Clinical validation of transrectal multiparametric ultrasound imaging strategy (PCaVision) for the detection of clinically significant prostate cancer: a head-to-head comparison with the MRI-based strategy

Clinical validation of transrectal multiparametric ultrasound imaging strategy (PCaVision) for the detection of clinically significant prostate cancer: a head-to-head comparison with the MRI-based strategy

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

2D	Two-dimensional
3D	Three-dimensional
3D mpUS	Three-dimensional mpUS
AE	Adverse Event
AR	Adverse Reaction
BPH	Benign prostate hyperplasia
CEUS	Contrast Enhanced Ultrasound
CI	Confidence Interval
csPCa	Clinically significant prostate cancer
CV	Curriculum Vitae
CUDI	Contrast Ultrasound Dispersion Imaging
DRE	Digital Rectal Examination
ESUR	European Society of Urogenital Radiology
GCP	Good Clinical Practice
GG	Grade Group
IC	Informed Consent
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
mpMRI	Multiparametric Magnetic Resonance Imaging (with or without contrast)
mpUS	Multiparametric Utrasound
MRI	Magnetic Resonance Imaging
NVU	Nederlandse vereniging voor Urologen
PCa	Prostate Cancer
PCaVision	Prostate Cancer Classifier that makes use of mpUS including CUDI
PIN	Prostatic Intraepithelial Neoplasia
PIRADS	Prostate Imaging-reporting and data system

PSA	Prostate-Specific Antigen
SAE	Serious Adverse Event
SBx	Systematic biopsy
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBx	Targeted Biopsy
RP	Radical Prostatectomy
US	Ultrasound

SUMMARY

Rationale:

The incidence of prostate cancer (PCa) has increased over the years. To diagnose a patient with PCa, histopathological confirmation is required. Different diagnostic pathways are currently available. Systematic biopsies have long been the cornerstone in the diagnostic work-up of men suspected for prostate cancer. Systematic biopsies, however, can lead to under-diagnosing of clinically significant prostate cancer (csPCa) and each biopsy is associated with the risk of infection and other side effects.

In the magnetic resonance imaging (MRI) pathway, targeted biopsies are only performed when suspicious lesions are detected on MRI. The MRI pathway purposely detects fewer clinically insignificant prostate cancer (ciPCa), but has an increased sensitivity for csPCa, and an increased localization accuracy of cancer suspicious regions. The MRI-based strategy is now recommended as the first-line investigation. However, reported sensitivities and specificities for MRI vary widely between studies which can be attributed to a difference in MRI equipment, study design related to the quality of reference standard procedure, and inter-observer variability. Moreover, MRI suffers from limited availability, and is a time-consuming and expensive imaging modality.

Transrectal ultrasound, which is widely available, cheaper to implement and familiar to the urologist could be a valid alternative. In an ultrasound pathway, 2D contrast-enhanced ultrasound (CEUS) in combination with contrast-US-dispersion imaging (CUDI) focuses on the detection of angiogenetic changes in the microvascular architecture, to localize lesions suspected for PCa, followed by target biopsies for histological confirmation. Promising results for 2D CEUS have been obtained and it is expected that 3D multiparametric ultrasound (mpUS) including 3D CUDI will allow more accurate identification of significant prostate cancer.

PCaVision software was developed for post-processing analysis of several modalities of ultrasound data of the prostate including 3D CEUS. PCaVision combines Image Analysis Software and Viewing Software developed as an algorithm based on machine learning trained in the detection of lesions suspect for cancer.

The overall aim of this study is to compare the diagnostic accuracy of two different diagnostic pathways to detect csPCa in biopsy-naïve men: (1) PCaVision targeted biopsy pathway; (2)

MRI targeted biopsy pathway. These pathways will be examined in this study using a fully paired design.

Objective:

The primary objective is to demonstrate non-inferiority of the detection rate of clinically significant prostate cancer in targeted biopsies based on PCaVision imaging (PCaVision pathway) in comparison with the detection rate of clinically significant cancer in targeted biopsies based on MRI (MRI pathway). In this comparison, clinically significant prostate cancer is defined as International Society of Urological Pathology (ISUP) Grade Group (GG) ≥ 2 in any of the biopsy cores taken from a lesion.

Secondary objectives are (1) to compare the proportion of men in whom targeted biopsies could be “safely” omitted in the PCaVision pathway, defined as the number of men in whom no lesions for target biopsies have been identified by PCaVision while no clinically significant cancer is detected in either MRI targeted biopsies or possible systematic biopsies, compared to the MRI pathway, (2) to perform the same diagnostic assessments described in the Primary objective for different definitions of the target condition, including (i) ISUP $\geq$ 3 in any of the biopsy cores taken from a lesion; (ii) ISUP $\geq$ 2 with cribriform growth and/or intraductal carcinoma (CR/IDC) in any of the biopsy cores taken from a lesion; (iii) ISUP = 1, (3) to compare the number of men in whom the PCaVision pathway provided insufficient quality images with the number of men with insufficient quality MRI images

Study design:

This study is a prospective, diagnostic accuracy study with a fully paired design.

Study population:

Biopsy-naïve men above the age of 18 years that are scheduled for evaluation by prostate MRI based on a suspicious DRE and/or elevated serum PSA will be included in the study. .

Intervention:

All patients undergo 3D mpUS using PCaVision and MRI. Suspicious lesions will be identified based on each imaging technique independently. Thereafter, an MRI targeted biopsy and/or a PCaVision targeted biopsy will be performed if suspicious lesions have been identified based on imaging. When lesions have been identified with both PCaVision and MRI in the same patient, the order of the targeted biopsies will be randomized.

Per imaging technique, the following approach is followed. When 1 suspicious lesion has been identified, 3 targeted biopsies will be taken. When 2 lesions have been identified, 3 targeted biopsies will be taken per lesion, so 6 biopsies in total. In case 3 or more suspicious

lesions have been found, 2 lesions will be selected based on: (1) PI- RADS and (2) size for MRI, and (1) severity indication and (2) size for PCaVision. Consecutively, 3 targeted biopsies will be taken from these 2 selected lesions.

So, the maximum number of targeted biopsies per imaging technique is 6, and the maximum number of study-driven biopsies per patient is 12. This is excluding potential systematic biopsies taken in line with the current care being delivered in the participating clinical centre. All biopsies will be performed in a single visit.

Main study parameter/endpoint:

The primary endpoint will be clinically significant cancer (GG ≥2 PCa) based on histological examination of study-driven biopsies. The primary aim of the study is to demonstrate non-inferiority in the detection of clinically significant cancer based on PCaVision imaging (PCaVision pathway) in comparison to MRI (MRI pathway) with a pre-defined non-inferiority margin of 5 percentage point.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

In most participants centres, local clinical care would consist of MRI-target and (potentially) systematic biopsies. Local clinical care can differ between hospitals with respect to systematic biopsies; varying from standard in all, only in men with one or more lesions on MRI, to no systematic biopsies at all. This part of usual care will not change in the current study. Systematic biopsies play no role in the formal head-to-head comparison between PCaVision and MRI. Evaluating whether PCaVision is non-inferior to MRI requires only the comparison of the findings from the targeted biopsies of both strategies. Consequently, there is no change in the quality of current care.

There could be a potential benefit for the participants in this study when PCaVision detects additional cancers missed by MRI. Furthermore, the study results may be important for future patients suspected for prostate cancer. If PCaVision is sufficiently accurate, it could coexist with MRI as the central imaging strategies which would lead to a more streamlined and more cost-effective diagnostic work-up, especially in centres with moderate MRI quality, or centres lacking MRI capacity.

In this study, additional targeted biopsies could be performed because of the identification of PCa on PCaVision imaging. These extra biopsies also carry a small risk of haemorrhage and infection. There is also a small, anticipated risk for participants associated with the administration of ultrasound contrast. However, adverse events caused by the ultrasound contrast agent appear to be transient, mild, and rare.

In conclusion, although the potential benefit for the patient is small, we also believe that the burden and risk associated with participation in this study is limited.

1. INTRODUCTION AND RATIONALE

The incidence of prostate cancer (PCa) has increased over the years. Prostate cancer is the second most common malignancy in men worldwide, with an incidence of 1,276,106 and mortality of 358,989 in 2018 [1]. In developed countries with a high percentage of elderly men in the population, it is deemed a major health concern. Changing demographics and an increase in elderly population will likely contribute to an increase in PCa diagnoses over the years and thus an increase in the disease's economic burden [2,3]. To be able to manage and treat PCa, it is important that the presence of clinically significant tumors, the corresponding tumor-grade, and the progression risk are accurately assessed and characterized. Accurately assessing these factors can avoid overtreatment in patients with a low progression risk and avoid delay or undertreatment for patients with clinically significant cancer [4,5].

MRI in which T2-weighted, diffusion weighted, and dynamic contrast enhancement are used to visualize tissue structure, density, and vascularity, is increasingly being used for the detection of clinically significant prostate cancer (csPCa). It is currently recommended as the first-line investigation by the European Association of Urology (EAU) guidelines on PCa based on the results from three prospective multicentre trials [6,7,8]. These studies have shown that MRI-targeted biopsy (TBx) decreased the number of biopsy procedures and reduced the detection of insignificant PCa (International Society of Urological Pathology [ISUP] Grade group [GG] = 1), while maintaining the detection of csPCa (GG ≥2), as compared to TRUS-guided systematic biopsy (SBx) [6,7,8]. However, MRI still misses 9% of csPCa (GG ≥2), detected by template biopsies [9]. Based on 12 studies with a total inclusion of 3091 patients, MRI has a sensitivity of 0.91 (95% Confidence Interval {CI}:
0.83-0.95) and a specificity of 0.37 (95% CI: 0.29-0.46) [9]. Per one-thousand men, with an expected prevalence of 30% of clinically relevant cancer, MRI scanning may result in 273 true positives, 259 true negatives, but may still lead to 441 false positives (unnecessary biopsies and potential for overtreatment) and 27 false negatives (missed relevant cancers by MRI) [9]. These results are from high quality, high volume centers, however, apart from these results, there was a large variation in the sensitivities and specificities for MRI between individual studies [10,11] which can be attributed to a difference in MRI equipment, study design and inter-observer variability [12,13,14]. Next to its limited availability, MRI is a time-consuming, expensive imaging modality which is not available at bedside and requires highly trained radiologists for interpretation. Moreover, the added value of the dynamic contrast-enhanced MRI (DCE-MRI) sequence in comparison with MRI without contrast enhancement in different risk scenarios in biopsy-naive men is being discussed.

However, greater evidence is needed to precisely define which patient groups benefit from contrast enhancement and who can safely avoid it [15].

