STUDY NUMBER: M2M-GlycoScore-1

EVALUATION OF THE PERFORMANCE OF THE GLYCOSCORE BIOMARKERS FOR THE DETECTION OF CLINICALLY SIGNIFICANT PROSTATE CANCER

SPONSORED BY

Medtechtomarket Consulting Ltd
Magazine House
6 Magazine Point
Riverbank Rd
Bromborough
CH62 3JP
UK
www.medtechtomarket.com.

RESEARCH ETHICS COMMITTEE APPROVAL DATE: 20/09/2024

PROTOCOL APPROVAL

This protocol has been read and approved by:

Principal Investigator: **Signature:** **Date:**	

This protocol has been read and approved by:

Sponsor representative: **Signature:** **Date:**	

INVESTIGATOR PROTOCOL APPROVAL PAGE

EVALUATION OF THE PERFORMANCE OF THE GLYCOSCORE BIOMARKERS FOR THE DETECTION OF CLINICALLY SIGNIFICANT PROSTATE CANCER

I, the undersigned, have read and understood the protocol and am aware of my responsibilities as an Investigator. I agree to conduct the study in accordance with this protocol and any subsequent amendments, the Declaration of Helsinki and the laws and regulations of the country in which the study is being conducted.

Principal Investigator Name:	**Mr Henry Lazarowicz**
Investigator Title:	**Consultant Urological Surgeon**
Investigator Address:	**Royal Liverpool University Hospital** **Mount Vernon Street** **Liverpool** **L7 8XP**

Investigator Signature: **Date:**	

CONTACT NAMES AND ADDRESSES

PRINCIPAL INVESTIGATOR:

Name:	Mr Henry Lazarowicz
Address:	Royal Liverpool University Hospital Mount Vernon Street Liverpool L7 8XP
Phone:	0151 706 6807
Email:	henry.lazarowicz@liverpoolft.nhs.uk

SPONSOR:

Name of Company:	Medtechtomarket Consulting Ltd
Address:	Magazine House, 6 Magazine Point, Riverbank Rd, Bromborough CH62 3JP UK
Email:	Rebecca.newman@medtechtomarket.com

TABLE OF CONTENTS

LIST OF ABBREVIATIONS/GLOSSARY

PSA	Prostate specific antigen
PCa	Prostate cancer
mpMRI	Multi-parametric magnetic resonance imaging
CPG	Cambridge Prognostic Group
DRE	Digital rectal examination
TRUS	Transrectal ultrasound guided biopsy
RUA	Research Use Only
ELISA	Enzyme linked immunosorbent assay
BPH	Benign prostatic hyperplasia
CRF	Case Report Form
GCP	Good Clinical Practice
ICH	International Conference of Harmonisation
AE	Adverse Event
SAE	Serious Adverse Event
SDV	Source Data Verification
PPA	Positive Percent Agreement
NPA	Negative Percent Agreement
ADT	Androgen Deprivation Therapy
LUB	Liverpool University Biobank

1 PROTOCOL SYNOPSIS

STUDY TITLE	Evaluation of the performance of the GlycoScore biomarkers for the detection of clinically significant prostate cancer
PURPOSE OF THE STUDY	GlycoScore Dx Limited, a UK-based diagnostics company have identified three markers, ST6GAL1, GCNT1 & GALNT7, that showed promise as biomarkers of prostate cancer in initial marker validation studies. The purpose of this study is to further evaluate the sensitivity and specificity of the GlycoScore biomarkers for the detection of clinically significant prostate cancer. Sensitivity and specificity will be determined for each marker, combinations of the three markers and combinations of the GlycoScore biomarkers with PSA. The results from this study will be used to identify the most suitable biomarker/biomarkers for use in the GlycoScore test.
SPONSOR	Medtechtomarket Consulting Ltd Magazine House 6 Magazine Point Riverbank Rd Bromborough CH62 3JP UK
STUDY DESIGN	This is a prospective, non-interventional study using venous blood samples taken from patients with suspected prostate cancer or on active surveillance, attending the hospital Urology department for a transperineal biopsy of prostate. Following informed consent, venous blood samples from at least 137 patients will be collected and sent to Medtechtomarket Laboratories for testing. The concentrations of ST6GAL1, GCNT1, GALNT7 in each sample will be measured by ELISA assays, and the sample PSA will be measured using a CE-marked PSA assay. Results from testing of the samples will not be used in the clinical management of enrolled patients. The Investigator will provide Medtechtomarket with all prostate cancer related results from each participant including PSA, mpMRI (Likert or PI-RADS® score) and biopsy result (if carried out). Where prostate cancer is diagnosed, an indication of severity (according to the hospital's preferred method) such as Gleason score or CPG classification[1] will also be provided. As part of the consenting process, patients will be asked permission for two additional blood samples to be taken at the same time as the study sample, alongside a urine sample which

	will be transferred to the Liverpool University Biobank to be frozen and archived for use in future prostate cancer research. Patients' will also be asked permission for utilisation of archived biopsy tissue samples for potential future research studies. The Urology Department at Liverpool University Hospitals NHS Foundation Trust has a track record of clinical and translational research, including studies that have looked at detecting prostate and bladder cancer using patient's urine. These additional samples would provide an opportunity for further validation samples that would complement the current work and would provide a valuable bio-resource for future research studies, both in-house and in collaboration with other centres.
STUDY PRIMARY OBJECTIVES	To evaluate the diagnostic performance of the GlycoScore biomarkers for detection of clinically significant prostate cancer (Gleason score of ≥7)[2].
SECONDARY OBJECTIVES	• To establish an algorithm for calculation of a 'GlycoScore' that converts the assay results from the optimal combination of biomarkers into a score that indicates the likelihood that a patient has clinically significant prostate cancer • To evaluate the ability of the 'GlycoScore' to distinguish between individual Gleason scores and Gleason grade groups • To evaluate the ability of the 'GlycoScore' to distinguish between CPG groups 1 to 5 (Cambridge Prognostic Group classification) • To compare the ability of the GlycoScore biomarkers to detect clinically significant prostate cancer (with and without PSA) to PSA alone
PRIMARY ENDPOINT	The primary endpoint will be a comparison of calculated sensitivity, specificity and positive and negative predictive values for detection of clinically significant prostate cancer for the following: • Each individual GlycoScore biomarker, alone and in combination with PSA (measured at Medtechtechtomarket using a CE-marked assay) • Each possible combination of GlycoScore biomarkers, alone and in combination with PSA (measured at Medtechtechtomarket using a CE-marked assay)

