With history of any clinically unstable cardiac condition including class III/IV cardiac failure or right-to-left shunts• Has had severe cardiac rhythm disorders within the last 7 d• Has severe pulmonary hypertension (pulmonary artery pressure >90 mm Hg) or uncontrolled systemic hypertension or respiratory distress syndrome• Has received a bioptic procedure within 30 days before admission into this study• Has any medical condition or other circumstances, which would significantly decrease the chances of obtaining reliable data, achieving study objectives, or completing the study• Is determined by the investigator that the patient is clinically unsuitable for the study• Is incapable of understanding the language in which the information for the patient is given• Participation in a concurrent clinical trial or in another trial within the past 30 d

EMA indicates European Medicines Agency.

FIGURE 2. Study safety protocol of the first-in-human trial of molecular ultrasound imaging with the targeted contrast agent BR55. ECG, electrocardiogram.

FIGURE 3. Example of ultrasound molecular imaging of the prostate after an injection of BR55. A–C, Left contrast-specific imaging, right grayscale B-mode. A, Peak enhancement of BR55 at 1 minute after injection. B, 7 minutes after injection. C, 17 minutes after injection. D, Histopathology after radical prostatectomy. Cancer areas are marked in red. Figure 3 can be viewed online in color at www.investigativeradiology.com.

regard to dosing of the MCA providing a visible enhancement, imaging technique, safety profile, and preliminary evaluation of the ability of the MCA to identify PCa lesions.

MATERIALS AND METHODS

In this single-center, open-label, prospective phase 0 study entitled “Exploratory Clinical Trial Using BR55 Targeted Ultrasound Molecular Contrast Agent in the Detection of Prostate Cancer by Molecular Imaging of VEGFR2” (NCT01253213; www.clinicaltrials.gov), initially, 12 patients with biopsy-proven PCa and scheduled (at least 30 days after the biopsy procedure) for a radical prostatectomy were enrolled. Thereafter, the study was amended to increase the number of included patients to a total of 24. Patients were enrolled in this study between July 2010 and October 2012. Main inclusion and exclusion criteria can be found in Table 1. The Institutional Review Board of the Academic Medical Center (Amsterdam, the Netherlands) approved the study, including the amendment, and all patients signed an informed consent before inclusion into the study.

Dosage and Safety

As this was the first time the MCA was administered in humans, a low dose was given initially that was subsequently increased, and patients received a maximum of 2 doses of the MCA. Following European Medicines Agency (EMA) guidelines for guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals, a NOAEL (no observed adverse effect level) of 5 mL/kg per day was determined during a 2-week rat repeated dose toxicity study and confirmed in a shorter repeated dose study in dogs. This NOAEL corresponds to approximately 1 mL/kg in humans based on body surface area. Therefore, dosing started at a level of 0.01 mL/kg (about 1/100th of the NOAEL) and was planned to reach a maximum of 0.08 mL/kg. The upper limit of the maximal cumulated dose of the microbubble peptide was set to 100 µg per patient following the guidelines from EMA for the exploratory phase 0 study.²⁸

Therefore, per patient, a maximum dose of 7.6 mL was allowed, which translates to a maximum weight of 95 kg.

Safety monitoring was done by physical examination, blood pressure and heart rate measurements, electrocardiogram (ECG), and blood sampling within 1 hour before intravenous injection and 15 minutes, 1 hour, and 24 hour after intravenous injection with the MCA (Fig. 2).

Imaging Protocol

Ultrasound imaging was performed using an iU22 ultrasound scanner (Philips, Bothell, WA) and a C8-4v rectal probe in the first 12 patients and a C10-3v probe for the 12 additional patients enrolled in the amended protocol. Imaging was performed using a contrast-specific mode (power modulation) at 3.5 MHz and a low mechanical index ($MI \leq 0.12$). After intravenous bolus injection of the MCA, imaging was performed for 1 minute at a frame rate of 1 Hz to record the inflow of contrast in a transverse plane. After peak enhancement, insonation was stopped. For 5 minutes, no imaging was performed, allowing binding and washout of the unbound microbubbles, followed by image acquisitions of 10 seconds at a frame rate of 4 Hz, every minute from 5 to 10 minutes after injection. The described imaging procedures were defined with the goal to minimize possible bubble destruction and detachment of bubbles caused by the applied acoustic pressure and to cover the prostate volume as much as possible.