Ultrasound (US), is widely available, cheaper to implement and familiar to the urologist, however, it is currently recommended for prostate biopsy guidance only [8]. New US

modalities including contrast-enhanced US (CEUS), micro-US and (shear wave) elastography have been introduced to improve US-based diagnosis of PCa [16,17,18].

In CEUS, an ultrasound contrast agent is injected intravenously to enhance imaging [19]. The dispersion of the ultrasound contrast agent in the vascular network is measured over time, enabling visualization of angiogenesis. PCa requires angiogenesis to be able to progress into clinically significant disease [20]. Moreover, the degree of angiogenesis has been shown to correlate to the aggressiveness of PCa [21-23].

In a previous study, CEUS quantification predicted 72.2% of biopsy locations as benign, resulting in a false negative rate of 2.6%. Thirty-one of eighty-two patients were classified as not having a malignant focus, resulting in a missed diagnosis in three patients. The sensitivity and specificity of CEUS were 91% and 56%, respectively [24].

As qualitative CEUS interpretation showed limited value [24,25,26], algorithms for quantitative interpretation have been developed to assist in the CEUS interpretation [27]. Particularly, contrast-US-dispersion imaging (CUDI), which focuses on the detection of angiogenetic changes in the microvascular architecture, provides several parameters that can be used for PCa localisation [25,27,28]. In a clinical validation of this technique, it was shown that two-dimensional (2D) CEUS in combination with CUDI yields a sensitivity and specificity of 83% and 55%, respectively, with an area under the curve (AUC) of 0.78 [29]. In another study, MRI-TBx, CUDI-TBx and SBx were compared to each other. Although it was shown in the interim analysis that both the MRI- and CUDI strategies were inferior to systematic biopsy, MRI and CUDI had comparable performance for the detection of, and localization of Grade Group ≥ 2 (a method of grading PCa) PCa. MRI detected 29% (41/142) and CUDI detected 28% (40/142) [30]. Moreover, Brock et al. carried out a study with a multiparametric approach, in which real-time elastography and contrast enhanced ultrasound were combined, that was able to reduce false positives from 34.9% to 10.3% [31]. Mannaerts et al., showed results regarding multiparametric ultrasound, in which the combination of greyscale, shear wave elastography and CEUS has a significantly increased sensitivity of 77% compared to 55%, 55% and 55% for greyscale, shear wave elastography and CEUS alone, respectively [32].

Even though these studies show promising results for 2D CEUS as an imaging technique, 2D CEUS carries several limitations. 2D CEUS only captures blood flow within the imaging plane while the tumour-associated blood vessel anomalies are a 3D phenomenon. It is expected that PCaVision, 3D mpUS with quantitative analysis using multiple parameters, allows for more accurate identification of significant prostate cancer [33].

To summarize, various diagnostic pathways are currently available which can be compared to a potential pathway based on PCaVision. The MRI pathway comprises the performance of an MRI followed by subsequent targeted biopsies if suspicious lesions are detected on these MRI images. An alternative would be the PCaVision pathway in which the performance is based on PCaVision imaging followed by target biopsies if suspicious

lesions are detected in these images. The systematic biopsy pathway is based on findings from systematic biopsies in all men. The overall aim of this study is to compare the diagnostic accuracy of the two different diagnostic pathways to detect csPCa in biopsy-naïve men: (1) PCaVision pathway; (2) MRI pathway. These pathways will be examined in this study using a fully paired design.

2. OBJECTIVES

The main interest of this study is in the comparison of an imaging pathway based on PCaVision with the pathway based on the currently preferred imaging technique of MRI.

Primary Objectives

- To demonstrate non-inferiority of the detection rate of clinically significant prostate cancer (defined as GG ≥ 2 in any of the biopsy cores taken from a lesion) in targeted biopsies based on PCaVision imaging (PCaVision pathway) in comparison with the detection rate of clinically significant cancer in targeted biopsies based on MRI (MRI pathway).

Secondary Objectives

- To compare the proportion of men in whom targeted biopsies could be safely omitted in the PCaVision pathway versus the MRI pathway. This is defined as the number of men in whom no lesions for target biopsies have been identified by PCaVision while no clinically significant cancer is detected in either MRI targeted biopsies or systematic biopsies. The combined findings of all types of biopsies will serve as the reference standard to define ‘safe’.
- To perform the same diagnostic accuracy analyses as described in the primary objectives for different definitions of the target condition. This includes: (i) ISUP $\geq$ 3 in any of the biopsy cores taken from a lesion; (ii) ISUP $\geq$ 2 with cribriform growth and/or intraductal carcinoma (CR/IDC) in any of the biopsy cores taken from a lesion; (iii) ISUP = 1.
- To compare the number of men in whom the PCaVision pathway generated insufficient quality images with the number of men with insufficient quality MRI images in the MRI pathway.
- To perform the same diagnostic accuracy analyses as described in the primary and secondary outcome with different levels of increased criteria for ultrasound image quality, automatically detected by PCaVision

3. STUDY DESIGN

This study is a prospective diagnostic accuracy study with a fully paired design in biopsy-naïve men suspected of prostate cancer. A flowchart illustrating the design is presented

in figure 1.

In summary, all patients will undergo imaging using MRI and PCaVision during which suspicious lesions will be identified based on each imaging technique independently with readers being blinded for the results of the other imaging technique. Thereafter, a MRI targeted 3-core biopsy per lesion (maximum of 2 lesions) and/ or a PCaVision targeted 3-core biopsy (maximum of 2 lesions) will be performed by a one physician if suspicious lesions have been identified based on imaging. If lesions have been identified with both PCaVision and MRI in the same patient, the order of the targeted biopsies will be randomized. If the same lesion has been identified on both MRI and PCaVision, both a MRI-targeted and a PCaVision targeted biopsy will be separately performed. Histological examination of the targeted biopsies will be performed to determine presence of clinically significant prostate cancer.

More details on each of these steps are provided in Chapter 5.

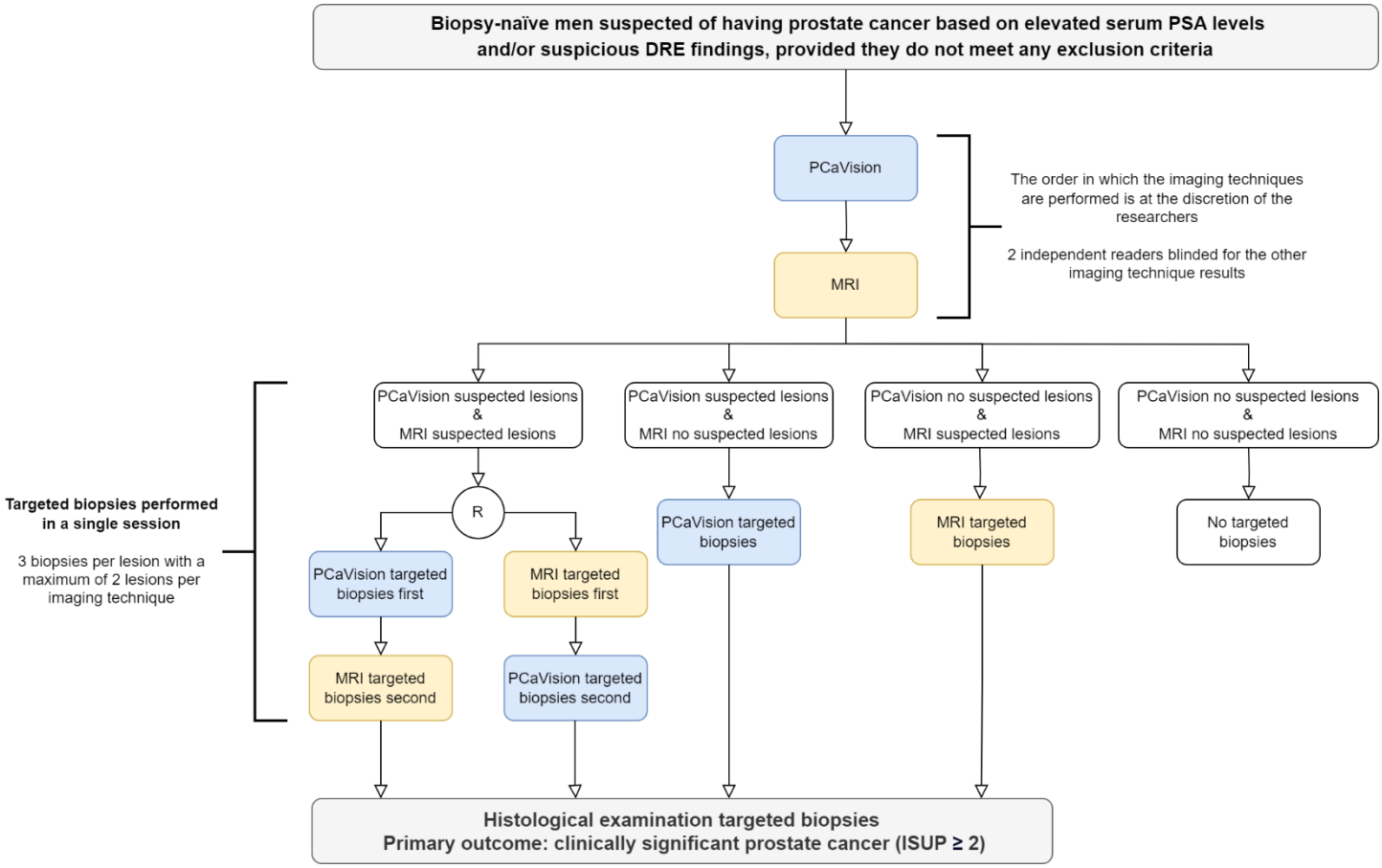


Figure 1: Study design of this prospective, paired, diagnostic study

4. STUDY POPULATION

4.1. Population (base)

Biopsy-naïve men above the age of 18 years that are scheduled for evaluation by prostate MRI based on a suspicious DRE and/or elevated serum PSA will be included in the study. Exclusion criteria are mostly related to MRI, the Ultrasound Contrast Agent (UCA) used for CEUS imaging and biopsy procedures.

4.2. Inclusion criteria

To be eligible to participate in this study, a subject must:

- be male
- have an age of 18 years or older
- be biopsy naïve
- have a clinical suspicion of prostate cancer
- be scheduled for evaluation by prostate MRI based on a suspicious DRE and/or elevated serum PSA
- have signed informed consent

4.3. Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- active (urinary tract) infection or prostatitis
- a patient history with a cardiac right-to-left shunt.
- allergic to sulphur hexafluoride or any of the other ingredients of the ultrasound contrast agent SonoVue
- current treatment with dobutamine
- known severe pulmonary hypertension (pulmonary artery pressure >90 mmHg), uncontrolled systemic hypertension or respiratory distress syndrome
- any (further) contraindication to undergo MRI or 3D mpUS imaging
- medical history of prostate surgery
- current treatment of 5 alpha-reductase inhibitors for at least 3 months
- incapable of understanding the language in which the patient information is given.