	The reference method for diagnosis of prostate cancer will be biopsy, and the reference method for identifying patients who do not have cancer will be by a combination of mpMRI and/or biopsy.
SECONDARY ENDPOINT	Secondary endpoints will be: • Establishment of an updated 'GlycoScore' algorithm • Assessment of the correlation between the 'GlycoScore' for each sample and Gleason score at biopsy • Assessment of the correlation between the 'GlycoScore' and the Cambridge Prognostic Group classification • A comparison of the diagnostic performance of the GlycoScore biomarkers (with and without PSA) for the detection of clinically significant prostate cancer against the PSA test (hospital PSA result) alone
SAMPLE SIZE	A minimum of 137 male adult patients aged 18 and over.
SUBJECT POPULATION	The subject population will consist of male patients who are attending the Urology Department for a transperineal prostate biopsy for suspected prostate cancer or are on active surveillance (patients diagnosed with low-grade, slow-growing localised prostate cancer). **<u>Patient Group</u>** **Inclusion Criteria** • Male patients aged 18 years and over • The patient is being investigated for suspected prostate cancer or is on active surveillance • The patient is able to give informed consent to take part in the study **Exclusion Criteria** • A patient who has already taken part in the study • A patient with an active urinary tract infection • The patient has a prior diagnosis of any other cancer or receiving any cancer treatment (including ADT) • The patient is currently or within the last 4 months enrolled on another study involving an investigational medicinal product.
STUDY DURATION	The study will run for 12 months. The maximum duration of subject participation in this study is 1 hospital visit (informed

	consent, blood and urine sample collection will be carried out during the same visit).
STATISTICAL METHODS AND ANALYSIS SUMMARY	Data from the study will be analysed using the results of prostate biopsy or a combination of mpMRI and biopsy as the reference method for diagnosis of PCa. ROC curves will be plotted for: • Each individual GlycoScore biomarker, alone and in combination with PSA (measured at Medtechtomarket) • Each possible combination of GlycoScore biomarkers, alone and in combination with PSA (measured at Medtechtomarket) The ROC curves will be used to calculate sensitivity, specificity, likelihood ratio, and positive and negative predictive values for that method. This will also be repeated using the most recent PSA result provided by the hospital to compare the diagnostic performance of the current PSA test method with the GlycoScore biomarkers. Suitable correlation analysis will be used to evaluate the agreement between the 'GlycoScore' and • Gleason score & Gleason Grade Group • Cambridge Prognostic Group score
FOLLOW-UP EVALUATIONS	No planned follow up evaluations required.

2 REVISION HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
N/A	1.0	18/06/2024	N/A	Original Version submitted to IRAS
1	2.0	24/07/2024	Lindsay Traynor	Addition of 'End of Study' definition. Update to clarify that potential participants may be given the patient information sheet prior to attending the consent and donation visit. Updated specimen handling section to include processing of blood samples by LUB (also added to glossary) Study Procedures Flowchart updated. Clarification of participant information collected following consent.
2	3.0	13/09/2024	Rebecca Newman	Added consent for utilisation of archived tissue biopsy samples for future research purposes
3	4.0	20/09/2024	Lindsay Traynor	Added additional Paxgene tube to be collected for LUB. In addition to the trust, LUB to hold patient identifiable details for biobanking purposes only.
4	5.0	23/12/2024	Lindsay Traynor	Correction to state urine to be aliquoted by LUB and clarification of LUB process and collection kit

3 BACKGROUND

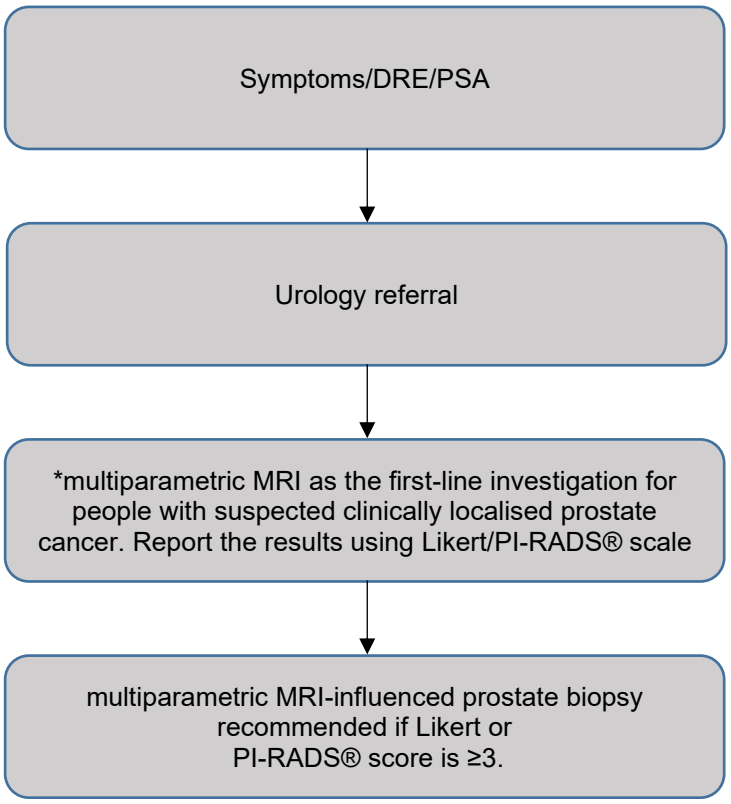
Prostate cancer (PCa) is the most common cancer in men in the UK and the second most common cancer in men worldwide. Affecting one in every eight men, more people live with a diagnosis of prostate cancer than any other type of cancer. It develops slowly and can remain undetected for many years. However, in some cases the cancer may be aggressive and, if undetected and unchecked, may spread to other areas of the body.

An urgent and unmet clinical need exists to develop more accurate diagnostic tests which can be applied at an earlier stage in the clinical pathway, and can help clinicians differentiate between clinically significant prostate cancers and slow-growing cancers that may never cause problems or shorten life.

Prostate-specific antigen (PSA) is a protein secreted by the prostate gland that can be detected in a patient's blood and can be used to help identify patients likely to have prostate cancer.

After presenting either with lower urinary tract symptoms or requesting screening for prostate cancer, patients typically undergo a digital rectal exam (DRE) and a PSA blood test. The current recommended diagnosis pathway for prostate cancer is outlined in Fig.1.

Fig. 1 NICE diagnostic pathway for suspected prostate cancer[,3]



*Not offered to patients who are unsuitable for radical treatment.

3.1 PSA

One of the best-known biomarkers in cancer biology is PSA[4,5]. It is a proteolytic glycoprotein enzyme belonging to the kallikrein family of serine proteases produced by normal prostate epithelium. PSA is organ-specific but not disease-specific and is detected in both normal prostate and benign prostatic hyperplasia, as well as primary and metastatic prostate cancer cells meaning levels can be elevated for reasons other than cancer (leading to false positive results).

