Protocol for spin off research project- Circulating tumour cells as biomarkers to predict prostate cancer metastasis for treatment stratification of localised cancer

Full Title	**Circulating tumour cells as biomarkers to predict prostate cancer metastasis for treatment stratification of localised cancer**
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Protocol for spin off research project- Circulating tumour cells as biomarkers to predict prostate cancer metastasis for treatment stratification of localised cancer

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1. Glossary/Abbreviations:

ADT	Androgen Deprivation Therapy
AUC	Area under the ROC Curve
BCI	Barts Cancer Institute
BCR	Biochemical Recurrence
CTC	Circulating Tumour Cell
CRF	Clinical Research Fellow
EAU	European Association of Urology
EDTA	Ethylenediamine Tetraacetic Acid
FTE	Full Time Equivalent
GCP	Good Clinical Practice
ID	Identification
Min	Minute
MHRA	Medicines and Health-care Products Regulatory Agency
MTA	Material Transfer Agreement
PBS	Phosphate Buffered Saline
PBMC	Peripheral Blood Mononuclear Cell

PCa	Prostate Cancer
PI	Principal Investigator
PIS	Patient Information Sheet
PSMA-PET	Prostate Specific Membrane Antigen – Positron Emission Tomography
QMUL	Queen Mary University of London
RBC	Red Blood Cells
RT	Room Temperature
ROC	Receiver Operating Curve
RP	Radical Prostatectomy
RLTB	Robert Lane Tissue Bank
SOP	Standard Operating Procedure
TBAO	Tissue Bank Acquisition Officer
UCLH	University College London Hospital
WBC	White Blood Cells

2. Introduction:

2.1. Rational: As many prostate cancer (PCa) cases diagnosed at early stage are indolent, PCa progression risk- stratification systems have been developed, based on biopsy characteristics, staging and PSA levels, and patients are classified into low- intermediate- and high-risk groups for treatment stratification. However, these systems are imperfect. Moreover, determining which curative treatment will be most effective for individual patients with localised PCa is challenging and remains a major obstacle in improving patient management and is recognised as an unmet clinical need. Cancer metastasis is the main cause of PCa death and determines the selection of therapeutic methods. Theoretically, localised PCa should be cured by radical prostatectomy (RP). However, more than half of apparently localised high-risk PCa cases treated by RP reoccur, indicating the presence of undetectable micro-metastasis at the time of surgery. These particular patients require additional treatment, usually with radiotherapy and systemic hormone therapy. The major challenge in managing aggressive localised PCa is differentiating truly localised PCa from those with micro-metastatic disease, which cannot be cured by RP. Pelvic lymph node dissection for high-risk localised disease is the current diagnostic test for assessing spread of disease but is invasive and performed during surgery. A test is required which can be performed before surgery to distinguish patients suitable for RP from those who would benefit from more extensive systemic treatment and radiotherapy. No current imaging test has the resolution to detect micro-metastases that may consist of just a few viable PCa cells. Circulating tumour cells (CTCs), the seeds of cancer metastasis, can occur at a very early stage of cancer development. We hypothesise that detection of CTCs provides an accurate indicator of cancer micro-metastasis and CTC gene expression may predict the potential of future metastasis development. We have established a promising CTC analysis method, by which we have detected CTCs in all patients with metastatic PCa and for the first time have demonstrated the value of using CTC analysis in predicting clinically significant post-biopsy ~~clinically significant~~ PCa outcome in pre-biopsy patients. Our CTC results may reflect the existence of micro-metastasis and predict PCa progression risk better than the current imperfect risk-stratification systems. Therefore, a prospective study with

clinical outcome follow- up over a long period is required to confirm the value of this CTC analysis in determining micro- metastasis, i.e. predicting post-RP PSA failure and future metastasis development.

2.2. Aims:

I. To establish the value of CTC positivity in predicting post-RP treatment failure, including BCR and new lesions detected by cancer imaging.

II. To establish the value of a combined CTC test (including CTC enumeration and gene expression) in predicting post-RP treatment failure and the time of the development of BCR and new lesions detected by cancer imaging.

III. To provide a preliminary assessment of the prognostic value of CTC data (positivity and gene expression) in predicting post-RP PCa metastasis development, overall survival and cancer specific survival and the generation of CTC data for future longer-term follow-up.