4.4. Relationship of investigation population to target population

The subset of the population that will participate in the study (the investigation population = all the included subjects) directly relates to the actual population for which the product is intended. In- and exclusion criteria are related to the clinical procedures used in prostate cancer diagnosis and treatment; MRI, the Ultrasound Contrast Agent (UCA) used for CEUS imaging and biopsy procedures. As a result, the investigation population is a good representation of the total population and the in- and exclusion criteria do not result in a bias making the data less generally applicable.

4.5. Sample size calculation

The sample size calculation is based on demonstrating non-inferiority between the detection rate of clinically significant cancer from the PCaVision pathway and the MRI pathway using patients as the unit of analysis and data from the fully paired design.

The detection rates of PCa (GG≥2) based on MRI and PCaVision targeted biopsies are expected to be around 30%. These values are based on the detection rates of PCa (GG≥2) reported by Mannaerts et al., 2020 [30] and Grey et al., 2022 [43], which were found to be 29% and 30% with MRI and 28% and 26% with 2D CUDI (older version of PCaVision), respectively.

The non-inferiority margin is set to 5% absolute difference in detection rate. The expected proportion of discordant pairs is 10%. This proportion expresses the degree in which PCaVision and MRI disagree with respect to detecting and missing significant cancers in a paired design. This parameter is relevant when analysing data from a paired study design. A smaller percentage of discordant pairs will lower the required sample size. In the studies of Mannaerts et al., 2020 [30] and Grey et al., 2022 [43], the percentage discordant pairs were 10.1% and 8.9%, respectively.

Our assumptions of the sample size calculations are therefore the following: an expected detection rate of 30% for both imaging techniques, a non-inferiority margin of 5% points and a percentage discordance between both imaging techniques of 10%, a one-sided type 1 error of 2,5% (as recommended by the European Medicines Agency) and a power of 80%. This leads to a required total sample size of 350 patients.. This is the total number of men who need to undergo both PCaVision and MRI, and produce images of sufficient quality, followed by targeted biopsies if indicated. This sample size calculation was performed according to Liu et al., 2002 [47] and Hintze, 2008 [48].

With both imaging pathways, we expect a certain percentage of men in whom either PCaVision or MRI or both produce image quality that is insufficient to perform targeted biopsies upon, or technical issues when performing targeted biopsies. The primary, paired analyses will be based on men having sufficient quality images and targeted biopsies from both imaging techniques. During the development of the PCaVision algorithm, insufficient scans were manually excluded to ensure optimal quality for training purposes. Currently, PCaVision incorporates an automated image quality check to guarantee sufficient signal and quality. To compensate for discarded scans due to quality issues, 25% additional patients will be included. The total sample size will therefore be 438 subjects to obtain 350 evaluable patients in the paired analysis.

As we would like to include PCa patients from 5 clinical centres, the patient number target per centre is 80, with a range of 40-120.

4.6. Expected duration of the study

The expected duration of the participation of each subject is 1-2 months. This time starts from the moment of consenting to the study (See section 11.2) and will cover 2 or 3 visits. The participation of each subject ends after completion of the biopsy procedure(s) (1-2 months) and the follow-up of possible adverse events resulting from the biopsies (2-6 weeks follow-up).

It is expected that the enrolment of all patients will take around 14 months. This is based on the information that each clinical site sees between 10-15 eligible patients per month and an expected participation rate of 50%.

The completion of a clinical investigation shall be deemed to coincide with the last visit of the last subject and when follow-up has been completed, whether the clinical investigation was concluded according to the pre-specified clinical investigation plan or was terminated prematurely.

Therefore, the total clinical study duration is expected to be 14-18 months.

5. TREATMENT OF SUBJECTS

5.1. Investigational treatment

In this study, all men will undergo both MRI and PCaVision imaging. Two different specialists will perform these imaging who are unaware of the results of the other imaging technique.

MRI images will be read by an experienced uro-radiologist and suspicious lesions will be marked. An experienced urologist will read the PCaVision images and mark suspicious lesions. This will ensure that suspicious lesions will be identified based on each imaging technique independently (i.e., blinded for the other imaging technique).

A MRI targeted 3-core biopsy per lesion (with a maximum of 2 lesions) and/or a PCaVision targeted 3-core biopsy per lesion (with a maximum of 2 lesions) will be performed by a urologist if suspicious lesions have been identified based on imaging. The maximum number of targeted biopsies per imaging technique is 6. Each biopsy sample will be collected in a separate container. As a result, there will be maximally 6 containers with samples from the PCaVision targeted biopsy, and 6 containers with samples from the MRI targeted biopsy.

If no suspicious lesions have been identified by an imaging technique, no targeted biopsies based on that imaging technique will be performed. It is up to the local physician whether routine systematic biopsies will be taken.. If suspicious lesions have been identified with both PCaVision and MRI in the same patient, the order of the targeted biopsies will be randomized. Randomization of the order will prevent any systematic advantage of performing the targeted biopsies of one imaging technique always before the other technique.

Moreover, if the clinician suspects that the same lesion has been identified by both PCaVision and MRI, both PCaVision and MRI targeted biopsies of this lesion will be performed. This event will be documented. The outcome of the evaluation of the collected samples obtained by both PCaVision and MRI targeted biopsies will be compared to ensure that there is no deviation in outcome resulting from the order of targeting the same lesion. It is up to the local physician whether routine systematic biopsies will be taken beside these target biopsies. All biopsies will be performed in a single visit.

Overall, patients will undergo minimally 1 and maximally 3 visits to the hospital. In the 1-visit approach, patients will undergo the two imaging techniques (MRI and PCaVision) and potential biopsy session at the same day. In the 2-visits approach, patients will have both MRI and PCaVision imaging in the first hospital visit, and both targeted biopsy procedures in the second visit. The amount of visits in these two approaches is comparable to the standard of care. In the 3-visits approach, MRI and PCaVision imaging will be separately performed in 2 visits followed by the 3rd visit in which all biopsy procedures will be done. In the latter situation, patients will have 1 visit extra compared to the standard of care.

Histological examination of the targeted biopsies will be the reference standard to determine whether clinically significant prostate cancer is present or not. The detection rate of clinically significant cancer per imaging strategy on a patient level will be the primary outcome.

So, the MRI and PCaVision imaging techniques plus subsequent targeted biopsies (as a package) are the main interventions in this study. As a reference standard for these biopsy strategies, a histological examination of all biopsies is performed.

Of these interventions, the PCaVision imaging technique with the use of US contrast agent plus the targeted biopsy procedure based on this imaging technique are considered extra in relation to the Standard of Care which consists of MRI, its targeted biopsy procedure, and (potentially) systematic biopsies. Depending on the local organization of the study this might lead to 1 extra hospital visit.

5.2. Factors that may compromise the outcome of clinical study

By randomisation, blinding and treatment allocation, compromise of the outcome of the clinical study or interpretation of its results are prevented. The following measures are taken.

MRI and PCaVision reading

The MRI images and PCaVision images are evaluated by two separate readers. The reader of the MRI images is blinded for the PCaVision images and the PCaVision reader is blinded for the MRI images.

PCaVision versus MRI targeted biopsy procedures

When suspicious lesions have been identified on both PCaVision and MRI, all targeted biopsy procedures are performed by one physician in a randomized order by applying block randomization (with block sizes varying between 6 and 10) using a computer-generated list, to prevent potential bias. Due to the nature of the image fusion which is recognizable for both pathways (MRI vs PCaVision), the physician performing the targeted biopsy procedures cannot be blinded for the image modality on which the suspected lesions were identified.

Interpretation of biopsy results

Each biopsy sample from the (PCaVision and MRI) targeted biopsy procedures, will be put in a separate container and analyzed by the pathologists. Pathologists are blinded to the modality from which the target biopsies are derived.

5.3. Follow up Activities

After completion of the biopsy procedure(s), it is expected that the only complications possibly experienced by the study participants encompass the adverse events that are related to the biopsy procedures. From clinical practice it is known that these events will

manifest within 2 weeks, and will be resolved within 6 weeks. If there are no complications within 2 weeks, the patient is considered to have completed the study. The study will be closed when all patients have completed the study, with a maximum follow-up of 6 weeks if complications occur.

5.4. Post clinical study medical care and follow up

Not applicable (see 5.3) as no specific medical care is required for the study participants.

6. INVESTIGATIONAL PRODUCT

Below is a summary of the information on the product which consists of a computer module, PCaVision and a probe fixture that will be used in this study.

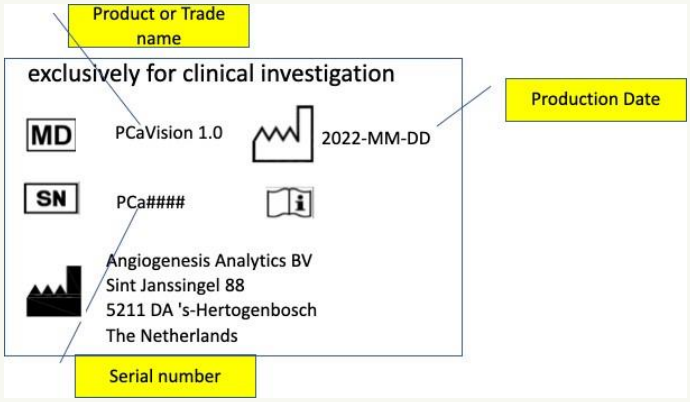
- The computing module is an off-the-shelf embedded computer which is enclosed in a medical-certified housing and is attached to the ultrasound machine. A wired network cable connects the computer module with the ultrasound machine enabling PCaVision to communicate and acquire data.
- PCaVision is the Image Analysis and Viewing Software for analyzing 3D contrast enhanced and other modality ultrasound data of the prostate.
- The probe fixture is required to provide fixation of the transrectal ultrasound imaging probe during prostate diagnostic imaging.

Only PCaVision is an investigational product.

6.1. Name and description of the investigational product

Summary device description	PCaVision is the Image Analysis and Viewing Software for analyzing 3D contrast-enhanced and other non-contrast ultrasound modalities of the prostate. PCaVision consists of Image Analysis Software and Viewing Software developed for use as a post-processing application to aid the physician in analyzing three-dimensional contrast-enhanced ultrasound and other modality ultrasound data of the prostate.
Summary intended purpose	Support the physician in analyzing three-dimensional contrast-enhanced ultrasound and other modality ultrasound data of the prostate to detect suspicious lesions of prostate cancer.