Two thirds of men with a raised PSA level will not have prostate cancer, meaning many men undergo unnecessary invasive investigations including prostate biopsy.

Similarly, 1 in 7 men with prostate cancer do not have elevated PSA levels, so a significant number of patients with prostate cancer are not diagnosed by PSA also (leading to false negative results).

Thus, PSA alone has a poor predictive value for diagnosing or excluding clinically significant prostate cancer.

3.2 mpMRI

Multi-parametric magnetic resonance imaging (mpMRI) creates detailed images of the prostate that enables the clinician to better detect suspected prostate cancer. mpMRI combines the morphological assessment of T2-weighted imaging (T2WI) with diffusion-weighted imaging (DWI), dynamic contrast-enhanced (DCE) perfusion imaging and spectroscopic imaging (MRSI), which prior to biopsy can improve the detection rate of clinically significant PCa by allowing targeted samples to be taken. MpMRI can provide valuable clues about the histopathological aggressiveness of a PCa lesion and tumour stage. However, approximately 40% of patients have an inconclusive mpMRI result, which can lead to further examinations and repeat biopsies, with the associated anxiety, discomfort, risk and cost for both the patient and the NHS[6].

2.3 BIOPSY

Prostate biopsies provide a definitive method of confirming prostate cancer, and determining its severity by taking samples of tissue from the prostate gland, either by transperineal biopsy (TPB) or transrectal ultrasound-guided biopsy (TRUS).

These samples are collected and analysed by a pathologist who assigns a Gleason Score to the detected prostate cancer, which is based upon its morphological appearances under a microscope. The Gleason score helps indicate the clinical significance of the prostate cancer, with high-grade (potentially aggressive) prostate cancers having Gleason scores greater than 7.

Prostate biopsy however, is an invasive and uncomfortable procedure associated with significant risks of bleeding, infection, sepsis and occasional death. Of those patients undergoing prostate biopsy, a significant proportion will have a negative result, meaning they have undergone an unnecessary procedure and incurred risks and costs to the NHS that could have been avoided.

The availability of PCa-specific biomarkers could potentially facilitate a more accurate diagnosis, prognostication, risk stratification for treatment and reduce the need for unnecessary biopsies and their associated risks.

4 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVE

The primary objective of this study is to evaluate the diagnostic performance of the GlycoScore biomarkers for clinically significant prostate cancer in blood samples taken from 137 male participants with suspected prostate cancer or on active surveillance. In this context, clinical significance would be taken as a Gleason score of ≥7[2].

3.2 SECONDARY OBJECTIVES

The secondary objectives of the study are:

- To establish an algorithm for calculating a 'GlycoScore' that converts the assay results from the optimal combination of biomarkers into a score that indicates the likelihood that a patient has clinically significant prostate cancer)
- To evaluate the ability of the 'GlycoScore' to distinguish between individual Gleason scores and Gleason grade groups
- To evaluate the ability of the 'GlycoScore' to distinguish between CPG groups 1 to 5 (Cambridge Prognostic Group classification)[1]
- To evaluate the ability of the GlycoScore biomarkers (with and without PSA) to detect clinically significant PCa against that of PSA alone

3.3 PRIMARY ENDPOINT

The primary endpoint will be a comparison of calculated sensitivity, specificity and positive and negative predictive values for clinically significant prostate cancer for the following:

- Each individual GlycoScore biomarker, alone and in combination with PSA
- Each possible combination of GlycoScore biomarkers, alone and in combination with PSA

The reference method for diagnosis of prostate cancer will be biopsy, and the reference method for identifying patients who do not have cancer will be by a combination of mpMRI and/or biopsy.

3.4 SECONDARY ENDPOINTS

Secondary endpoints will be the following:

- Establishment of an updated 'GlycoScore' algorithm
- Assessment of the correlation between the 'GlycoScore' for each sample and Gleason score and Gleason Grade Group at biopsy
- Assessment of the correlation between the 'GlycoScore' and the Cambridge Prognostic Group classification
- Comparison of the sensitivity, specificity and positive and negative predictive value of the GlycoScore biomarkers (with and without PSA measured at Medtechtomarket) against that of PSA alone

The reference method for diagnosis of prostate cancer will be biopsy, and the reference method for identifying patients who do not have cancer will be mpMRI or biopsy.

3.5 STUDY RISKS AND BENEFITS

3.5.1 Risks

No anticipated adverse device effects related to this study have been identified.
As this is an observational study, participant risks are limited to those relating to the collection of the blood samples. Participants in this study will be subject to risks that are no greater than, or are equivalent to the risks associated with undergoing a peripheral venous blood test. Anticipated whole blood volume sampling should be ≤22.5ml (2 x 10ml and 1 x 2.5ml tubes). The use of standard precautions will minimise risk. Since blood sampling is a normal part of routine patient care, participating in this study should not cause any additional emotional stress or cause additional risks.

Possible side effects from blood drawing include faintness, inflammation of the vein, pain, bruising, haematoma or bleeding at the site of the puncture. A peripheral site infection may occur in rare circumstances.

Risks to study staff are those relating to contact with blood products. The use of standard precautions will minimise this risk.

Urine collection is a routine part of the procedure prior to the transperineal biopsy and providing a urine sample for use in future ethically approved research projects should result in no additional risk to the participant. Collection of biopsy tissue samples is the purpose of the transperineal biopsy, no additional tissue samples will be required therefore there should be no additional risk to the participant. There are no other residual risks associated with the study.

3.5.2 Benefits

The clinical benefit of the GlycoScore Prostate Cancer Test is being able to detect prostate cancer and to be able to distinguish between clinically significant and non-significant prostate cancer using a blood sample. This could reduce the number of unnecessary biopsies carried out and allow faster diagnosis of prostate cancer than the current clinical pathway. There are no direct benefits to patients participating in the study and results from the GlycoScore Test will not be used to influence patient management or decision making in any way. However, if the study leads to the clinical availability of the GlycoScore Prostate Cancer Test, then there may be a potential significant benefit to patients in the future.

For patients that agree for their samples to be used in future research, the storage of urine, blood and use of archived tissue samples would provide the hosting trust a valuable clinical resource for future ethically approved clinical research and material for validating current research studies.

5 CLINICAL STUDY DESIGN

5.1 STUDY PURPOSE

Initial studies carried out by Newcastle University using RUO ELISA kits showed significant correlation between PCa and the three biomarkers ST6GAL1, GCNT1 and GALNT7. A further cohort of plasma samples from a range of low and high Gleason grade samples as well as BPH

(benign prostatic hyperplasia) and healthy patients (supplied by York biobank) was used to generate a 'GlycoScore' algorithm[7].