For the first 3 patients enrolled, after peak enhancement, imaging was performed as consecutive transverse sweeps every minute up to 10 minutes after administration of the MCA. However, when visualizing 1 plane for a time, bound microbubbles do not move, while unbound bubbles do move. When performing sweeps, bound bubbles also move out of plane, making it harder to differentiate between bound or unbound. Therefore, for the following 9 patients, a refined imaging protocol was used; recording of the inflow was performed, focusing on 1 imaging plane (apex, mid or base) viewing most biopsy locations containing PCa. Then, 10 seconds' acquisitions were performed in 3 planes of the prostate (apex, mid, and base alternatively) every minute up to 10 minutes' postinjection. After completion of the first 12 dosed

TABLE 2. Patient and Tumor Characteristics

Variables	n = 24
Age, mean (range), y	62 (43–70)
Ethnicity, n (%)	
White	17 (70.8)
Non-white	7 (29.2)
Mean PSA in ng/mL (median; range)	9.9 (7.9; 3.8–31.9)
Mean prostate volume (median; range)	43 (36; 15–103)
Digital rectal examination, n (%)	
Benign	12 (50)
Suspicious	12 (50)
Biopsy cores during last biopsy procedure, n (%)	
8 cores	10 (41.7)
10 cores	1 (4.2)
12 cores	13 (54.2)
Mean positive biopsy cores per biopsy procedure (median; range)	4 (3; 1–12)
Biopsy Gleason score, n (%)	
3 + 3 = 6	10 (41.7)
3 + 4 = 7	8 (33.3)
4 + 3 = 7	2 (8.3)
4 + 4 = 8	2 (8.3)
4 + 5 = 9	2 (8.3)
Pathological T stage, n (%)	n = 23
pT2a-b	2 (8.7)
pT2c	19 (78.3)
pT3	3 (13.0)
Radical prostatectomy Gleason score	n = 23
3 + 3 = 6	9 (39.1)
3 + 4 = 7	10 (43.5)
4 + 3 = 7	2 (8.7)
3 + 5 = 8	1 (4.3)
4 + 5 = 9	1 (4.3)

patients, the study protocol was amended and the imaging procedure was further adapted. Because strong contrast enhancement was still visible at 10 minutes, the imaging period was extended to up to 30 minutes after injection in the last 12 patients to investigate for how long contrast enhancement would stay visible. For all patients, a low MI (0.10 or 0.11) was used during MCA imaging, with the exception of 1 patient (MI = 0.12) where it accidentally was set higher.

Histopathology

Patients underwent radical (laparoscopic) prostatectomy. The prostate was fixed in formalin, sectioned into slices, and paraffin embedded. The slices were 4 to 8 mm thick, resulting in 6 to 13 slices per prostate, depending on prostate size. The entire specimen was hematoxylin-eosin-stained and evaluated by 2 uropathologists with no information in regard to imaging results. All slides containing tumor were identified, and tumor areas were marked and outlined on the slides; from the 6 to 13 slices, 3 were selected representing the basal, mid, and apical plane, for comparison with the imaging planes. Pathological TNM staging and Gleason score were also performed. An example of imaging and histopathology is presented in Figure 3.

Data Analysis

Imaging data were examined by 2 examiners (M.S. and H.W.). The examiners were aware that patients had biopsy-proven PCa but

were blinded for radical prostatectomy histopathology findings. The contrast enhancement with the MCA was evaluated by the examiners, scores were dichotomized into 2 categories for analysis—enhanced and not enhanced, respectively. Enhanced suspected areas were manually mapped on a prostate study map with the prostate gland divided into 3 axial planes (apex, mid, and base), and each of these planes were further divided into 4 sections, thus into 12 different regions. To determine the ability of MCA imaging to correctly identify PCa lesions, these predetermined enhanced regions were correlated with pathological tumor localization that were also manually mapped into the same 12 region-based map in a one-to-one correlation. Patient characteristics and imaging results were analyzed descriptively using SPSS Statistics 22.0 (IBM Corp, Armonk, NY).