Population description	Patients with clinical and laboratory signs suspect of prostate cancer.
Manufacturer	Angiogenesis-Analytics B.V. Sint Janssingel 88 5211BA, ‘s-Hertogenbosch The Netherlands
Device model/type	PCaVision 1.0 The unique product code (serial number will be different for each hospital).
Software version	PCaVision 1.0
Accessories	Probe fixture: To hold US probe in place during imaging to free Operator. PCaVisio Probe Fixture P684.NNN (serial number will be different for each hospital) Off the shelve Computer: Mac Mini attached to the ultrasound machine via a wired cable, enabling PCaVision to communicate and acquire data from the ultrasound machine
Traceability procedure	The computer running the PCaVision application will be labelled with unique serial number (see device labelling), the device will be placed by Angiogenesis Analytics staff at the start of the study. The computer and the SW will be removed from the site at the end of the study by Angiogenesis Analytics staff.
Required training	PCaVision should be used by urologists medically trained in ultrasound imaging and trained in working according to the PCaVision procedures. For the study the training consists of at least 3-5 patients imaged under supervision of Angiogenesis Analysis staff (see section 8.3.2 for details).
Specific procedure acts	Performance of an ultrasound prostate scan of the patient as input for the PCaVision analysis. Execution of a systematic or targeted prostate biopsy procedure as intervention after PCa diagnosis Full or focal prostate therapy dependent on whether clinically significant PCa tissue has been revealed.

Parts of investigational device coming in contact with tissues or body fluids	Not Applicable, the PCaVision investigational device is a SW only medical device, not making any contact with patient or body fluids.
Device Labeling	
Number of investigational devices required.	The study will be performed in 5 centres (see section 4.4), each site will require 1 investigational device to be present.

Additional information on the investigational device can be found in the Investigator Brochure and the Instructions for Use (IFU) of the device.

6.1.1 PCaVision

PCaVision consists of Image Analysis Software and Viewing Software developed for use as a post-processing application to aid the physician in analyzing three-dimensional contrast-enhanced ultrasound and other modality ultrasound data of the prostate. In particular, the PCaVision system is intended to:

- Indicate the contour of the prostate and calculate the volume of the prostate based on this contour
- Provide images and analysis of the prostate to aid the physician in identifying the absence or presence of at least one suspected clinically significant prostate cancer lesion
- Provide images and analysis of the prostate to aid the physician in localizing the position of one or more suspected clinically relevant prostate cancer lesions
- Export datasets, including the contour, images and analysis results described above, to facilitate targeted and full-prostate clinical interventions

The input ultrasound data for PCaVision needs to be acquired under specific contrast-enhanced settings with a 3D US rectal probe. The recorded data-set shall then be exported to the PCaVision system.

6.1.2 PCaVision quality control

PCaVision is using automated detection of ultrasound image quality by measuring parameters of CEUS and patient motion. Patients will not be processed by PCaVision if they do not fulfil these criteria. Loose criteria are default and used for the primary outcome. A combination of stricter criteria is used for additional (secondary outcome) diagnostic accuracy analysis.

Quality parameter	Specification	Loose	Strict
CEUS signal	Minimum peak intensity value (dB)	43	50
Patient motion	Minimum IoU (intersection over union) of B-mode prostate contours	0.60	0.70
	Maximum root mean square magnitude of detected motion vectors (mm)	8.1	3.0

6.2. Summary of findings from (non) clinical studies

6.2.1 PCaVision

PCaVision is based on contrast ultrasound dispersion imaging (CUDI), a computer-aided quantification algorithm developed at the Eindhoven University of Technology. It can aid in the accurate interpretation of CEUS by constructing per-pixel time-intensity curves during the Ultrasound Contrast Agent inflow phase. Using these time-intensity curves, various flow and dispersion related parameters can be deduced from the CEUS recordings and presented to the reader in the form of color-coded maps. Combinations of these parameters have also been used as input for a “classifier”, an algorithm that marks prostate zones as benign or suspect. CUDI parametric maps and especially the classifier can therefore increase accuracy, decrease user dependence and speed up the reading process [36].
In a clinical validation of this technique, it was shown that two-dimensional (2D) CEUS in combination with CUDI yields a sensitivity and specificity of 83% and 55%, respectively, with an area under the curve (AUC) of 0.78 [29]. In another study, MRI targeted biopsies, 2D-CEUS targeted biopsies and systematic biopsies were compared to each other. An interim analysis after inclusion of 150 patients in this study showed that MRI and 2D-CEUS had comparable performance for the detection of and localization of ≥Gleason Group 2 (a method of grading PCa) PCa. MRI detected 29% (41/142) and 2D-CEUS detected 28% (40/142) [30]. Moreover, Brock et al. carried out a study with a multiparametric approach, in which real-time elastography and contrast enhanced ultrasound are combined, that was able to reduce false positives from 34.9% to 10.3% [31]. The results of above-mentioned studies demonstrate that with improved computer-aided diagnosis algorithms and combination with

other modalities such as elastography and greyscale, CEUS could become a useful imaging tool in the diagnosis of PCa.
Even though these studies show promising results for 2D CEUS as an imaging technique, 2D CEUS carries several limitations. 2D CEUS only captures blood flow within the imaging plane while the tumor-associated blood vessel anomalies are a 3D phenomenon. Recently, ultrasound scanners have become available that are capable of 3D CEUS, allowing capturing the blood flow anomalies in 3D [37]. It is expected that 3D CEUS with quantitative analysis using multiple flow parameters (multiparametric) by CUDI will allow more accurate identification of significant prostate cancers. Furthermore, 3D CEUS allows scanning the whole prostate in 2 minutes after one single bolus of contrast, whereas 2D CEUS requires a contrast bolus and 5 minutes per plane of visualization.
The results of our previous studies in combination with the expected improvement from the transition from 2D to 3D can lead to an accurate 3D multiparametric ultrasound classification algorithm for the detection, grading and localization of PCa. Therefore, a multiparametric ultrasound classifier in 3D, named PCaVision has been developed by collection of high-quality 3D multiparametric images, loops and histology data to train, validate and asses the initial performance of this algorithm [33].

6.3. Summary of known and potential risks and benefits

See info in paragraph 11.3: Benefits and risks assessment, group relatedness

7. NON-INVESTIGATIONAL PRODUCT

In the study, the following non-investigational products will be used:

- Probe fixture
- Ultrasound contrast agent Sonovue® (sulphur hexafluoride, Bracco Imaging S.p.A., Colleretto Giacosa, Italy)
- 3D ultrasound machine LOGIQTM E10 (GE Heathcare, Milwaukee, WI, USA)
- Multiparametric magnetic resonance imaging (MRI)
- Fusion biopsy system

Below is a summary of the information of these products.

7.1. Name and description of non-investigational products

7.1.1 Probe fixture

The probe fixture is a device that is intended to provide fixation of a transrectal ultrasound imaging probe during prostate diagnostic imaging. The device is not intended to be used other than for ultrasound imaging, such as minimally invasive puncture procedures. The probe fixture will assist the ultrasound operator during image acquisition during this trial. The fixture can be attached to a DIN rail on the operating table. The ultrasound probe can be placed in the fixture and inserted. Once the probe is in the desired position the fixture can be

fixed in place. Fixing the probe releases the operator from holding the probe during the scanning procedure, while providing stable image acquisition.
The probe fixture should be used by a urologist medically trained in ultrasound imaging and PCaVision. The probe fixture features a central single-point locking mechanism to rapidly lock the fixture position without transducer migration (Figure 2).

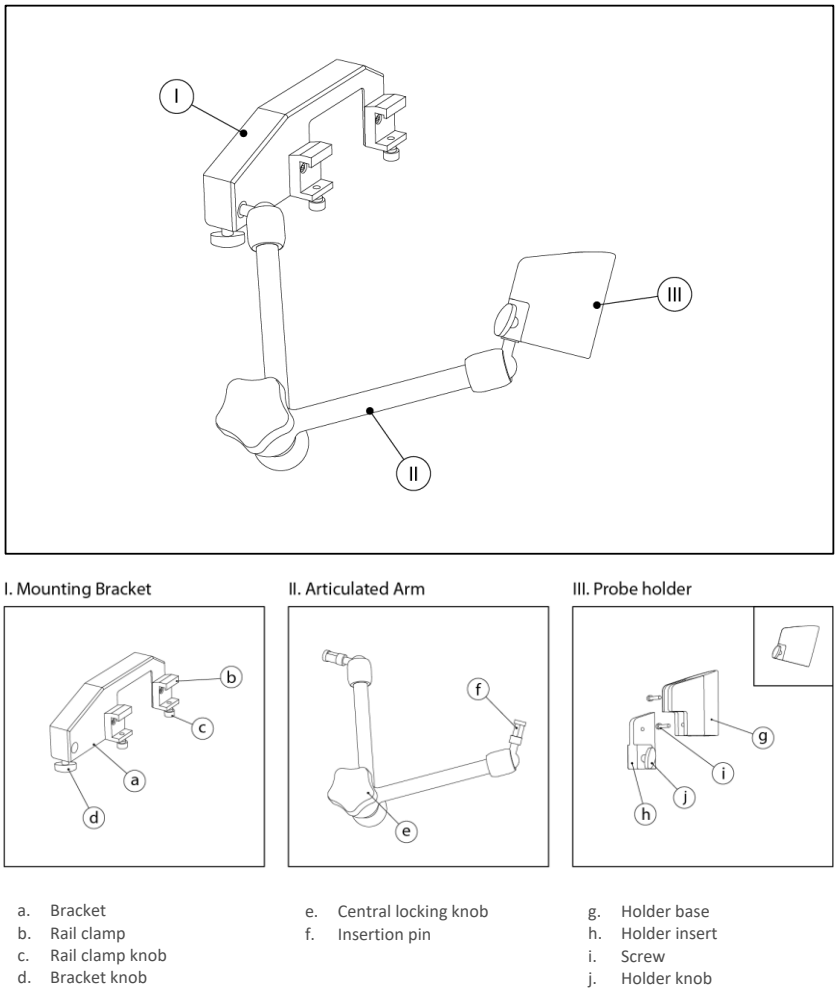


Figure 2: Probe fixture components

7.1.2 Ultrasound contrast agent

Ultrasound contrast agent Sonovue® (sulphur hexafluoride, Bracco Imaging S.p.A., Colleretto Giacosa, Italy) is registered for use in:

- Echocardiography
- Doppler of macrovasculature

- Doppler of microvasculature

Ultrasonography of excretory urinary tract
The use of the SonoVue in the current study is outside of its registered use (off-label).

7.1.3 3D ultrasound machine

LOGIQ™ E10 (GE Heathcare, Milwaukee, WI, USA) complies with regulatory requirements of the following European Directive 93/42/EEC concerning medical devices. First CE Marked in 2018. The LOGIQ E10 is intended for use by a qualified physician for ultrasound evaluation.
Specific clinical applications and exam types include:

- Fetal/Obstetrics
- Abdominal (includes Renal, Gynecology/Pelvic)
- Pediatric
- Small Organ (Breast, Testes, Thyroid)
- Neonatal Cephalic
- Adult Cephalic
- Cardiac (Adult and Pediatric)
- Peripheral Vascular
- Musculo-skeletal Conventional and Superficial
- Urology (including Prostate)
- Transrectal
- Transvaginal
- Transesophageal

Intraoperative (Vascular) Image Acquisition is for diagnostic purposes, including measurements on acquired images.