IV. To provide evidence and data which can be used to design a future clinical trial of a CTC-based intervention to be used to guide therapeutic choice in the management of high-risk localised PCa.

3. Summary and synopsis

This study will require 10-year patient follow up for analyses of the primary and secondary endpoints to have statistically significant power. Nevertheless, extensive research activities will be mainly conducted in the first 5-years where research funding has been secured. After the first 5-years, further research activity will only include annual patient clinical follow-up, data collection and statistical analysis. A renewal application for further funding will be submitted by the end of the first 5-year funding period. The overall study set up is summarised in **Table 1.**

Table 1. Overall study summary.

Methodology	Single site, double-blinded, prospective, paired cohort study. Participating patients and clinicians involved in treatment or management will be blinded to the CTC results, to avoid influencing standard patient treatment, management, and progression outcomes after RP.
Study objectives and endpoints	Objectives are set in the context of high-risk patients diagnosed with localised prostate cancer scheduled for robot-assisted RP who have not had neoadjuvant ADT, and are listed below. **Primary objectives:** To establish the value of CTC positivity in predicting post-RP treatment failure, including BCR and new lesions detected by cancer imaging. To establish the value of a combined CTC test (including CTC enumeration and gene expression) in predicting post-RP treatment failure and the time of the development of BCR and new lesions detected by cancer imaging. **Secondary objectives** To investigate the potential of CTCs in predicting positive lymph node metastasis, which affects the selection and scale of RP as the initial treatment. To assess potential for convenient non-invasive disease monitoring

	To provide a preliminary assessment of the prognostic value of CTC data (positivity and gene expression) in predicting post-RP PCa metastasis development, overall survival and cancer specific survival. To provide evidence and data which can be used to design a future clinical trial of a CTC- based intervention to be used to guide therapeutic choice in the management of high-risk localised PCa. To evaluate the longer-term prognostic value of CTC detection and CTC gene expression for metastases and cancer-specific survival. **Primary endpoint** The primary outcome is **post-RP treatment failure** during the first 4.5 years of follow up from start of recruitment. Post-RP treatment failure is defined as a PSA ≥ 0.2mg/ml at the routine PSA test 3 months after RP (commonly called 'failure to nadir') and remaining at this level or further increase afterwards without further treatment, or imaging detected appearance of cancer lesions. Cancer lesions detected by imaging without a PSA rise might include neuroendocrine PCa and lesions detected by PSAM-PET. This combined post-RP treatment failure primary endpoint will maximally capture all the clinically significant cancer appearance events. **Secondary endpoints** BCR during the first 4.5 years of follow up: PSA ≥ 0.2ng/ml at any time post-RP and remaining at this level or further increase afterwards without further treatment. Metastasis (any location)-free survival during the first 4.5 years of follow up. Only 5% of subjects with distant metastasis event (based on traditional imaging technologies) within this time frame (4-6). Metastasis (any location)-free survival at 10 years follow up. to confirm that metastatic event rates have increased among the positives, i.e. a declining rate of "false positives". Deaths from any cause during the first 4.5 years of follow up Overall survival at 10 years of follow up. Prostate cancer specific deaths during the first 4.5 years of follow up. Expected to be 2% or less based on previous studies in the post RP context. Prostate cancer specific survival at 10 years of follow up. Our hypothesis is that most if not all of the patients dying of PCa will be in the CTC positive group.
Target sample size	294
Eligibility	**Inclusion criteria:** High/High intermediate risk non-metastatic risk localised PCa based on the EAU stratification system Scheduled for robot-assisted RP Informed consent **Exclusion criteria:** With other cooccurring cancers Neo-adjuvant ADT Adjuvant ADT
Statistical methodology and analysis (if applicable)	The primary analysis is the estimation of the sensitivity of the pre-surgery CTCs for detection of RP treatment failure, i.e. BCR or progression detected by imaging. Observed specificity, positive and negative prediction values will be reported. Prevalence of events and specificity will be presented over time. BCR-free survival will be estimated and Kaplan-Meier curves presented. Preliminary investigation of the prognostic value of pre-surgery CTC data in terms of predicting survival outcomes will be investigated. CTC data will be explored alongside other pre-surgery risk factors using multivariate analysis. Recurrence events and follow up CTC results will be reported and compared over time.