RESULTS

Study Population

A total of 26 patients agreed to participate in the study. Two patients were excluded from the study; one of them was not suitable for the study per investigator's assessment and the other met the exclusion criterion of body weight greater than 95 kg (resulting in exceeding the maximum limit of the maximal cumulative dose). Therefore, a total of 24 patients were included in the study. Patient and tumor characteristics are shown in Table 2. The mean age of the patients was 62 years (range, 43–70 years), and the mean level of PSA was 9.9 ng/mL (range, 3.8–31.9). All patients had a systematic 8-, 10-, or 12-core TRUS-guided biopsy-proven PCa, and 84% (20/24) of the patients had a biopsy Gleason score of 6 or 7. The studied group would therefore generally fit in the low-moderate risk group of the European Association of Urology Risk group classification.¹ In 1 patient that was included, a bone metastasis was detected by MRI and prostatectomy was not performed. All other included patients completed all protocol-specified procedures and underwent a radical prostatectomy. Ninety-one percent (21/23) of the patients had a Gleason score of 6 or 7 in the radical prostatectomy specimen.

Dosage and Safety

Dosage, imaging, and safety results are shown in Table 3. Because this was the first time the MCA was administered in humans, the first 2 patients received only 1 injection. All others, with the exception of 1 patient, received 2 injections. The first 2 patients received 0.01 mL/kg, and the next 2, 0.01 and 0.02 mL/kg, resulting in no or poor enhancement and binding. Therefore, the dose in the next group of patients was increased to 0.03 and 0.05 mL/kg sequentially leading to a cumulative dose of 0.08 mL/kg. Enhancement differences are shown in Figure 4. The latter doses resulted in enhancements judged as adequate and sustainable, bubbles were still visible at imaging after 5 to 10 minutes. Adequate enhancement was judged as clear delineation of an enhanced area from surrounding areas.

No clinically meaningful changes from baseline in vital signs (systolic and diastolic blood pressure, heart rate) and qualitative and quantitative 12-lead ECG assessments and no clinically significant changes in laboratory values (hematology and clinical chemistry) were observed. No serious adverse events were reported, and no patient discontinued as a result of an adverse event. Four patients experienced a nonserious adverse event after administration of the MCA: rash at 50 minutes, cold shivers at more than 10 hours, headache at approximately 4 hours, and back pain at 8 hours. All nonserious adverse events were reported as mild to moderate in intensity, and all patients recovered without medical action or sequelae at the 24-hour follow-up.

Detection of PCa Lesions

Molecular contrast agent dosage and ultrasound imaging acquisition method and duration were fine-tuned along the enrolment in the

TABLE 3. Dosage, Imaging, and Safety Data

Patient ID	Dosage		Imaging				Safety					
	Injection 1, mL/kg	Injection 2, mL/kg	Mechanical Index Used for Imaging 1	Mechanical Index Used for Imaging 2	Acquisition Method	Acquisition Time, min	Yes/No	Nonserious Adverse Event				
								Type	Time After BR55	Intensity	Action Needed	Outcome
1	0.01	—	0.12	—	Sweep	10	No	—	—	—	—	—
2	0.01	—	0.10	—	Sweep	10	No	—	—	—	—	—
3	0.01	0.02	0.10	0.10	Sweep	10	No	—	—	—	—	—
4	0.01	0.02	0.10	0.10	Planes	10	No	—	—	—	—	—
5	0.03	0.05	0.11	0.11	Planes	10	No	—	—	—	—	—
6	0.03	0.05	0.11	0.11	Planes	10	No	—	—	—	—	—
7	0.03	0.05	0.10	0.10	Planes	10	No	—	—	—	—	—
8	0.03	0.05	0.11	0.11	Planes	10	Yes	Rash	50 min	Mild	None	Full recovery
9	0.03	0.05	0.10	0.11	Planes	10	No	—	—	—	—	—
10	0.03	—	0.11	—	Planes	10	No	—	—	—	—	—
11	0.03	0.05	0.10	0.10	Planes	10	Yes	Cold shivers	10 h	Mild	None	Full recovery
12	0.03	0.05	0.11	0.11	Planes	10	No	—	—	—	—	—
13	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
14	0.03	0.05	0.10	0.10	Planes	30	Yes	Headache	4 h	Mild	None	Full recovery
15	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
16	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
17	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
18	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
19	0.03	0.05	0.10	0.10	Planes	30	Yes	Back pain	9 h	Moderate	None	Full recovery
20	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
21	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
22	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
23	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—
24	0.03	0.05	0.10	0.10	Planes	30	No	—	—	—	—	—