7.1.4 Magnetic resonance imaging (MRI)

The following requirements have been defined for the performance of MRI in this study:

- 3T MRI
- PI-RADS training for the operator
- PI-RADS technical requirements
- use of MRI contrast agent is pending local standard of care
- use of rectal coil probe is optional

NB: in general: we don’t want to compromise the local standard of care

7.1.5 Fusion biopsy system

A MRI-TRUS fusion biopsy system provides 2D and 3D visualization of Ultrasound (US) images and the ability to fuse and register these images with those from other imaging modalities such as Magnetic Resonance. It is intended for treatment planning and guidance

for clinical, interventional and/or diagnostic procedures, and to be used in interventional and diagnostic procedures in a clinical setting. Example procedures include, but are not limited to image fusion for diagnostic clinical examinations and procedures, soft tissue biopsies, soft tissue ablations and placement of fiducial markers.
In this study, each participating clinical centre will use the same fusion system to prevent problems related to US fusion. Consequently, each centre needs to be adequately trained on this system.

7.2. Summary of findings from non-clinical studies

7.2.1 Probe fixture

The probe fixture is a CE-marked class I medical device. An extensive risk assessment was performed, and all potential risks were deemed acceptable. Ex vivo testing and analysis concluded that the probe fixture provides all desired functionalities.

7.2.2 Ultrasound contrast agent

Sonovue® (sulphur hexafluoride, Bracco Imaging S.p.A., Colleretto Giacosa, Italy)

Information extracted from the European public assessment report (EPAR) for SonoVue (available from http://www.ema.europa.eu/ema/). The EPAR was last updated on 14/09/2021.

SonoVue is a medicine that contains the active substance sulphur hexafluoride (a gas). It is available as a kit including one vial of gas and powder and one prefilled syringe containing 5 ml of solvent. When made up into a solution, SonoVue contains sulphur hexafluoride gas as ‘microbubbles’ in suspension in a liquid. SonoVue is for diagnostic use only. It is a contrast agent (it helps make internal body structures visible during imaging tests). SonoVue is used in tests that measure how ultrasound travels within the body because it improves the ability of the blood to create an echo. The medicine can only be obtained with a prescription. SonoVue should only be used by doctors who have experience in diagnostic ultrasound imaging. It is injected intravenously (into a vein) before the test is carried out, as a 2- or 2.4-ml dose depending on which test is being carried out. The dose can be repeated. The active substance in SonoVue, sulphur hexafluoride, is a gas that is not soluble in the blood. When SonoVue is made up into a suspension, the gas is trapped in tiny bubbles called microbubbles. After injection, the microbubbles travel in the blood, where they reflect ultrasound waves more than the surrounding tissues. This helps to enhance the results of

tests that rely on measuring ultrasound, such as echocardiography and Doppler tests. The gas is removed naturally from the body through the lungs.

The most common side effects with SonoVue (seen in between 1 in 100 and 1 in 1,000 patients) are headache, paraesthesia (unusual sensations like pins and needles), dizziness, dysgeusia (taste disturbances), flushing (reddening of the skin), pharyngitis (sore throat), nausea (feeling sick), abdominal pain, pruritus (itching), rash, back pain, chest discomfort, reactions at the injection site, feeling hot and raised blood sugar levels. For the full list of all side effects reported with SonoVue, see the package leaflet. SonoVue must not be used in patients known to have right-to-left shunts (abnormal movement of blood within the heart), severe pulmonary hypertension (high blood pressure in the pulmonary artery, the blood vessel that leads from the heart to the lungs), uncontrolled hypertension (high blood pressure) or adult respiratory distress syndrome (severe fluid build-up in both lungs). SonoVue must also not be used together with the medicine dobutamine (used for heart failure) in patients for whom dobutamine is not suitable. For the full list of restrictions, see the package leaflet. The CHMP decided that SonoVue's benefits are greater than its risks and recommended that it be given marketing authorisation. The European Commission granted a marketing authorisation valid throughout the European Union for SonoVue on 26 March 2001.

7.2.3 3D ultrasound machine

LOGIQ™ E10 (GE Heathcare, Milwaukee, WI, USA)
Information extracted from the Technical Publications
Direction 5750001-1EN Rev. 3.
(available from https://customer-doc.cloud.gehealthcare.com/#/cdp/dashboard)
The document was last updated on 15/06/2019.

Medical ultrasound images are created by computer and digital memory from the transmission and reception of mechanical high-frequency waves applied through a transducer. The mechanical ultrasound waves spread through the body, producing an echo where density changes occur. For example, in the case of human tissue, an echo is created where a signal passes from an adipose tissue (fat) region to a muscular tissue region. The echoes return to the transducer where they are converted back into electrical signals. These echo signals are highly amplified and processed by several analog and digital circuits having filters with many frequency and time response options, transforming the high-frequency electrical signals into a series of digital image signals which are stored in memory. Once in memory, the image can be displayed in real-time on the image monitor. All signal transmission, reception and processing characteristics are controlled by the main computer. By selection from the system control panel, the user can alter the characteristics and features of the system, allowing a wide

range of uses, from obstetrics to peripheral vascular examinations. Transducers are accurate, solid-state devices, providing multiple image formats. The digital design and use of solid-state components provides highly stable and consistent imaging performance with minimal required maintenance. Sophisticated design with computer control offers a system with extensive features and functions which is user-friendly and easy to use.

7.2.4 Multiparametric magnetic resonance imaging (MRI)

(tbd)

7.2.5 Fusion biopsy system

(tbd)

7.3. Dosages, dosage modifications and method of administration

7.3.1 Ultrasound contrast agent

In this study, the recommended dose of 2.4 mL will be used.
Intravenous use
The recommended doses of SonoVue in adults are:

- B-mode imaging of cardiac chambers, at rest or with stress: 2 mL.
- Vascular Doppler imaging: 2.4 mL.

SonoVue should be administered after drawing into the syringe by injection into a peripheral vein. Every injection should be followed by a flush with 5 mL of sodium chloride 9 mg/mL (0.9%) solution for injection.

7.4. Preparation and labelling of Non-Investigational Medicinal Product - ultrasound contrast agent

7.4.1 Preparation

Pack size: 1 vial + 1 pre-filled syringe + 1 'MiniSpike' rec. device

If SonoVue is not used immediately after reconstitution the dispersion will be shaken again before being drawn up into a syringe. The product is for a single examination only. Any unused liquid remaining at the end of an examination must be discarded.

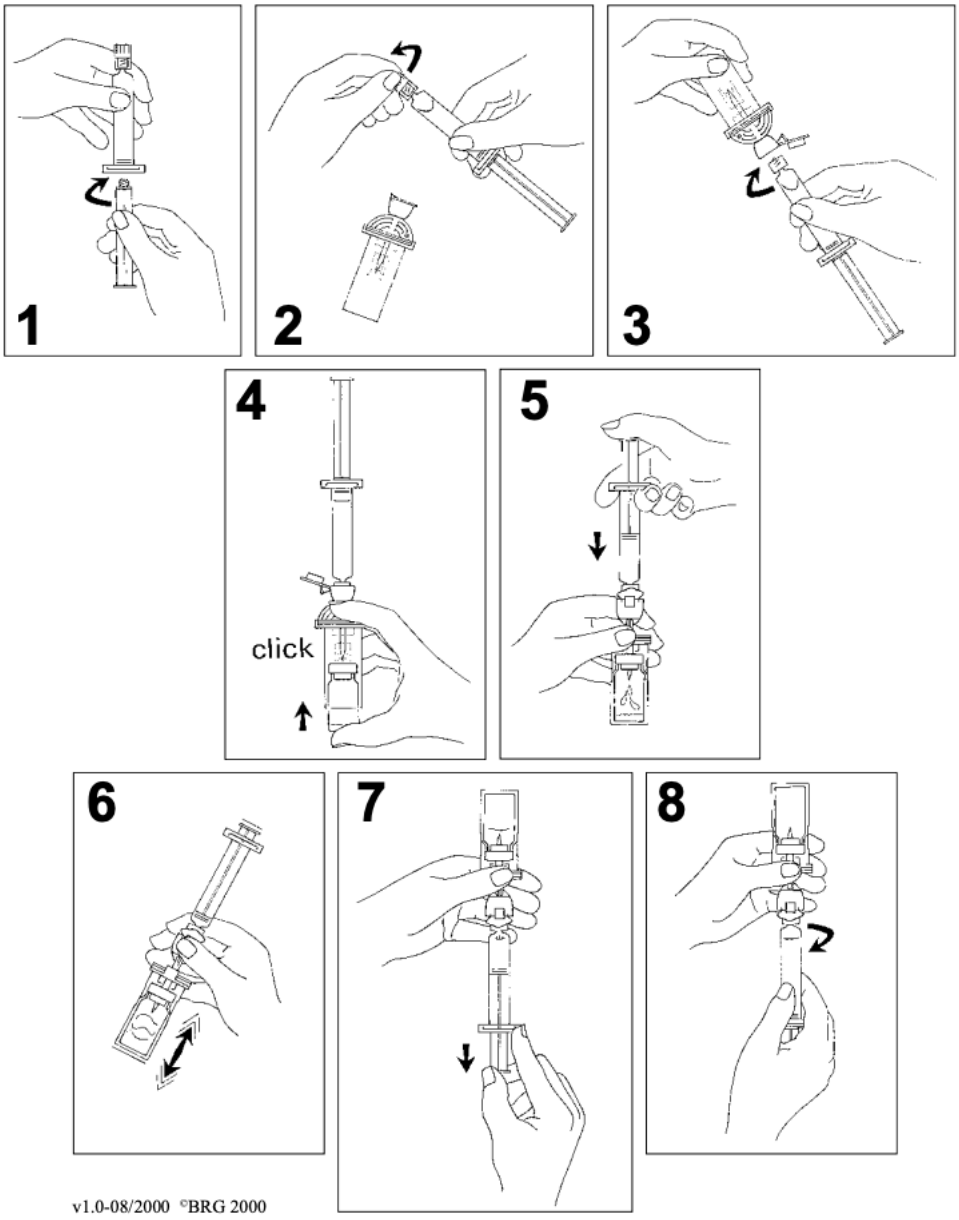


Figure 3: Reconstitution instructions

7.4.2 Reconstitution instructions

The reconstitution instructions below are supported by images in figure 3.