The purpose of this study is to evaluate the three biomarkers ST6GAL1, GCNT1 and GALNT7 for their ability to identify clinically significant prostate cancer, either alone, as a combination of the biomarkers or in combination with the PSA (measurement obtained at Medtechtomarket). ST6GAL1, GCNT1 and GALNT7 concentrations from each plasma sample will be measured using the GlycoScore ELISA assay developed by Medtechtomarket.

The diagnostic presence of prostate cancer will be determined by comparison with biopsy results, and controls determined by a combination of mpMRI and negative biopsy results.

Assessment of the correlation between GlycoScore results with the full range of biopsy Gleason scores/Gleason Grade group/CPG grade will allow an assessment of the prognostic value of the GlycoScore test and its potential use in the clinical management of prostate cancer.

5.2 STUDY DESIGN

This is a prospective, non-interventional study using venous blood samples taken from patients with suspected prostate cancer or on active surveillance. A venous blood sample for use in the study will be obtained from a minimum of 137 patients referred to the Urology Clinic for transperineal biopsy for the investigation of suspected PCa or patients undergoing active surveillance. The venous blood and urine samples will be taken during the patient's routine hospital visit, prior to the biopsy procedure taking place. The blood samples will be collected according to the study protocol and sent to Medtechtomarket Laboratories where they will be tested using the GlycoScore ELISA assays for all three biomarkers and a PSA measurement recorded using a CE marked PSA test.

All blood and urine samples will be transferred to Liverpool University Biobank for processing. The GlycoScore study samples will then be transferred to Medtechtomarket for testing and the additional blood and urine samples will be frozen and archived at LUB. Tissue samples will be stored in the pathology lab as standard.

The following test results will be supplied by the hospital for each study participant:

- Diagnosis or otherwise of PCa
- Hospital PSA test result and date
- mpMRI report including Likert or PI-RADS® score
- Gleason score
- Gleason grade group
- CPG group (if available)

Results obtained at Medtechtomarket from testing of the samples will not be used in the clinical management of enrolled study participants.

5.3 JUSTIFICATION FOR SPECIMEN COLLECTION

The ELISAs used to measure the ST6GAL1, GCNT1 and GALNT7 biomarker concentrations require a venous whole blood sample, and therefore whole blood samples are essential for the completion of this study. Whenever possible this will be obtained if any blood is required as part of the patient's journey. In cases where blood samples have already been taken prior to study recruitment, a separate venous blood sample will be required for the purpose of the study.

5.4 METHODS TO AVOID BIAS

All patients referred to secondary care with suspected PCa or on active surveillance and meeting the study criteria will be invited to join the study. No further selection or grouping of participants will take place during the study.

Samples and clinical data will be pseudonymised, GlycoScore results from each participant will be blinded to the clinician managing their healthcare, so as not to influence their diagnosis and/or treatment.

There is unlikely to be any operator bias when performing the GlycoScore assays as each test incorporates a calibration curve. However, a minimum of 2 study staff (laboratory staff) will be fully trained to run the GlycoScore immunoassays to minimise further any potential risk of Operator bias. All Medtechtomarket study staff will be blinded to patient diagnosis until after the samples have been analysed for biomarker concentration.

6 DIAGNOSTIC METHOD

6.1 THE GLYCOSCORE PROSTATE CANCER BIOMARKERS

The aim of the study is to identify whether the three prostate cancer markers identified by Glycoscore can be used individually, or in combination, to diagnose significant prostate cancer (The GlycoScore Prostate Cancer biomarkers will be measured using quantitative immunoassays, with labelled antibodies against the biomarkers ST6GAL1, GCNT1 & GALNT7). The immunoassays will measure and report the concentration of the selected biomarkers (or combination of) in plasma. Once the most suitable biomarkers have been identified the aim will be to generate an algorithm that will convert the assay results into a ‘GlycoScore’ which can be used to give an indication of disease severity.

The GlycoScore biomarkers are intended to be used for the detection of clinically significant prostate cancer and could potentially be used to distinguish between different grades of PCa. The test is intended to be used by trained professionals in a pathology laboratory setting.

6.2 DIAGNOSTIC METHODS USED FOR COMPARISON

The methods used for comparison in this study will be mpMRI and biopsy.

7 SPECIMENS AND SUBJECTS PROVIDING SPECIMENS

7.1 PATIENT PARTICIPANTS

A minimum of 137 adult male patients will be recruited for the study.

7.1.1 Patient Inclusion Criteria

A patient is considered eligible for participation in this part of the study if all the following inclusion criteria are satisfied before enrolment:

- Male patients aged 18 years and over
- Patient is being investigated for suspected prostate cancer or is on active surveillance
- The patient is able to give consent to take part in the study

7.1.2 Patient Exclusion Criteria

A patient is considered ineligible for participation in this part of the trial if any of the following exclusion criteria are met prior to enrolment:

- A patient who has already taken part in the study
- A patient with an active urinary tract infection
- The patient has a prior diagnosis of any other cancer or is receiving any cancer treatment (including ADT)
- The patient is currently or within the last 4 months enrolled on another study involving an investigational medicinal product.

7.2 SPECIMEN COLLECTION

All specimen collection will be carried out by trained study staff using standard site procedures (Appendix A – Study Procedures Flowchart). Any study blood (and urine) samples must be drawn/taken prior to any invasive procedure, such as prostate biopsy.

Two (≤10ml) K2 EDTA and one (≤2.5ml) Paxgene tube of venous blood will be taken on the patient's 1st study visit (this will be during their pre-biopsy check prior to their biopsy); one EDTA tube for the study and the other EDTA and Paxgene tube for the University of Liverpool Biobank. Study samples will only be collected on a week day.

The 20ml sample of urine obtained from clinic, pre-biopsy and pre-dipstick testing will be decanted then transferred, processed and stored at -80°C at the University of Liverpool Biobank.

Following routine processing biopsy tissue samples will be stored in the pathology lab as standard practice.

7.3 EXPECTED DURATION OF SUBJECT PARTICIPATION

The maximum duration of subject participation in this trial is one hospital visit, with screening, consent and sample collection taking place on the same visit. Potential participants may be given the patient information sheet prior to attending the consent and donation visit.

7.4 EXPECTED DURATION OF STUDY

The study is expected to take approximately 12 months to fulfil recruitment targets.

7.5 END OF STUDY

The end of the study, will be the date that the associated clinical data is collected from the last participant recruited onto the trial.