first 12 patients (group 1). For the 12 patients that enrolled under the protocol amendment (group 2), dosage and imaging protocol were equal to each other—imaging acquisition in 3 planes up to 30 minutes after the MCA injections of 0.03 and 0.05 mL/kg, respectively. Results are shown in Table 4. For the first 12 patients, a total of 24 malignant lesions were identified from histopathology results. Seven (29%) of the 24 malignant lesions were detected during MCA ultrasound molecular imaging while 17 (71%) were not seen. For the 11 patients under the protocol amendment, 28 malignant lesions were identified from histopathology results; 19 (68%) of the malignant lesions were seen during MCA ultrasound molecular imaging, and 9 (32%) were not identified. In total, 52 lesions were determined to be malignant by histopathology and 26 (50%) were seen during MCA imaging after the first and/or second injection of the MCA.

DISCUSSION

The objective of this exploratory study reporting on the use of the targeted ultrasound contrast agent BR55 was to address questions around safety profile, dosage, imaging technique, and a preliminary evaluation of the ability of the MCA to identify PCa lesions. Because that was the first-in-human molecular ultrasound imaging trial with

intravenous injection of targeted microbubbles, these questions were of particular relevance to consider further clinical developments of this new imaging mode.

The safety profile of conventional nonmolecular microbubble ultrasound contrast agents is well recognized after its use in thousands of patients. Adverse reactions are transient, mild, and rare with altered sensation at the injection site, flushing, taste perversion, and headache most commonly mentioned.^{29–33} In our study, no serious adverse events or unanticipated events were reported after administration of the MCA. Four patients (17%) reported each 1 nonserious adverse events; 3 of them were mild, 1 moderate, and all 4 patients recovered without medical action. Determination of causality of each nonserious adverse event to the MCA was difficult as different factors such as preexisting medical conditions, concomitant medication, and the patient's clinical state also play their role. For example, patient 19 with back pain as moderate nonserious adverse event 9 hours after MCA imaging had already a documented ongoing chronic low back pain during medical history interrogation. Furthermore, 2 mild nonserious adverse events (cold shivers and headache) started at 4 and 10 hours after the MCA imaging, raising questions of reasonable temporal sequence following the MCA injection. With no serious adverse events, no clinically meaningful changes from baseline in vital signs or ECG results, no clinically significant changes in laboratory values, good toleration with no patient

FIGURE 4. Example of enhancement using different BR55 concentrations, images taken about 5 minutes after injection. A–D, Left contrast-specific imaging, right grayscale B-mode. A, 0.01 mL/kg. B, 0.02 mL/kg. C, 0.03 mL/kg. D, 0.05 mL/kg. Figure 4 can be viewed online in color at www.investigativeradiology.com.

discontinuation, and only mild self-limiting nonserious adverse events, the overall safety profile of the MCA in this study was considered as good. However, the safety profile still needs to be assessed in future studies with larger sample sizes.

The study provides a preliminary indication that the lowest tested doses of the MCA, 0.01 mL/kg and 0.02 mL/kg, cannot provide adequate and sustainable contrast enhancement in humans during inflow and in the imaging intervals between 5 and 10 minutes. This is most likely due to a limited number of bubbles flowing in the vessels resulting in a low binding, at least below the detection capacity of ultrasound molecular imaging. Conversely, the highest doses tested, 0.03 mL/kg and 0.05 mL/kg, were judged as adequate to obtain a sustainable delayed contrast enhancement of the whole prostate and a significant portion of suspected lesions. We suggest starting with the dose of 0.03 mL/kg in following the MCA studies; however, advice on the optimal dosage cannot be provided because doses higher than 0.05 mL/kg were not injected. We acknowledge that this is a user-dependent interpretation calling for quantitative measuring methods to be used in future studies.

A bolus injection was used, achieving a high intravascular peak concentration, therefore increasing the likely interaction between these microbubbles and the VEGFR2 receptors located at the surface of the vessel wall endothelium, facilitating receptor binding.

The inflow of contrast was clearly visualized using a low frame rate of 1 frame per second with a low MI to prevent bubble destruction and possible detachment. The imaging protocol was optimized first to limit bubble destruction, second, to provide an objective difference between flowing and bound bubbles, and finally, to cover as much as possible the whole prostate volume. This explains why, in the absence of previous experience in human, a significant effort has been made to continuously fine-tune the imaging protocol during the trial to identify the best conditions in terms of timing and imaging planes for reaching our objectives.