1. Connect the plunger rod by screwing it clockwise into the syringe.
2. Open the MiniSpike transfer system blister and remove syringe tip cap.
3. Open the transfer system cap and connect the syringe to the transfer system by screwing it in clockwise.

4. Remove the protective disk from the vial. Slide the vial into the transparent sleeve of the transfer system and press firmly to lock the vial in place.
5. Empty the contents of the syringe into the vial by pushing on the plunger rod.
6. Shake vigorously for 20 seconds to mix all the contents in the vial to obtain a white milky homogeneous liquid.
7. Invert the system and carefully withdraw SonoVue into the syringe.
8. Unscrew the syringe from the system.
After reconstitution, SonoVue is a homogeneous white milky dispersion. Do not use if the liquid obtained is clear and/or if solid parts of the lyophilisate are seen in the suspension. SonoVue dispersion should be administered within six hours of its preparation. Do not throw away any medicines via wastewater. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

7.4.3 Labelling

1. Name of the medicinal product: SonoVue 8 microlitres/mL powder and solvent for dispersion for injection sulphur hexafluoride
2. statement of active substance(s): Each mL of the dispersion contains 8 µLsulphur hexafluoride microbubbles, equivalent to 45 micrograms.
3. List of excipients: Macrogol 4000, distearoylphosphatidylcholine, dipalmitoylphosphatidylglycerol sodium, palmitic acid, Solvent: sodium chloride 9 mg/mL
4. Pharmaceutical form and contents: 1 powder vial, 1 pre-filled syringe of solvent, 1 transfer system
5. Method and route(s) of administration: Intravenous or intravesical use. Read the package leaflet before use. For single use only
6. Special warning that the medicinal product must be stored out of the reach and sight of children: Keep out of the sight and reach of children
7. Name and address of the marketing authorization holder: Bracco International B.V., Strawinskylaan 3051, NL - 1077 ZX Amsterdam, The Netherlands

8. METHODS

8.1. Study parameters/endpoints

This paragraph describes the study endpoints of the clinical study.

8.1.1 Main study parameter/endpoint

The primary endpoint of this study is clinically significant (GG ≥2) PCa. The detection rate of this endpoint in any core for each biopsy strategy will be assessed to demonstrate the non-inferiority of PCaVision targeted biopsy strategy in comparison with MRI targeted biopsy strategy. The detection rate is expressed with patients as the unit of analysis.

8.1.2 Secondary study parameters/endpoints

Secondary endpoints:

- proportion of men in whom targeted biopsies could have been safely omitted in the PCaVision pathway compared to the MRI pathway. This is defined as the number of men in whom no lesions for target biopsies have been identified by PCaVision while no clinically significant cancer is detected in either MRI targeted biopsies or systematic biopsies. The combined findings of all types of biopsies will serve as the reference standard to define ‘safe’.
- same diagnostic accuracy analyses as described in the primary endpoint for different definitions of the target condition. This includes: (i) ISUP ≥ 3 in any of the biopsy cores taken from a lesion; (ii) ISUP ≥ 2 with cribriform growth and/or intraductal carcinoma (CR/IDC) in any of the biopsy cores taken from a lesion; (iii) ISUP = 1.
- comparison of the number of men in whom the PCaVision pathway provided insufficient quality images or targeted biopsies with the number of men with insufficient quality MRI images or target biopsies in the MRI pathway.
- same diagnostic accuracy analysis as described in the primary and secondary outcome but using increased ultrasound image quality requirements (predefined and automated in PCaVision)

8.2. Randomisation, blinding and treatment allocation

All patients will undergo both PCaVision and MRI imaging before the scheduled biopsy appointment (fully paired diagnostic accuracy design). The MRI – based biopsy targets and PCaVision - based biopsy targets are appointed independently from, and blinded for each other by 2 specialized readers.

After the imaging has been recorded, 0 to 2 different biopsies procedures are performed:

(1) a maximum of 6 targeted biopsies in maximum 2 different lesions are taken by one physician, from the MRI lesions delineated by the radiologist. The following biopsy approach is used. When 1 suspicious lesion has been identified, 3 targeted biopsies

will be taken. When 2 lesions have been identified, 3 targeted biopsies will be taken per lesion, so 6 biopsies in total. In case 3 or more suspicious lesions have been found, 2 lesions will be selected based on: (1) PI-RADS and (2) size. Consecutively, 3 targeted biopsies will be taken from these 2 selected lesions. So, the maximum number of targeted biopsies per imaging technique is 6. The physician is blinded for the PCaVision results. When there are no suspicious lesions identified on MRI, this biopsy will not be performed.

(2) a maximum of 6 targeted biopsies in maximum 2 different lesions are also taken by this same physician from the lesions identified on PCaVision by the CEUS expert. The same biopsy approach as for MRI is used. When 1 suspicious lesion has been identified, 3 targeted biopsies will be taken. When 2 lesions have been identified, 3 targeted biopsies will be taken per lesion, so 6 biopsies in total. In case 3 or more suspicious lesions have been found, 2 lesions will be selected based on: (1) severity indication and (2) size. Consecutively, 3 targeted biopsies will be taken from these 2 selected lesions. So, the maximum number of targeted biopsies per imaging technique is 6. The physician is blinded for the MRI results and the systematic biopsy approach and outcomes. When there are no suspicious lesions identified on PCaVision, this biopsy will not be performed.

When suspicious lesions have been identified on both PCaVision and MRI, the targeted biopsy procedures are performed in a randomized order by applying block randomization (with block sizes varying between 6 and 10) using a computer-generated list, to prevent potential bias by post-biopsy haemorrhage.

Due to the nature of the image fusion which is recognisable for both pathways (MRI vs PCaVision), the physician performing the targeted biopsies cannot be blinded for the image modality on which the suspected lesions were identified.
The pathologists interpreting the biopsies (targeted and systematic if available) will be blinded to which modality the target biopsies are based on (PCaVision and MRI results).

8.3. Study procedures

8.3.1 Prostate magnetic resonance imaging (MRI)

MRI will be performed in supine position on a 3 Tesla MRI. Pre-biopsy prostate MRI image acquisition will be performed according to the most recent Prostate Imaging Reporting And Data System (PI-RADS) guidelines [33]. MRI sequences will include at least T1-weighted, T2-weighted, Diffusion-weighted imaging (DWI) and calculation of apparent diffusion coefficient (ADC) maps.
MRI will be evaluated by a adequately trained uro-radiologist with prostate MRI experience (blinded for 3D mpUS results) on prostate volume and area's suspicious for PCa. Scoring of suspicion will be performed using the European Society of Urogenital Radiology (ESUR) PI-

RADS standardized scoring system [33]. All lesions will be marked and delineated for MRI-TRUS fusion.3D multiparametric ultrasound (mpUS).

8.3.2 3D multiparametric ultrasound (mpUS)

The procedure of 3D multiparametric ultrasound consists of 3 components: (1) intravenous administration of ultrasound contrast; (2) rectal multiparametric ultrasound imaging; (3) PCaVision: artificial intelligence algorithm analyzing the images.

Ad 1. Intravenous administration of ultrasound contrast

All patients will undergo 3D multiparametric ultrasound imaging before or during the targeted biopsy that is scheduled. To be able to carry out 3D multiparametric ultrasound, intravenous administration of an ultrasound contrast agent must be carried out. SonoVue® (sulphur hexafluoride, Bracco Imaging S.p.A., Colleretto Graciosa, Italy) will be used as the ultrasound contrast agent in this study. This is an additional procedure for patients participating in the study. In usual care, transrectal ultrasound examination is without contrast. SonoVue® is a registered product used in various ultrasound examinations of the heart, vasculature and of the excretory urinary tract. Details of this product and its usage can be found in Chapter 7.
In the procedure, an endorectal TRUS probe will be placed in the rectal cavity of the patient. Consecutively patients will undergo: 3D Doppler, 3D-SWE, 3D-B-mode and 4D-CEUS. For 4D-CEUS one 2.4mL bolus of SonoVue® will be administered intravenously. A two-minute recording in the contrast enhanced mode will be made following the administration of SonoVue®. The entire 3D mpUS imaging protocol will take approximately 10 minutes.

Ad 2. Transrectal ultrasound imaging

The clinically approved ultrasound system LOGIQ™ E10 (GE Heathcare, Milwaukee, WI, USA) consisting of all necessary requirements will be used for the purpose of this study. Three-dimensional (3D) prostate CEUS recordings will be captured including 3D CUDI with the 2.4 mL bolus injection of SonoVue® (Bracco, Geneva, Switzerland).

Ad 3. PCaVision

The 3D multiparametric ultrasound images will be stored and transported to an image processing system for 3D multiparametric ultrasound analysis. The CEUS recordings will be subjected to PCaVision quantification analyses based on the pharmacokinetic modelling of the contrast bolus transport through the microvasculature of the prostate as a convective-dispersion process. A PCaVision parametric map will be generated. So, different analyses will be done for each modality (B-mode, CEUS, SWE) with the 3D multiparametric ultrasound classifier parametric image map as end result.

A training consisting of at least 3-5 patients will be performed under supervision of an expert teacher for each investigator in each participating center to optimize the protocol, local

procedures, and preparation prior to the start of the study. The obtained results from these patients will be incorporated in the final statistical analysis and are normal part of the study. The training will be divided into 2 parts: 1) performance of the PCaVision examination 2) interpretation of the PCaVision examination. During this training period, the expert teacher will be on-site in each center to monitor study procedures, identify problems, and provide direct feedback where possible and needed. The expert teacher will ensure sufficient quality of the obtained results by the learners, and finally certify the learners. Training records will be maintained to document the training of the staff.

A total of 50 patients will be asked to undergo an additional ultrasound scan in the same session, as detailed in Appendix I.

8.3.3 Biopsy procedure and histological examination

An urologist will perform software-assisted fusion TBx or cognitive TBx based on the standard of care of each participating centre. For each imaging modality (e.g. MRI and 3D mpUS), a maximum of 2 lesion will be targeted. Each lesion will be sampled using 3 biopsy cores. In case both imaging techniques have identified lesions to be biopsied, the targeted biopsy procedures are performed in a randomized order to prevent potential bias.
The applied prostate biopsy method; transperineal will be selected based on the standard of care of each participating clinical centre.
Individualbiopsy cores from targeted biopsies will be separately analysed by an uropathologist for the presence of PCa, ISUP, Gleason score (GS), percentage tumour core involvement and morphological patterns including cribriform growth pattern and intraductal carcinoma (CR/IDC) in accordance with the 2019 ISUP guidelines [34]. A work order instruction for the exact sample labelling of the biopsies and histopathological workup will be provided and agreed on with each clinical site.

8.4. Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Furthermore, when subjects experience poor diagnostic imaging or other technical issues related to US, MRI or biopsy, they will be excluded for analysis.

8.5. Replacement of individual subjects after withdrawal

Subjects will be replaced after the withdrawal.