7.6 SPECIMEN HANDLING AND PROCESSING

Blood samples will be handled, transported and disposed of according to site-specific guidelines. Liverpool University Biobank will provide Sample Collection Kits with a specific kit ID number, Pathoseal and sample transfer bag, pre-labelled blood and urine tubes, a Sample Information Sheet, GlycoScore Consent Form, to be completed for each donor. The Sample Collection Kits will be transferred to LUB for processing and archiving. Whole blood will be collected into the pre-labelled K_2 EDTA vacutainer/Paxgene blood tubes and processed as follows;[8,9]

7.6.1 GlycoScore Study Blood Samples (One EDTA tube collected for Medtechtomarket)

For the first 5 samples, Medtechtomarket will be notified and fresh blood samples will be collected from LUB on the same day and transported back to Medtechtomarket. Medtechtomarket will process and run a fresh versus frozen comparison study. It is anticipated that following the comparison study all remaining blood samples will then be processed by Liverpool University Biobank and stored frozen for collection by Medtechtomarket once a suitable number of samples have been collected. The blood samples will be processed using the following sample procedure (either at Medtechtomarket or Liverpool Biobank):

- After collection, the blood will be gently mixed by inverting the tube 8 to 10 times
- The pre-labelled tubes will be stored at 2-8ºC
- Liverpool biobank / Medtechtomarket will be notified, and the samples will be collected and processed the same day
- The blood sample will be centrifuged for 10 minutes at 2,000 x g using a refrigerated centrifuge (samples should be centrifuged on the same day as sample collection)
- The plasma layer will be carefully collected with an appropriate transfer pipette
- Aliquots (0.2ml) of plasma will be pipetted into appropriately sized pre-labelled cryovials. This process will be completed within 1 hour of centrifugation
- Aliquots will be stored in a specimen box or rack in a -80ºC freezer
- Plasma samples will be tested once sufficient samples have been collected

7.6.2 Liverpool University Biobank Urine Sample (storage at LUB for future research)

- After collection, half of the urine sample will be decanted and given to the research team
- The urine will then be decanted into the pre-labelled urine tube supplied in the Sample Collection Kit by the research nurse

Samples will be collected by a member of the LUB team for processing and storage.

7.6.3 Liverpool University Biobank Blood Sample (one EDTA and one Paxgene tube storage at LUB for future research)

- After collection, the blood will be gently mixed by inverting the tube 8 to 10 times.
- The pre-labelled tubes will be stored in the sample bag provided by LUB until collection.

- Samples will be collected by the Liverpool University Biobank Team who will spin down the samples and aliquot the plasma into 1ml cryovials, alongside the cell pellet, prior to freezing at -80°C.

- The Paxgene blood sample will be processed according to Biobank site procedures

Plasma samples will be destroyed at the end of the study unless the participant has indicated they would be happy for samples to be used in future ethically approved research, in which case the remaining samples will be stored at Medtechtomarket laboratories and the additional blood samples at the Liverpool University Biobank alongside their urine samples.

7.7 WITHDRAWAL OF PARTICIPANTS

Participants will be advised that they have the right to withdraw from the study at any time without prejudice, and may be withdrawn at the Investigator's discretion at any time. In the event that a patient drops out of the study or is withdrawn, the withdrawal section of the eCRF/database should be completed. The Investigator should record the date of the withdrawal, who initiated the withdrawal, and the reason for the withdrawal (if the patient provided a reason) within the additional information. The Investigator should inform Medtechtomarket of the participant study number of any withdrawn patients. Any data obtained from a withdrawn participant will be excluded from the study and any stored plasma or urine samples will be destroyed. Additional participants will be recruited to replace any participants who withdraw from the study.

8 STUDY OPERATOR SELECTION AND TRAINING

Study Operators will be Medtechtomarket laboratory staff trained in how to run the PSA test (Dialab PSA ELISA Z00338) and the GlycoScore immunoassays. All testing will be carried out at Medtechtomarket Laboratories. A minimum of 2 study operators will be required to conduct the study to ensure data is available from several operators.

9 STUDY PROCEDURES

9.1 PARTICIPANT RECRUITMENT

Potential participants will be sent a letter about the study with their biopsy appointment letter.

Potential participants will be identified by the NHS staff member looking after the patients attending for their biopsy. Potential participants will then be introduced to the research/study nurse to discuss the study and whether they wish to participate. They will also be provided with a patient information sheet which explains the study. Potential participants will be asked if they are willing to donate three tubes of venous blood, a urine sample and asked permission for utilisation of archived biopsy tissue samples for future research purposes. A nurse associated with the study and trained to obtain informed consent for research projects will be available to answer any questions a potential participant might have and will be present to administer and witness consent before blood is taken. A copy of the signed and dated written informed consent form and any other written information will be provided to the participant prior to donating (see Section 18 for further details). Bloods will be taken by the research nurse or by clinic staff trained in phlebotomy.

Patients that meet the eligibility criteria will be enrolled onto the study.

The following information shall be recorded for all patients on the eCRF/database:

- Demographics, to include race, ethnicity, age.
- Dietary preference (required for patients consenting for future research)
- Concomitant medications and dosages
- Date of PSA test (if possible) and result
- Date of mpMRI, mpMRI report and Likert or PI-RADS® score (if available)
- Gleason grade and score (when available)
- CPG grade (if available)
- Additional relevant scan information, e.g. Bone Scan, CT scan or PSMA scan.

9.2 SAMPLE COLLECTION

Following informed consent, three tubes of venous blood will be collected from the participant. The procedure for collecting and processing of the plasma samples is detailed in Section 7.6. Once sufficient plasma samples have been received by Medtechtomarket they will be tested on the GlycoScore immunoassays and the PSA test (Dialab PSA ELISA Z00338).

If consent for sample use in future research has taken place for the urine and biopsy tissue samples, the sample of urine obtained from the clinic, pre-biopsy and pre-dipstick testing will be transferred to LUB with the additional blood samples for processing and storage. The biopsy tissue sample will be processed according to standard procedures following the biopsy and stored in the pathology lab.
A flowchart summarising each step of the study procedures can be seen in Appendix A.

9.3 QUALITY CONTROL PROCEDURES

A 5-point standard curve will be run with each biomarker immunoassay test according to the Instructions for Use**.** 3 replicates per sample will be run**.** Data will be analysed for outliers using Grubbs test for quantitative data. All outliers will be investigated to determine the cause. The investigation and its conclusions will be documented in the report.

9.4 PSA TEST QUALITY CONTROL PROCEDURES

Quality controls will be run as per the Instructions for Use accompanying the kit.

10 STUDY MONITORING

Medtechtomarket Consulting Ltd will monitor the trial in accordance with ICH-GCP guidelines, ISO 20916:2019 and applicable regulatory requirements, and ensure that trial initiation, conduct, and closure are adequate. The Investigator and site staff are expected to cooperate with Medtechtomarket Consulting Ltd personnel or agents of Medtechtomarket Consulting Ltd and to be available during the monitoring visits to answer questions and to provide any missing information. The Investigator(s)/institution(s) will permit direct access to source data/documents for trial-related source data verification, audits, Ethics Committee review, and regulatory inspection(s).