Study duration	The planned maximum duration is 12 years (Target duration for patient recruitment is 2 years. Each patient provides blood samples for CTC analysis and target duration for completion of CTC analysis is 1 year after end of recruitment. Target duration of long-term patient follow-up from point of recruitment is 10 years)

4. Study design (first five years)

The study flow diagram for the first 5 years is shown in **figure 1.**

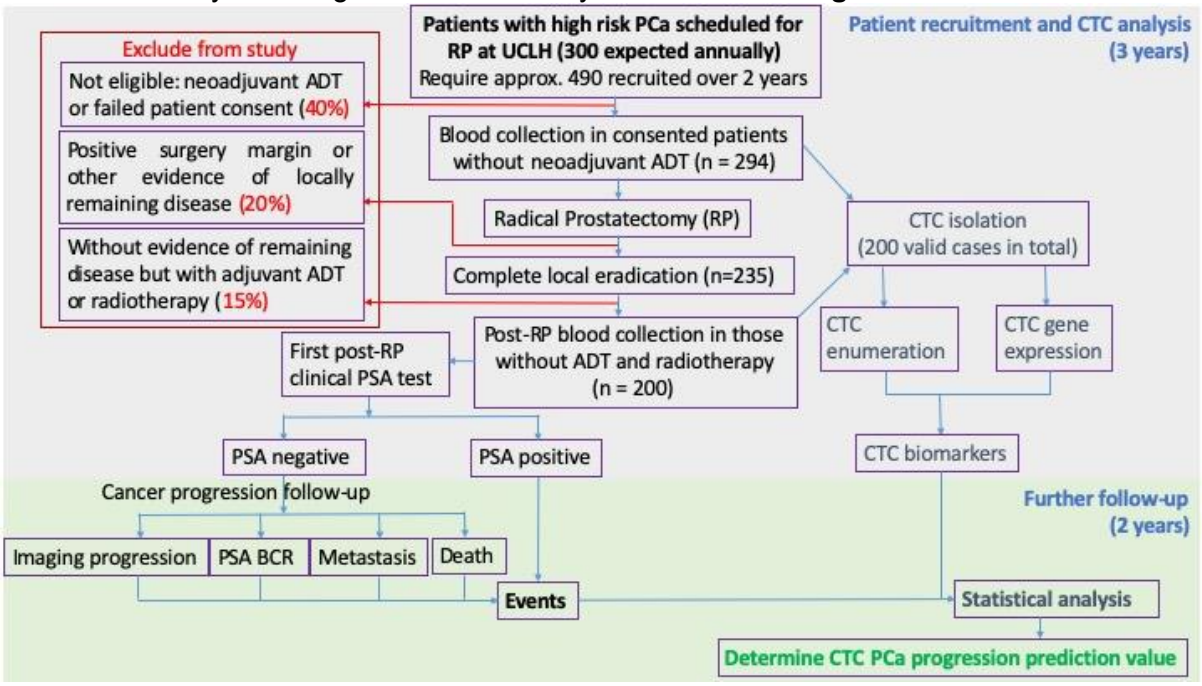

Figure 1. The study flow diagram for the first 5 years.

The 5-year funded study will be taken with the planned milestones described in **Table 2** below.

Table 2: Planned study milestones:

Patient recruitment	years 0-2
Blood sample collection	years 0-2.25
CTC analysis	years 0-3
Follow up	years 0-4.5
Analysis	years 4-5*

*The statistical analysis will start after 4.5 year and takes 6 months to complete within the initial 5-year funded period.

5. Study population

Potential participants will be identified, approached and screened by the study team if they are interested in participating as described in the study design above. In order to recruit patients meeting the eligibility criteria, it will be necessary to screen all patients in the participating centre who are diagnosed with high risk localised PCa based on the current European Urology Association classification system who have been deemed eligible for radical prostatectomy by a specialist multi-disciplinary committee, and who have been scheduled for surgery to completely remove the cancer in the prostate gland.

Based on past patient numbers at UCLH, it is anticipated that approximately 600 patients will meet these initial criteria over the 2-year recruitment period. However, to meet our target

recruitment we anticipate contacting approximately 490 of these potential participants (**Figure 1**). Potential participants will be identified via MDT meetings and in clinics. Potential participants who meet eligibility criteria will be posted, emailed or physically provided with a patient information sheet (PIS) approved by the ethics committee, prior to their next clinic appointment in order to give sufficient time to consider the information. At their clinical consultation (remote or face to face), they will be approached regarding the study, and given the opportunity to ask any questions and confirm eligibility checks. Inclusion and exclusion criteria are given below.