8.6. Follow-up of subjects withdrawn from treatment

As this trial does not interfere with the standard care, subjects withdrawn from treatment will receive the clinical standard follow up after a prostate cancer biopsy procedure.

8.7. Premature termination of the study

The MRI and TRUS guided prostate biopsies are already performed routinely in clinical care. The extra targeted prostate biopsies are considered to convey minimal additional risk over the systematic biopsy cores that were already planned. Therefore, premature termination of the study based on risk convened to participants is very low. As is mandatory, every serious adverse event will be discussed with the accredited METC. In the case of several adverse events (Grade 3; CTCAE v3.0) caused by mpUS imaging of the prostate with the UCA Sonovue®, the principal investigator can decide in consultation with the accredited METC to terminate the study. The METC and/or CCMO will be informed. After premature termination of the study, subjects will still receive the standard clinical treatment or follow-up for prostate cancer.

9. SAFETY REPORTING

9.1. Ultrasound and ultrasound contrast agent

Ultrasound is considered a safe diagnostic tool because of the use of non-ionizing radiation. In general, few side-effects are attributed to ultrasound microbubble contrast agents. Complications like nephrotoxicity, which can occur with iodine containing contrast media in use for CT, have never been reported. Adverse events of microbubble contrast media appear to be transient, mild and rare [38,39,40]. The most frequently mentioned minor side-effects of microbubble contrast agents are transient alteration of taste, local pain at the injection site and facial or general flush (1-5%) [41]. Serious adverse events, which consists of hypersensitivity allergic reactions, are rare (<0.01%) [42]. Because the gaseous content of the microbubble agents is eliminated by the lungs, it is of importance to evaluate whether impaired pulmonary function could be a contraindication for the use of microbubbles. In a study with SonoVue® (Bracco, Geneva) in patients with Chronic Obstructive Pulmonary Disease, CEUS appeared to be as safe and well tolerated as in a healthy control group [38]. The Committee for Medicinal Products for Human Use (CHMP) has granted SonoVue® a marketing authorization, but it should not be used together with the medicine dobutamine and in pregnant or breastfeeding women (www.emea.eu).

9.2. Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.3. AEs, SAEs and SUSARs

9.3.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.3.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Excessive hematuria, acute urinary retention, prostatitis, hospitalization due to prostatitis and/or urosepsis will be recorded but not considered as SAE, as these adverse events are attributed to the regular prostate biopsy procedure and are not expected to be aggravated or occur more frequently due to participation in this study.

Emergency contact details for reporting serious adverse events and serious adverse device effects:

Mark Bloemendaal

+31 652304577

Mark.bloemendaal@angiogenesis-analytics.nl

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.3.3 Device deficiency

Device deficiencies are defined as inadequacy of a medical device with respect to its identity, quality, durability, reliability, usability, safety or performance. These events are reported to the study manager (principal investigator) who shall report these to the accredited METC and manufacturer and assess reportability to Competent Authorities.

9.4. Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5. Data Safety Monitoring Board (DSMB)

Since participants will receive standard of care, with additional biopsies based on PCaVision for this study which can be considered of minimal risk and without infringement in clinical decision making, no DSMB will be established.

10. STATISTICAL ANALYSIS

In the analyses, we will compare the histological findings from two main diagnostic pathways: (1) PCaVision imaging in all men followed by targeted biopsies in men with suspicious lesions; (2) MRI imaging in all men followed by targeted biopsies in men with suspicious lesions. The findings will be analysed and reported at the per-patient rather than per-lesion level because the per-patient level of reporting has the greatest clinical relevance. In the analysis, we differentiate between the following two datasets:

- Total study population, all men included in the study to undergo PCaVision and MRI imaging.
- Paired dataset with sufficient quality images and subsequent targeted biopsies for both PCaVision and MRI.

The last dataset will be used in the paired analysis when comparing the diagnostic accuracy of different imaging pathways.

In the descriptive part of the analysis we will show the cross- tabulation of histological findings (ranging from no cancer, GG=1 to GG=5) comparing the two main diagnostic pathways: PCaVision based targeted biopsies against MRI based targeted biopsies.

10.1. Primary study endpoint analysis

The main analysis is the comparison of the detection rate of clinically significant cancer (defined as GG≥2) between the PCaVision targeted biopsy pathway and the MRI targeted pathway. We will calculate the difference in these detection rates and its corresponding 97.5% confidence interval. Because of the paired design of our study, we will use the approach of Liu which accounts for the correlation between the detection rates of both pathways [44,45,46]. A one-sided 2.5% equivalence test will be performed to formally evaluate whether the lower bound of the confidence interval will not exceed the non-inferiority margin specified at an absolute difference in detection rates of 5%.

10.2. Secondary study endpoint analysis

As a secondary analysis, the comparison of the proportion of men in whom targeted biopsies could have been safely omitted in the PCaVision pathway compared to the MRI pathway. This analysis is only necessary if the PCaVision pathway is non-inferior with respect to detection rate (see above) does not come at the price of fewer patients in whom targeted biopsies can be prevented (e.g., more unnecessary targeted biopsies). For PCaVision this proportion is the number of men in whom there are no lesions to be biopsied based on PCaVision while the MRI biopsy strategy and systematic biopsy (when performed) did not detect clinically significant cancer divided by the total number of men in the study. Likewise, this proportion for MRI is defined as the number of men in whom there are no lesions to be biopsied based on MRI while the PCaVision biopsy strategy and systematic biopsy (when performed) did not

detect clinically significant cancer divided by the total number of men in the study. Given the fully paired nature of the design, the proportions will be tested for being statistically significance different using the McNemar test (two-sided alpha of 5%). The approach of Liu which accounts for the correlation [44,45,46] will be used to calculate a 95% confidence interval for the difference between these proportions.

Furthermore, the same effect measures and statistical approach will be used as in the previous analyses, only the definition of the target condition will change. The following alternative definitions of the target condition (rather than ISUP ≥ 2) will be used: (i) ISUP ≥ 3; (ii) ISUP ≥ 1; (iii) ISUP = 1. The reason also assessing alternative definitions is that there is still ongoing discussion about the clinical relevance of these histological findings.

Both imaging techniques (PCaVision and MRI) can produce images of insufficient quality impeding the process of taking targeted biopsies. This hampers the clinical usefulness of the imaging technique in practice. Therefore, we will compare the proportion of men in whom the PCaVision pathway produces insufficient-quality images or targeted biopsies with the proportion of men with insufficient-quality MRI images or target biopsies in the MRI pathway. The same statistical approach for paired proportions will be used in the comparison of the detection rate.

The PCaVision software includes automated detection of ultrasound image quality by analysing patient motion and CEUS signal. We will analyse diagnostic accuracy of PCaVision using the same statistical approach with increased requirements of ultrasound images quality with regards to motion and CEUS signal. The exact levels of these increased requirements are described in section 6.1.2.

10.3. Interim analysis

Since participants will receive standard of care, with additional biopsies based on PCaVision for this study which can be considered of minimal risk and without infringement in clinical decision making, no interim analysis will be performed.
An interim analysis will be performed when the data of 50% of the planned total sample size is available for review. We will determine the prevalence of csPCa in our cohort and re-perform the sample size calculation while leaving all other parameters the same. Moreover, we will evaluate the frequency of discarded scans due to quality issues. If more patients are needed based on one of these recalculations, the total sample size will be adjusted accordingly. The total sample size will not be lowered as a result of this re-calculation.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (Fortaleza, Brazil, October 2013), and in accordance with the Medical Research Involving

Human Subjects ACT (WMO), Good Clinical Practice (ICH-GCP) and in compliance with the approved research protocol.
The clinical investigation shall not begin until the required approval/ favourable opinion from the EC and regulatory authority have been obtained.
Any additional requirements imposed by the EC or regulatory authority shall also be followed.

11.2 Recruitment and consent

Patients will present themselves at the outpatient clinic where their urologist will assess the possibility of study participation. When a patient is eligible for participation, study information will be provided both orally and in writing (participant information and consent form) by the treating physician. Next to informing the patient about the study, this physician also provides the Patient Information Folder (PIF) to the patient. The local investigator will then perform the Informed Consent Form (ICF) procedure. If the patient is willing to participate in the study, informed consent will be asked by the local investigator and signed by both the local investigator and the patient. Patients will be informed that they can withdraw at any time for any reason if they wish to do so without any consequences. The patient will have two days or more to consider their consent.

11.3 Benefits and risks assessment, group relatedness

There could be a potential benefit for the patients participating in this study when a clinically significant cancer is detected by targeted biopsies based on PCaVision that would otherwise have been missed by both MRI and systematic biopsies. Furthermore, the results of this study may be important for future patients suspected of prostate cancer. If PCaVision is sufficiently accurate, it could coexist with MRI as the central imaging strategies which would lead to a more streamlined and more cost-effective diagnostic work-up as the ultrasound examination is less costly and can be performed by urologists followed by targeted biopsies in the same session. If PCaVision is sufficiently sensitive, systematic biopsies after a negative ultrasound examination could even be omitted in the future, thereby reducing the burden associated with unnecessary biopsies.
Additional targeted biopsies could be performed because of the identification of PCa based on PCaVision imaging. These extra biopsies carry a small risk of haemorrhage and infection. There is also a small, anticipated risk for participants (with a risk classification of negligible risk) associated with the administration of ultrasound contrast agent. However, adverse events caused by the ultrasound contrast agent appear to be transient, mild, and rare. These mostly consist of transient alteration of taste, local pain at the injection site and facial or general flush. In rare cases allergic reaction is described.
In conclusion, although the potential benefit for the patient is small, we also believe that the burden and risk associated with participation in this study is limited. Patients will be informed about this risk before the study, and it will be described in the study information.

11.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.5 Incentives

This study is free of charge for every patient. Angiogenesis Analytics will reimburse patients participating in this study with travel expenses incurred by participation in the study. This involves maximally 1 extra study-required visit at any of the participating centres. Otherwise, there are no special incentives.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data collection and analysis will be monitored according to GCP. Data will be collected in an electronic data management system: Castor Electronic Data Capture, from CIWIT BV, Amsterdam, the Netherlands, website https://www.castoredc.com. All data collected in Castor will be stored on servers located the Netherlands hosted by TRUE (www.true.nl).
All data will be handled confidentially. Each participant will be recoded and be given a case number. A subject identification code list can be used if it is necessary to trace data to an individual subject. The principal investigator will safeguard the key to the code. All handling of personal data complies with the GDPR (general data protection regulation) (in Dutch: Algemene verordening gegevensbescherming (AVG)).