A detailed study monitoring plan will be provided separately to this study protocol.

10.1 SOURCE DATA VERIFICATION

Source data verification (SDV) ensures accuracy and credibility of the data obtained. During monitoring visits, reported data will be reviewed with regard to being accurate, complete and verifiable from source documents (e. g., electronic clinical databases/applications including case notes (PENS), blood / pathology / scan results (ICE results server) alongside clinic letters (EPRO) and GP referral forms (available via eXchange). All data reported on the eCRF/database should be supported by source documents.

10.2 DEFINITION OF SOURCE DATA

Source data includes all information in source documents (original records and certified copies of original records, data, laboratory records) and includes all clinical findings or observations relevant to the study – as outlined within this protocol. In this study the following information will be supplied on the eCRF/database and therefore may require verifying from source documents:

- Diagnosis (or otherwise) of prostate cancer
- Hospital derived PSA test results (recorded on referral)
- mpMRI report and Likert or PI-RADS® score
- Gleason grade and score

10.3 AUDITING

As a quality assurance measure, Medtechtomarket Consulting Ltd or regulatory authorities may undertake audits before, during and after the trial. Regulatory authorities and representatives of the relevant Ethics Committee will be allowed to conduct inspections at the site. The Investigator will notify Medtechtomarket Consulting Ltd if regulatory authorities contact them to schedule an inspection.

11 DATA MANAGEMENT

11.1 DATA HANDLING

The Investigator is responsible for prompt reporting of accurate, complete and legible data in the eCRF/database and in all reports. Whenever possible, clinical data will be entered directly into an eCRF/database held locally on the host trust site. Pseudonymised data will be transferred to Medtechtomarket with appropriate linkage to the study sample ID. The eCRF/database is designed to provide a fully paperless solution to data entry, but will permit printed versions of the eCRF in real time, in a readable format, should a paper archive be required or necessary, The Investigator should maintain a list of personnel authorised to enter data into the eCRF. The Investigator will keep a list containing all participants approached and enrolled into the trial. This list remains with the Investigator and is used for unambiguous identification of each subject. The list contains the participants' study ID, full name, date of birth, date of enrolment in the trial, and the hospital number and National Health Service number, if applicable.

The subject's consent and enrolment in the trial must be recorded in the participant's medical record. This data should identify the trial and document the dates of the participant's trial participation.

11.2 CASE REPORT FORM

CRF data will be entered onto a bespoke backend password protected MS Access database which allows data to be entered into clearly defined portals. The data is backed up by the Trust

on a twice-daily basis. Screening CRF data which will hold the patient identifiable details is stored on a separate database. The eCRF/database will contain the following information:

- The study participants' identification number
- Most recent PSA measurement obtained
- Details of any current medication the patient is taking including medication name and dosage
- Dietary preference (required for patients consenting for future research)
- mpMRI result (Likert or PI-RADS® score)
- Gleason grade
- Gleason score
- Diagnosis/outcome

11.3 RECORD KEEPING

To comply with international regulations, the Investigator will arrange for the retention of essential trial documents for a minimum period of 10 years after the end of the study.

The Investigator should take measures to prevent accidental or premature destruction of records. If archiving can no longer be maintained at the site, the Investigator should notify Medtechtomarket Consulting Ltd.

Data from the eCRF/database will be exported to Medtechtomarket which will contain all of the clinical information (see Section 11.2) using the participant study ID to link to the sample. participant's name and any other identifying detail will NOT be included in any study data electronic file. Study data from the electronic file will be manually inputted into a clinical data management system by double data entry cross-checking for accuracy. Post verification, the database will be locked.

12 STATISTICAL CONSIDERATIONS

12.1 DATA QUALITY CONTROL

Direct entry of data into a clinical database will be the preferred mode of data entry. All data accepted as valid will be included.

All procedural errors, instrument malfunctions and control failures will be investigated to determine the cause. The investigation and its conclusions will be documented in the report.

Data will be analysed for outliers using Grubbs Test. All outliers will be investigated to determine the cause. The investigation and its conclusions will be documented in the report.

12.2 STATISTICAL ANALYSIS OF PRIMARY OBJECTIVE

The diagnostic performance of the individual biomarkers will be analysed by the calculation and comparison of the sensitivity and specificity, likelihood ratio, PPV and NPV and ROC analysis along with confidence intervals and significance levels. Analysis will be carried out for:

Each biomarker in combination with PSA

Each possible combination of biomarker with and without PSA

12.3 STATISTICAL ANALYSIS OF SECONDARY OBJECTIVES

Graphical plots and correlation statistics will be used to assess the correlation between the 'GlycoScore' and Gleason score, Gleason grade and CPG grade.

12.4 DETERMINATION OF SAMPLE SIZE

12.4.1 Sample Size Calculations

Calculations were performed in order to calculate the sample numbers required to achieve a statistical power of 80% for the primary objective of evaluating the diagnostic performance of the GlycoScore biomarkers for detection of clinically significant prostate cancer (Gleason score of ≥7). The main measures of diagnostic performance used in the power calculations were sensitivity and specificity.

The reference method for diagnosis of prostate cancer will be biopsy, and the reference method for identifying patients who do not have cancer will be by a combination of mpMRI and biopsy.

A pilot study involving two cohorts (York & Exeter) showed the following performance characteristics:

Statistic	Training set	Test set
Sensitivity	85%	67%
Sensitivity confidence interval	6%	25%
Specificity	67%	100%
Specificity confidence interval	10%	28%

From this pilot data, the following sensitivity and specificity estimates have been proposed for the calculation of sample size required to power a prospective study at 80%:

Estimated Performance

Statistic	Estimate	95% CI (lower)	95% CI (upper)
Sensitivity	0.8	0.65	0.95
Specificity	0.8	0.65	0.95

The study population has been confirmed to contain 356 clinically significant cases, and 430 non-clinically significant/PCa negative controls, giving a disease prevalence of 45.3%.

Sample size calculations were undertaken in R (version 4.1.2) using the MKmisc package (version 1.8) with formulae derived from Machin[10] and Flahault et al[11], and the previously outlined target values for sensitivity, specificity, and prevalence, along with listed confidence intervals, to derive the following sample size guidelines:

Sample Size for Sensitivity Optimised cut-offs

alpha	0.05	Level of significance (one-sided)
1-beta	0.8	Power
Diseased patient cohort size	62	Estimated minimum number of subjects with disease condition for sensitivity
Subjects without disease	75	Estimated minimum number of subjects without disease condition for sensitivity

Sample size for SN	137	Total sample size for confirming sensitivity
Total subjects (N)	**137**	**Recommended minimum sample size**

Sample Size for Specificity Optimised cut-offs

alpha	0.05	Level of significance (one-sided)
1-beta	0.8	Power
Diseased patient cohort size	52	Estimated minimum number of subjects with disease condition for specificity
Subjects without disease	62	Estimated minimum number of subjects without disease condition for specificity
Sample size for SP	114	Total sample size for confirming specificity
Total subjects (N)	**137**	**Recommended minimum sample size**

A minimum of 62 cases are required to provide 80% power at a 1-sided significance level of 0.05, and at a prevalence of 45.3%, it is predicted a minimum of 137 subjects in total would be required.