Inclusion criteria

- High/high intermediate risk non-metastatic PCa based on the EAU stratification system
- Scheduled for robot-assisted RP
- Informed consent

Exclusion criteria

- With other cooccurring cancers
- Neo-adjuvant ADT
- Adjuvant ADT

Withdrawal Criteria

- Unable or unwilling to undergo RP
- Patient changes their mind and withdraws their consent

With an anticipated drop out of 40% due to either use of neo-adjuvant ADT or failure to consent in patients sent the PIS, we further anticipate 294 patients will contribute blood samples prior to RP. There is a target of 200 blood samples from eligible participants, who subsequent to having had RP do not have a positive surgery margin or other evidence of locally remaining disease, and do not subsequently have ADT (adjuvant ADT) or radiotherapy. It is intended that recruitment will continue until this target has been met.

6. Patient recruitment, sample collection, lab CTC analysis and follow-up procedures

Patients will be recruited (months 1-24) at UCLH, where the UK largest urological surgery centre is located and performs robot-assisted RP on PCa patients referred from several regional hospitals. The clinical team at UCLH will identify eligible patients who will be approached by the clinical research fellow (CRF) employed on the research project or the clinical care team for informed consent using the consent forms specifically designed for this project for blood collection and future research. Non-metastatic disease will be based on the current standard diagnostic imaging methods including CT/MRI and PSMA-PET/bone scan. A pre-surgery PSA test will be performed routinely at UCLH.

2 x 10 ml blood samples will be collected (months 1-27) using the lavender cap EDTA tube according to our established method from each consented patient by the CRF or the clinical care team at UCLH during the pre- and post-RP PSA test blood sampling, and taken to the laboratory at Barts Cancer Institute, John Vane Science Centre, Charterhouse Square either by the CRF, a tissue bank acquisition officer (TBAO)(in the absence of the CRF) or the postdoc (anonymise samples transfer in the absence of CRF and TBAO) under the signed material transfer agreement (MTA), at room temperature. The samples will be transported in

designated sample carrier using a taxi service. No public transport is to be used for moving samples between sites.

Each patient will be allocated a unique ID and will be used by the lab research team. This unique ID will be recorded in the tissue bank database for future correlation analysis of the CTC results with clinical follow-up data. Sample processing will be performed in tissue culture hood in the designated lab. The whole blood sample will be processed within 4hrs for CTC analysis after collection. Standard Operating Procedure (SOP) (C-ProMeta-1) that is generated describing sample process and analyses need to be followed. There will be fewer patients for the post-RP than the pre-surgery blood collection based on patient exclusion criteria after RP.

Based on the sample size calculation 200 patients will be recruited for both pre- and post-surgery blood sample collections for CTC analysis, which are expected to be achieved within the first two years of the project.

Patient informed consent (including the permission to conduct remote longer-term follow-up) and blood sample collection will be performed by the CRF or the clinical care team.

All patients will have a PSA test at 3 months after their RP during a routine follow-up at UCLH. A PSA ≥ 0.2 will be recorded as a treatment failure event and PSMA-PET scans will be performed as standard clinical care at the study hospitals to detect small PCa lesions. After 3 months, patients are referred back to their original referring centres. Patients with negative PSA results at three months will be followed up by telephone or online app by the study CRF in order to ascertain whether subsequent PSA tests are positive (PSA ≥ 0.2), indicating BCR, metastasis occurs as detected by cancer imaging including PSMA-PET scan or if they have received salvage treatment with hormones/radiotherapy. In addition, details of long term follow up, such as post-operative PSA results and record of adjuvant therapies, will sourced from patients' hospital electronic medical records, or requested from their local GP or health centre. all patients will be followed-up until the end of the project for metastasis progression as detected by imaging, PCa-specific death and death from any causes. In addition to data collected within the 5-year funded period, we plan conduct remote longer-term follow up with fully informed consent from patients at point of enrolment, including:

- Contacting patients on an annual basis for 10 years so they may report their PSA and relevant further treatments. Methods of contact will be decided upon with PPI and may consist of list of preferred options including phone call by the CRF and/or use of secure electronic means via automated email prompts, secure website or app data entry. Mr. Shaw is currently collaborating with primary care colleagues to develop a patient support app under a separate funding support.
- Flagging and linkage to the patients' relevant electronic health data using their NHS number and date of birth. Under the Confidentiality Advisory Group (CAG), we will request treatment, imaging and cancer outcome data from NHS Digital based on ONS-HES-DID (Data Imaging Data) and NCRAS data sets.