The results indicate that BR55 ultrasound molecular imaging might help in detecting prostate lesions. Among all 52 lesions identified as malignant by histopathology, 26 (50%) were seen during MCA-enhanced ultrasound. Meanwhile, 19 (67.9%) of the 28 malignant

lesions were seen for the 12 patients that were enrolled under the protocol amendment with an identical dosage and imaging protocol. In this study, Gleason score of 3 + 3 was the most missed tumor. Gleason scores of 3 + 3 are probably more difficult to visualize and are expected to have less VEGFR2-binding potential compared with tumors with higher Gleason grade. Overall, the MCA enhancement was reported more frequently in those patients having more than 1 plane imaged and extended imaging time, and/or with the administration of the highest dose. Because this was the first-in-human study, the dose for adequate enhancement and the optimal imaging technique were investigated, resulting in the use of different doses and imaging procedures, therefore limiting further statistical analysis of the results. A larger study with a set dosage and imaging protocol is needed to determine the additional benefit of the MCA in PCa.

In our study, a 3-plane, 12-region division of the prostate was used; most prostate biopsy schemes use a 4-plane, 16-region division. Image acquisition following the aforementioned method will therefore take even longer. A major limiting factor is the use of 2-dimensional (2D) ultrasound imaging.

The use of 2D technology has limited use in covering the whole prostate volume. As a consequence, it was impossible to detect all potential areas of binding. On top of that, misalignment between oblique imaging planes and orthogonal histology planes has limited our capacity to correctly match enhance areas with histology. Future studies should address this point and should preferably be performed with a 3-dimensional (3D) ultrasound imaging technology. On that note, Wang et al³⁴ recently demonstrated in a murine model of human colon cancer that 3D ultrasound molecular imaging is feasible, highly reproducible, and allows accurate assessment and monitoring of VEGFR2 expression in tumor angiogenesis.

Correlating imaging with histopathology is difficult. Because of few landmarks, prostate deformation by the ultrasound probe, and histological fixation, potential mismatch between planes on imaging and histopathology is present. Another factor contributing to this mismatch is the positioning of DCE-US and pathology planes, which are acquired at different angles. A technique where imaging data and histopathology are coregistered within a computerized 3D model of the prostate would

TABLE 4. Detection of PCa Lesions

Patient ID*	Biopsy Cores		Gleason Score		PCa Lesion		
	PCa Positive Cores	Total Biopsy Cores	Biopsy	Radical Prostatectomy	Detected on BR55 Imaging (Both Procedures)	Identified on Histopathology	Detection Rate
Group 1							
1	2	12	3 + 4 = 7	3 + 3 = 6	1	1	100% (1/1)
2	6	10	3 + 3 = 6	3 + 3 = 6	0	2	0% (0/2)
3	2	8	3 + 4 = 7	3 + 4 = 7	0	2	0% (0/2)
4	3	8	3 + 3 = 6	3 + 4 = 7	1	5	20% (1/5)
5	1	12	3 + 3 = 6	3 + 3 = 6	1	1	100% (1/1)
6	3	8	3 + 4 = 7	3 + 4 = 7	1	3	33% (1/3)
7	1	8	4 + 5 = 9	3 + 4 = 7	1	2	50% (1/2)
8	2	8	3 + 4 = 7	3 + 4 = 7	1	2	50% (1/2)
9	2	8	3 + 3 = 6	3 + 3 = 6	0	2	0% (0/2)
10	1	12	3 + 3 = 6	3 + 3 = 6	0	2	0% (0/2)
11	4	8	4 + 4 = 8	3 + 5 = 8	0	1	0% (0/1)
12	2	8	3 + 3 = 6	3 + 3 = 6	1	1	100% (1/1)
Total group 1					7	24	29.2% (7/24)
Group 2							
13	6	8	3 + 4 = 7	4 + 3 = 7	2	2	100% (2/2)
14	6	12	3 + 3 = 6	3 + 3 = 6	0	3	0% (0/3)
15	6	8	3 + 3 = 6	3 + 4 = 7	2	2	100% (2/2)
16	3	12	3 + 3 = 6	3 + 3 = 6	2	3	67% (2/3)
17†	1	12	3 + 3 = 6	3 + 3 = 6	0	0	—
18	3	12	4 + 5 = 9	4 + 5 = 9	2	2	100% (2/2)
20	4	12	3 + 4 = 7	3 + 4 = 7	2	4	50% (2/4)
21	5	12	3 + 4 = 7	3 + 4 = 7	2	3	67% (2/3)
22	9	12	3 + 4 = 7	3 + 4 = 7	2	2	100% (2/2)
23	5	12	4 + 3 = 7	4 + 3 = 7	2	4	50% (2/4)
24	12	12	4 + 3 = 7	3 + 4 = 7	3	3	100% (3/3)
Total group 2					19	28	67.9% (19/28)
Whole group					26	52	50% (26/52)

*Patient ID 19 did not undergo a radical prostatectomy and is therefore not presented.