Statistical analysis of secondary objectives will be performed but not necessarily with a defined statistical power.

12.5 PROCEDURE FOR ACCOUNTING FOR MISSING, UNUSED OR SPURIOUS DATA

All missing, unused and spurious data will be documented in the study report with an explanation.

Data from participants who withdraw or drop out of the study will be excluded from the study and replaced.

Only complete data sets (PSA, mpMRI and/or biopsy results) will be used in the data analysis.

13 STUDY PROTOCOL AMENDMENTS

Protocol changes may affect the legal and ethical status of the study and may also affect the statistical evaluations of sample size and the likelihood of the study fulfilling its primary objective. Protocol amendment(s) are distributed to Investigators with instructions on how to implement them.

All protocol amendments, except non-substantial amendments i.e., logistical/administrative implications (e.g., change of monitor(s), change of telephone number) are submitted to the ethics committee for review and approval prior to implementation.

14 DEVIATIONS FROM THE CLINICAL STUDY PROTOCOL

The Investigator will not implement any deviation from, or changes of, the protocol without agreement by the Medtechtomarket Consulting Ltd and prior review and documented approval/favourable opinion from the ethics committee of an amendment, except where necessary to protect study participants rights safety and well-being, or the scientific integrity of the clinical performance study.

In the event that an emergency or other medical event occurs that requires a protocol deviation, the Investigator or designee will inform Medtechtomarket Consulting Ltd as soon as is possible. The protocol deviation will be documented in the eCRF/database.

Any deviation from the protocol will be recorded and assessed as minor or major by Medtechtomarket Consulting Ltd. For repeated and/or major deviations corrective and preventative actions will be developed by the site and Medtechtomarket which will be implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report protocol deviations.

Medtechtomarket Consulting Ltd has the right to discontinue the study at any time. In the event of premature discontinuation of the study, all study materials must be returned to Medtechtomarket Consulting Ltd.

15 STATEMENTS OF CONFORMITY

This protocol will be conducted under the relevant ICH guidelines commonly known as Good Clinical Practices, the applicable national requirements, and ethical principles that have their origins in the Declaration of Helsinki.

The work described in this protocol will be undertaken following Research and Ethics Committee review. The study will not be initiated until written and dated full approval is obtained.

16 INSURANCE

Insurance coverage will be handled according to local requirements.
Finance, insurance, and publication rights are addressed in separate agreements, as appropriate.

17 ADVERSE EVENTS

17.1 ADVERSE EVENT DEFINITION AND CATEGORISATION

The GlycoScore immunoassays will be run at Medtechtomarket laboratories therefore there will be no risk to patients or site study site staff of adverse device effects or device deficiencies.

The term adverse event (AE) describes events related to study activities. It refers to undesirable, negative events such as injuries or diseases observed over the course of the study.

Participants in this study are subject to risks that are no greater than, or are similar to the risks associated with undergoing a blood draw from a peripheral vein. There is a very small risk of infection with standard peripheral venepuncture. To minimize the risk, standard precautions will be taken and where possible additional blood tubes will be added to a routine blood draw as part of the standard procedure for investigating suspected prostate cancer. Since blood sampling is a normal part of routine patient care, participating in this study should not cause any emotional stress or additional risks to either the study staff or participants.

Urine collection is part of the pre-biopsy checks and not considered to pose any risk to the participants. Obtaining biopsy tissue samples is the purpose of the transperineal biopsy procedure. Patients are only consenting to their archived biopsy tissue samples being utilised for future research purposes and therefore there is no further to risk to the patient other than those outlined during consent for the biopsy procedure.

17.2 REVIEWING AND REPORTING ADVERSE EVENTS

For this study the duration of patient participation is one visit therefore adverse event reporting would only be relevant once the patient has been registered onto the study and during blood/urine collection. Any adverse events that could result in a serious adverse event should be recorded on the SAE forms held in the Study Site File. Adverse events must be reviewed and categorised by the Investigator as soon as possible. Any serious adverse events (SAE) should be reported by email to the Medtechtomarket immediately, if possible, but no later than 3 calendar days after site study staff awareness of the event. Any serious adverse events should be fully investigated with the findings recorded in a written report.

SAE must be reported to the REC in accordance with the clinical site's Research Governance policy and also reported to the Research and Development Manager at Medtechtomarket by email using the contact details below:

Hannah Martin
hannah.martin@medtechtomarket.com

18 INFORMED CONSENT PROCESS

A participant's informed consent must be obtained and documented in accordance with local regulations, ICH-GCP requirements, and the ethical principles that have their origin in the Declaration of Helsinki.

Prior to obtaining informed consent, a complete explanation (both verbally and in written format) of the nature and the purpose of the study will be provided to the participant in a language and at a level of complexity understandable to them. This shall take place under conditions where the participant has adequate time to consider the risks associated with their participation in the study.

Prior to participation in the study the participant and the person who conducted the informed consent discussion must sign and date the Ethics Committee approved informed consent form. As part of the consent process, each subject must consent to direct access to their medical records for study-related monitoring, auditing, Ethics Committee review and regulatory inspection. The participant will receive a copy of the signed and dated informed consent form.

18.1 VULNERABLE POPULATION - PATIENTS LACKING CAPACITY TO CONSENT

Patients lacking capacity to consent will not be recruited onto the study.

19 SUSPENSION OR PREMATURE TERMINATION OF THE STUDY

A principal Investigator, ethics committee, or regulatory authority can suspend or prematurely terminate participation in a clinical performance study at the study sites for which they are responsible.

Medtechtomarket may also suspend or discontinue the study at any time if, in the sponsor's opinion, there is a significant safety concern, relevant technical problems with the device or when monitoring or auditing identifies serious or repeated deviations from the protocol.

A justification in writing should be supplied by the terminating party and Medtechtomarket is responsible for informing the ethics committee and regulatory authority where necessary. All close-out activities shall be performed on termination of the study.

If there is suspicion of an unacceptable risk to participants during the study, recruitment can be suspended whilst the risk is being assessed.

20 PUBLICATION AND COMMUNICATION POLICY

Details of publication rights are addressed in a separate contract, as appropriate.