We would thus be able to plan analyses of the diagnostic accuracy and predictive value of the CTC-based tests in terms of metastases and progression-based outcomes at 7.5 years and 10 years of follow-up. Further longer-term follow-up after the 10-year will be performed by accessing the GP and NHS digital records if required and with further ethical approval.

Patient recruitment, sample collection and follow-up procedure were summarised in Table 2

Table 2. Patient recruitment, sample collection and follow-up procedure

Procedure	Screening/Baseline	Pre-RP	3 months post op	12 month post op	24 month post op	… each year…	120 month post op

Eligibility assessment	x						
Informed Consent	x						
Blood Sample collection		x	x				
PSA		x	x	x	x	x	x
Medical record review and online app/phone follow-up (PSA and/or Adjuvant therapies) rates of BCR, metastasis, other progression events and death				x	x	x	x

7. Evaluating the efficiency of CTC positive score and the gene panel in predicting RP treatment failure.

Accuracy of the CTC scores alone and in combination with CTC gene panel expression data in determining RP treatment failure, i.e. BCR and/or metastasis via imaging, in particular PSMA-PET, over the study period will be measured. The prognostic value of CTC data will also be investigated for BCR, PSMA-PET detected cancer lesions, metastasis event, overall survival and cancer specific survival outcomes (see below statistical analysis plan section). At the end of this project, we expect to generate robust data to determine whether CTCs detectable in patients with high-risk localised PCa at pre- and post-RP can predict RP treatment failure and a CTC gene panel will further enhance the prediction value of CTCs for post-RP cancer progression.

Statistical methodology and analysis

The primary analysis is the estimation of the sensitivity of the pre-surgery CTCs for detection of RP treatment failure, i.e. BCR or progression detected by imaging. Observed specificity, positive and negative prediction values will be reported. Prevalence of events and specificity will be presented over time. BCR-free survival will be estimated and Kaplan-Meier curves presented. Preliminary investigation of the prognostic value of pre-surgery CTC data in terms of predicting survival outcomes will be investigated. CTC data will be explored alongside other pre-surgery risk factors using multivariate analysis. Recurrence events and follow up CTC results will be reported and compared over time.

Statistical considerations

1). Definitions:

- **CTC score and result**: CTCs will be identified based on the immunofluorescence staining as $CK^+/VIM^-/CD45^-$, $CK^+/VIM^+/CD45^-$, and $CK^-/VIM^+/CD45^-$ CTCs as described above in lab procedure Part IV. Positive CTC score were defined based on our previous data as any $CK^+/VIM^+/CD45^-$, $CK^+/VIM^+/CD45^-$ and/or >3 $CK^-/VIM^+/CD45^-$ cells identified in the PCa patients.
- **CTC gene-expression result**: a binary (negative/positive) result based on the gene-expression profile from within the blood sample indicating the amount of genes expressed in CTCs from the PCa patients.

- **Combined CTC result**: a binary (negative/positive) result based on both CTC number and gene-expression profile where a positive result indicates a patient should be considered to have high metastatic potential.
- **MRI score**: Multi-parametric MRI Likert scale 1-5 where >3 indicates agressive cancer.
- **BCR**: PSA ≥ 0.2ng/ml at any time post-RP and remaining at this level or further increase afterwards without further treatment (see Table 4 for endpoint definitions)
- **Post-RP treatment failure**: (see Table 4 for endpoint definitions)

2). Sample size

The study hypothesis is that the presence of CTCs has close to 100% sensitivity and specificity for micro-metastasis, and thus RP treatment failure. Due to the long-term nature of recurrence events, there will be a proof of principle part to this study which occurs within the funded study timeframe of 5 years, and an intention to collect and analyse relevant longer-term events at 10 years (see section 4 for objectives and endpoints). The primary endpoint is **post-RP treatment failure**, the majority of which will be due to BCR. Despite the long-term nature of events of interest, it will be possible to provide a timely estimate of sensitivity, since of those who do have an observable BCR event, most if not all are expected to have a positive CTC result. Thus, consistent with previous work, this study estimates predicting post-RP cancer events with 95% sensitivity. During the first 4.5 years of follow up, the hypothesised prevalence of BCR events to be at least 40%.