12.1.1 Case Report Forms/Electronic Case Report Forms

An eCRF will be used in this study. A CRF is required and should be completed for each included subject. The investigator has ultimate responsibility for the collection and reporting of all clinical, and safety data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring and available when required. Any corrections to entries made in the CRFs, source documents must be dated, initialed and explained (if necessary) and should not obscure the original entry. In case of electronic data record, the corrections history will be maintained by an audit trail.
In most cases, the source documents are the hospital's or the physician's subject chart. In these cases data collected on the CRFs must match the data in those charts.
In some cases, the CRF, or part of the CRF, may also serve as source documents. In these cases, a document should be available at the investigator’s site as well as at Angiogenesis

Analytics and clearly identify those data that will be recorded in the CRF, and for which the CRF will stand as the source document.

12.1.2. Procedures

All data from the examinations and investigations in this study will be transferred to media provided by the subsidizing party and collected at the time of eCRF collection. The Principal Investigator will manage and maintain the study database throughout the investigation. At the conclusion of the investigation, the database will then be locked and data transferred for analysis. There will be a documented record of data transfer and measures in place for the recovery of original information after transfer.

The database maintained by the Principal Investigator, shall be validated and secured according to the sponsor standard operating procedures. The pseudonymized data to which the subsidizing party will have access include:

- age (in years)
- PSA value
- rectal examination results
- prostate volume
- dates of appointments and procedures
- US images + results
- MRI images + results
- pathology report of prostate biopsies

Any study data released shall be done according to the publication policy.
No tissue material originating from the subjects after the biopsy procedures shall be stored as part of the study and no tissue material shall be transferred to the sponsor. So, tissue storage common practice will not be affected by our study. Tissue storage will be according to local hospital protocol.

12.1.3. Privacy Procedure

Subject privacy is maintained and protected as follows.

- All personal data is encrypted and the coding is presented as “PCaVision -Study Site Acronym - Subject Number”
- Only the principal investigator has access to the key of this encryption
- Source documents and any other personally identifiable information can be accessed by:
    - principal investigators of participating centres
    - auditor
    - national authorities, for example: Inspectie Gezondheidszorg en Jeugd (IGJ)

- Test subjects will not be approached again after completion of the study (for example for further research or follow up)
- No tissue material will be transferred to the sponsor, only the pseudonymized pathology report

12.1.4. Procedure for locking and storing database

After study completion and data cleaning the Castor database will be locked.

Data will be preserved for the minimum duration of 15 years.

12.1.5. Data retention

All records pertaining to the study (e.g. data report forms, informed consent forms, source documents, IRB correspondence, study ID code list and other study documentation) will be retained at the site.
Data and all appropriate documentation will be stored according to guidelines for 15 years after the completion of the trial, depending on country-specific regulations.

12.2 Monitoring and Quality Assurance

The adherence to the protocol and the quality of completeness of the collected data will be monitored on a regular basis. We will plan an initiation monitor visit before the inclusion of the first patient in each center and an interim monitor visit each 3 months due to negligible patient risk.
Monitoring will be risk-based. In the Risk Management File, 5 out of 7 identified medium-level risks are related to an operator error; 1 is related to algorithm performance and 1 to an allergic response of the patient in response to the US contrast agent. As a result, there will be an extra focus in the monitoring visits on operator performance, algorithm performance and the (correct) use of the US contrast agent.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion. Non-substantial amendments will not be notified to the accredited METC and the competent authority but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

This is an investigator-initiated trial, all results all eligible for publication.
The study will be registered at ClinicalTrials.gov before the inclusion of the first patient.

12.7 Sponsor Activities

Sponsor representatives will perform all activities related to data collection.

12.8 Deviations

Protocol deviations are any alteration or deviation from the approved research plan including the original statistical analysis plan as defined in the study protocol. This includes equipment failures during study procedures.
The investigator is **not allowed to deviate from the CIP without prior approval** from the EC. However, deviations from the CIP may proceed without prior approval of the sponsor and the EC, if they are required to protect the rights, safety and well-being of human subjects under emergency circumstances. Such deviations shall be documented and reported to the sponsor and the EC as soon as possible;

If the researcher anticipates that there will be future requests for the same deviation, then the protocol will be amended (and such amendments must be approved by the REC/IRB). If the study objectives and procedure or cohort changes or the study changes from non-medical to medical, this generates a new study (not an amendment).

12.8.1 Corrective and preventive actions and principal disqualification criteria

All CIP deviations will be documented and assessed. If required, corrective and preventive action will be agreed upon and implemented with the relevant site. These decisions, activities and possible preventive actions will be documented.

A Principal Investigator can be disqualified if he has repeatedly or deliberately failed to comply with the requirements as specified in the CIP, including compliance with the relevant regulations, or if he has submitted false information in any required report. Corrective actions may include supplemental protocol training, discussions with PI and study staff for activities to prevent future recurrence, etc.. Misconduct can cause PI disqualification.

12.9 Investigational device accountability

The PCaVision investigational device will only be used on patients included in this study and following the study protocol.
The PCaVision device is SW running on an off the shelve computing unit (MacMini, Apple Inc.), the Computer Hardware will be labelled, including a serial number (See section 6.1) and will be installed by the staff of Angiogenesis Analytics on the clinical site. After the conclusion of patient inclusion at a specific clinical site the investigational device will be removed again.
Records will be kept of the identity (serial number) and location of each device as well as the data of installation and removal.
Due to the nature of the investigational device further device accountability (e.g. link to specific subject, expiry data and return of unused devices) is not appropriate.

13. STRUCTURED RISK ANALYSIS

A risk management plan, Policy template for Establishing Criteria for Safety Risk Acceptability, Risk Management Matrix, Risk Management Report and Benefit-Risk Determination have been created and approved. These documents are part of the Investigational Medical Device Dossier.

A summary overview of the risks and benefits of the investigational device and the clinical procedure applied in this clinical study are listed in the table below.

Anticipated clinical benefits	A potential benefit for the patients participating in this study when a clinically significant cancer is detected by targeted biopsies based on PCaVision that would otherwise have been missed by both MRI and systematic biopsies. Furthermore, the results of this study may be important for future patients suspected of prostate cancer. If PCaVision is sufficiently accurate, it could replace MRI as the central imaging strategy which would lead to a more streamlined and more cost-effective diagnostic work-up as the ultrasound examination is less costly and can be performed by urologists followed by targeted biopsies in the same session. If PCaVision is sufficiently sensitive, systematic biopsies after a negative ultrasound examination could even be omitted in the future, thereby reducing the burden associated with unnecessary biopsies.
Anticipated adverse device effects	There is a small, anticipated risk for participants (with a risk classification of negligible risk) associated with the administration of ultrasound contrast agent. However, adverse events caused by the ultrasound contrast agent appear to be transient, mild, and rare. These mostly consist of transient alteration of taste, local pain at the injection site and facial or general flush. In rare cases allergic reaction is described.
Residual risks associated with investigational device	In the risk analysis, in total 22 risks were identified of which 7 were classified as medium risks, and the other 15 as low risks. Of these 7 medium level risks, only 1 was not reduced to a low level after implementation of the risk mitigations. This risk involved an allergic response of the patient to the US contrast agent which could lead to an anaphylactic shock. In that situation, the standard of care protocol from the hospital should be followed to treat this anaphylactic shock. However, it is the responsibility of the clinic /hospital to act according to the standard of care in such situations.

Risks associated with participation in clinical study	Additional targeted biopsies could be performed because of the identification of PCa based on PCaVision imaging. These extra biopsies carry a small risk of haemorrhage and infection.
Possible interactions with concomitant medical treatments	There are no interactions with concomitant medical treatments
Steps that will be taken to control or mitigate risks	Risk are mitigated by specific mitigation actions as defined in the Risk Analysis File. For the 6 medium-level risks that were mitigated to low-level risk, adequate training and/or supervision of the operator, and detailed instructions in PCaVision 1.0' s IFU were the most often mentioned mitigation actions.
Risk-to-benefit rationale	The investigational device "PCaVision 1.0" has individual residual risks that are all acceptable and reduced as far as possible without introducing new risks or modifying the probability or severity levels of already defined risks. During the clinical investigation, the patient will receive standard of care and there will be no diagnosis nor clinical decisions based on the output of PCaVision, the diagnosis is always based on the outcome of the biopsy results. Therefore, the investigational device will not contribute to any hazardous situation that could result in unacceptable risk after implementing risk control measures. There could be a potential benefit for the patients participating in this study when a clinically significant cancer is detected by targeted biopsies based on PCaVision that would otherwise have been missed by both MRI and (potentially) systematic biopsies. However, even in the case where the patient do not receive immediate clinical benefit, the results of this study may be important for future patients suspected of prostate cancer. If PCaVision is sufficiently accurate, it could replace MRI as the central imaging strategy which would lead to a more streamlined and more cost-effective diagnostic work-up as the ultrasound examination is less costly and can be performed by urologists followed by targeted biopsies in the same session. If PCaVision is sufficiently sensitive, systematic biopsies after a negative ultrasound examination could even be omitted in the future, thereby reducing the burden associated with unnecessary biopsies.

None of the previous trials performing prostate imaging with CEUS reported procedure- or device-related adverse events. Apart from the aforementioned risks related to the

administration of contrast ultrasound there are no anticipated additional risks for participants in this study. The risk is negligible (“verwaarloosbaar risico”).

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Appendix I

Comparative analysis of the LOGIQ E10 R3 and LOGIQ E10 R4 ultrasound machines

Rationale

This study compares the PCa detection rate based on MRI with that of ultrasound using PCaVision. PCaVision has been developed based on ultrasound images created with a GE LOGIQ E10 ultrasound device, version R3. A more recent iteration of the device (GE Logic E10, version R4) is available for use in patient care. To ensure that PCaVision operates safely with the input from this newer ultrasound device (R4), an in-patient comparison with both devices is necessary. New study patients who are willing to participate in the current study will be asked to undergo an additional scan with both devices.

Procedure

The procedure will be fully explained to the patient during the informed consent consultation. The patient will undergo two 10-minute scans in a single session. The ultrasound scans will be performed using a probe that is inserted transrectally only once. First, the R3 scan will be conducted, then the probe will be disconnected from the R3 device and connected to the R4 device. This way, the probe does not need to be reinserted in the rectum of the patient. In both scans, a bolus of contrast fluid (SonoVue) will be administered. There are no adverse effects associated with administering a second bolus, and it is described as safe by the manufacturer. The total procedure will take approximately 10 minutes longer than usual.

Analysis

The primary and secondary analyses will be conducted using only the images obtained from the R3 device in order to ensure a uniform comparison between MRI and the R3 ultrasound images with PCaVision. A comparison of the R4 images with the R3 images will be conducted to ascertain whether software modifications are necessary to guarantee that PCaVision can be utilised with the R4 ultrasound system. The measured CEUS signal, SWE metrics and B-Mode will be compared. To validate the comparable output signals of R3 and R4, 50 patients will be required to undergo an additional R4 ultrasound scan.