21 REFERENCES

1. https://www.nice.org.uk/guidance/ng131. (15 December 2021)

2. https://www.nice.org.uk/guidance/ng131/evidence/d-diagnosing-and-identifying-clinically-significant-prostate-cancer-pdf-6779081777. Page 17

3. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/prostate-cancer/incidence

4. https://www.nhs.uk/conditions/prostate-cancer/diagnosis/

5. Roddam AW, Duffy MJ, Hamdy FC, et al. Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2-10 ng/ml: systematic review and meta-analysis. Eur Urol. 2005;48:386–99. doi:10.1016/j.eururo.2005.04.015. discussion 398–9.

6. Catalona WJ, Hudson MA, Scardino PT, et al. Selection of optimal prostate specific antigen cutoffs for early detection of prostate cancer: receiver operating characteristic curves. J Urol. 1994;152:2037–42.

7. Johnston E, Pye H, Bonet-Carne E, Panagiotaki E, Patel D, Galazi M, Heavey S, Carmona L, Freeman A, Trevisan G, Allen C, Kirkham A, Burling K, Stevens N, Hawkes D, Emberton M, Moore C, Ahmed HU, Atkinson D, Rodriguez-Justo M, Ng T, Alexander D, Whitaker H, Punwani S. INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. BMC Cancer. 2016 Oct 21;16(1):816. doi: 10.1186/s12885-016-2856-2. PMID: 27769214; PMCID: PMC5073433.

8. Henry, JB (1979) Clinical Diagnosis and Management by Laboratory Methods, Volume 1, W.B Saunders Company, Philadelphia, PA, p 60.

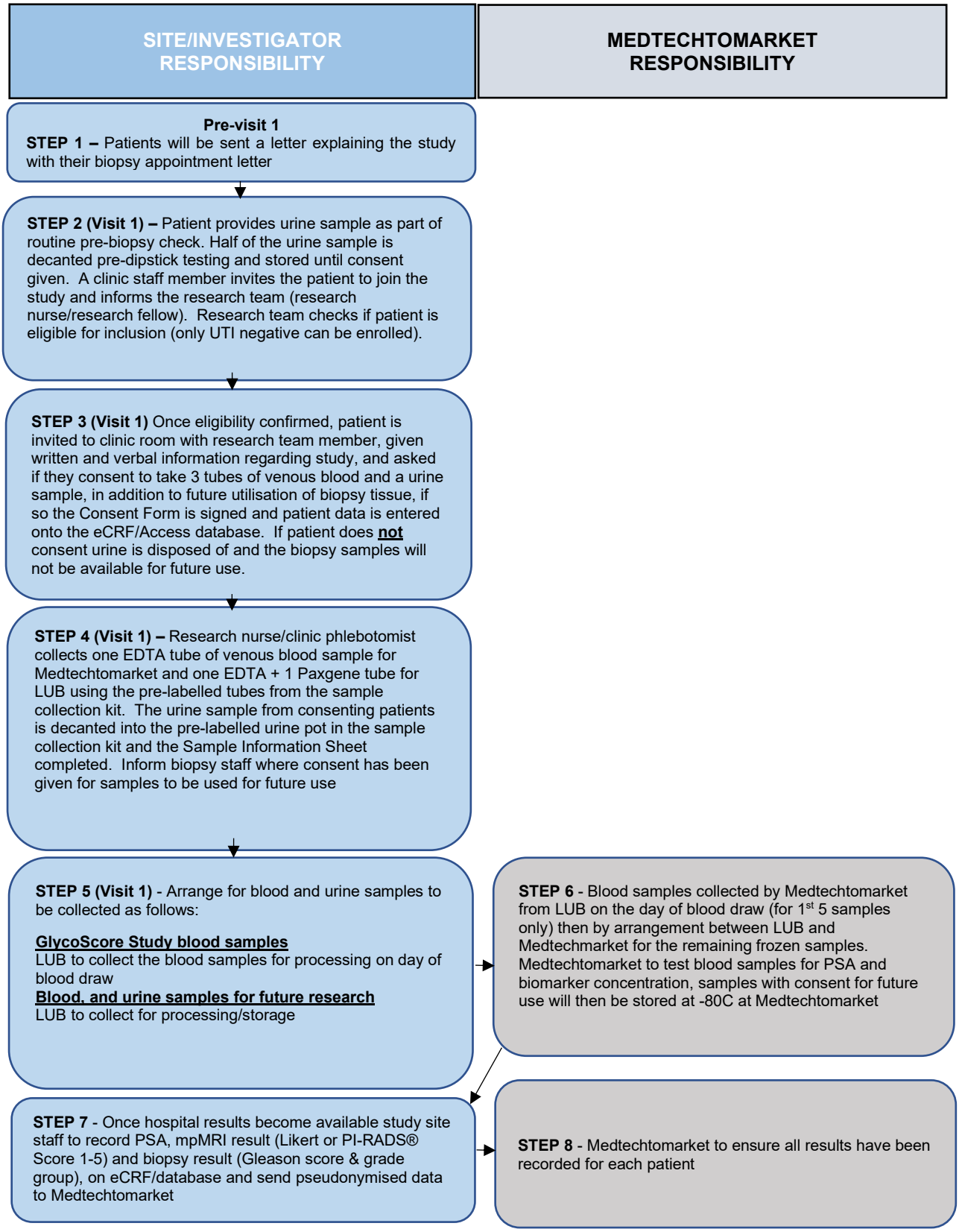
9. J. Proteome Res. 2009, 8, 1, 113–117 Publication Date:December 12, 2008 https://doi.org/10.1021/pr800545q - Supporting Info

10. Machin D, Wiley I. Sample size tables for clinical studies [electronic resource] / David Machin ... [et al.]. 3rd ed. Chichester: Chichester: Wiley-Blackwell; 2009.

11. Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. Journal of Clinical Epidemiology. 2005;58(8):859-62.

22 APPENDICES

22.1 APPENDIX A – STUDY PROCEDURES FLOWCHART



22.2 APPENDIX B – SCHEDULE OF EVENTS

Procedures	Total number of participant visits - 1	
	Pre-visit 1	Visit 1
Send out introduction letter/information leaflet to all transperineal biopsy patients with their biopsy appointment letter	✓	
Informed consent (+ core questions)		✓
Medical history (case note review)		✓
Concomitant medication check (at screening)		✓
Blood sample collection		✓
Urine collection processing for Liverpool Biobank		✓
Blood sample collection processing – 1 tube for GlycoScore study, 2 tubes for Liverpool Biobank (1 x EDTA, 1 x Paxgene tube)		✓
CRF/eCRF completion including data transfer and query resolution		When results are available