†No PCa lesion(s) identified on histopathology in the 3 planes used for BR55 imaging analysis.

enable comparison in the same plane.³⁵ Hopefully, this can be a future help in solving the angulation and plane correlation problem. In addition, 3D ultrasound enables image acquisition of the whole prostate in 1 image recording, compared with 3 or 4 different planes in 2D. Not only does 3D provide more information, by not having missing areas between planes, it also greatly reducing procedure time.³⁶ An additional technique reducing procedure was recently proposed by Turco et al. They show a method for quantitative assessment of MCA binding by modeling the microbubble binding rate during the MCA first pass, reducing the acquisition time to 1 minute.³⁷

The acoustic pressure typically used during imaging can potentially destroy the MCA bubbles and/or can induce detachment from the VEGFR2 receptors. In the present study, imaging was limited to short periods using a low MI and a low frame rate. In future trials, binding might be improved by applying ultrasound radiation force to “push” gently the bubbles toward the vessel wall with no destruction resulting in a possible increased binding. This effect was demonstrated to be effective in a prostate tumor model in rats showing approximately a 7-fold increase in specific binding in the tumor.³⁸ A future confirmation of a similar effect in patients will open further opportunities for molecular ultrasound imaging.

In the last 12 patients, imaging time was extended until 30 minutes after the MCA injection, because in the first patients,

microbubbles were still seen at 10 minutes. Nontargeted ultrasound contrast agents are only visible for 3 to 5 minutes. Possibly, bubbles stay intact for a longer period when receptor bound. The extension to 30 minutes was to see how long bubbles would stay visible.

As mentioned before, using ultrasound-guided biopsies over MRI-guided biopsies has advantages; ultrasound is portable and has no confined working space, and biopsy-needle position can be monitored in real time. Therefore, when reliable PCa detection using targeted microbubbles is available, ultrasound guidance could be preferable over MRI guidance.

In this phase 0 study, we used the MCA for prostate imaging; however, the VEGFR2 is present and overexpressed in many other cancer types and, therefore, could be used for ultrasound molecular imaging in, for example, breast or liver cancer.^{7,26,27}

In follow-up (phase 1 or 2) studies, we propose to focus on finding the optimal dosage, using 0.03 mL/kg and higher, attaining maximal receptor binding. Correlation of imaging with histology should be performed using radical prostatectomy specimens. VEGFR2 staining needs to be performed in all PCa areas, and enhanced areas where no PCa is present, to compare VEGFR2 expression differences. In addition, 3D ultrasound is preferred over 2D, allowing whole prostate imaging in 1 recording and enabling comparison in exactly the same plane. Image analysis would benefit

from enhancement quantification supplemented with subtraction tools, filtering bound from unbound bubbles.

The results of this exploratory clinical trial demonstrate that PCa ultrasound molecular imaging with BR55 is feasible with clinical standard technology, had a good safety profile, and is well tolerated. Because of the limitations of this exploratory study, only preliminary clinical results have been presented. Future studies with a fixed imaging protocol are needed to determine the additional clinical benefit.

CONCLUSIONS

This first-in-human ultrasound molecular imaging trial with BR55 as a targeted contrast agent demonstrated a good safety profile and tolerability at doses up to 0.05 mL/kg (cumulative doses up to 0.08 mL/kg) with no abnormal findings, significant changes, or trends measured in the safety parameters. Successful binding of the MCA was observed in this exploratory clinical trial with encouraging preliminary PCa detection results in the patients after necessary dosage and imaging fine-tuning. A minimum dose of 0.03 mL/kg is required to obtain a sustainable contrast enhancement of the whole prostate and of suspected lesions.

However, further studies are needed to determine the exact additional clinical value while addressing the various limitations here reported.

ACKNOWLEDGMENT

The authors thank Alice Bakker and Paola Pianezzola for their appreciated assistance and safety and adverse event monitoring during the conduct of the clinical trial.

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