In order to confirm an expected sensitivity of 95% within 5% precision either side of a 95% confidence interval (CI) in a sample with an underlying prevalence of 40%, requires at least 73 events, that is at least 183 recruited individuals. Inflate this by 8.5% to a target recruitment of 200 to ensure suitability of samples and measurable outcomes.

The recruitment plan will be 200 patients in the first two years of the 5-year project to allow an approximate 3.5 to 4 year median follow up. Specificity is important considering the future circumstance of potentially denying patients curative surgery, if a CTC test indicates metastasis. A study size of 200 and 120 true negatives would give a 97.5% lower confidence limit on a specificity of 99% (1% false positive rate). However, it is anticipated the specificity will be low due to positive results for those who recur beyond the 5-year study. Assuming these events are due to micro-metastases at the time of surgery, observed sensitivity and specificity will be used, plus additional analyses assuming up to 20% of the events occur after the funded 5-year project[4-11] to estimate the real false positive and negative rates.

3). Method of analysis

Study flow and patient characteristics will be reported. Measures of accuracy will be presented with 95% confidence intervals (CIs). The primary analysis is the estimation of the sensitivity of the pre-surgery existence of CTCs for detection of RP treatment failure, i.e. BCR or progression detected by any imaging at the 4.5-year follow-up time point. Observed specificity, positive and negative prediction values will also be reported at 4.5 years of follow up, with a note to indicate that these estimates are subject to change given the long-term nature of recurrence events. Specificity will be presented over time and additional analysis accounting for artificial inflation of false positives based on increasing prevalence of events. BCR-free survival will be estimated and Kaplan-Meier curves presented.

The number of participants with recorded metastases, lymph node metastases, deaths from any cause and PCa deaths during the first 4.5 years of follow up

will be reported alongside the number of these participants with a positive by CTC result, and positive combined CTC result. Metastases-free, PCa-specific and overall survival will be reported during the 5 years study time frame and it is intended to perform a later analysis at 10 years of follow up. Preliminary investigation of the prognostic value of pre-surgery CTC data in terms of predicting survival outcomes will be investigated using Cox regression, with hazard ratios reported with associated 95% CIs. CTC data will be explored alongside other pre-surgery risk factors such as plasma PSA using multivariate analysis. Recurrence events and follow up CTC results will be reported and compared over time. The above analyses will be applied to the CTC gene panel. The gene panel will also be correlated with the pre- and post- RP CTC number changes and cancer progression events.

CTC results will be presented for the whole cohort who provided analysable blood samples and stratified by those included in the above analyses, and those excluded due to reasons such as a positive surgery margin, other evidence of locally remaining disease, and who subsequently have adjuvant ADT or radiotherapy.

8. **Ethics**

   The study is a spin out project of the parent project authorised under RLTB ethics, where the main research tissue bank approval provided by the London City & East Research Ethics Committee.

9. **Data handling, storage and record keeping**- to describe who and how the data will be handled, which data nodes will be used, where it will be stored and security of the data

   The CRF (funded by QMUL, but with contract at UCLH) will be part of the study management group and will act as the contact point between UCLH and QMUL. Under the MTA and data sharing agreement (DSA) between the two institutions and with patient consent, the CRF will collect blood samples during pre-surgery operation and after the surgery during the first PSA follow-up visit at UCLH.

   The following clinical data will be collected for each patient by the CRF:

   - Histology reports
   - Clinical stage
   - Prostate Specific Antigen (PAS) (pre- and post-surgery)
   - Imaging (MRI, PSMA-PET/CT and bone scan)
   - Results and record of adjuvant therapies
   - Treatment date and method for post-surgery cancer recurrence

   The data will be obtained using either electronic medical records at UCLH NHS Trust system or will be requested from patient's local GP or health centres. PCa recurrence, metastasis and the starting of new treatment will also be directly collected from the patients by phone or secure online system/app during the post-operation follow-up period by the CRF. Collection of follow up data from GP and NHS digital records will be with approval from the Confidentiality Advisory Group (CAG)

   Each patient will be assigned a unique ID, and this ID will be used for identifying the patient. All samples will be anonymised using this ID. Completed consent forms will be handed over to the RLTB for secure storage. Patient identifiable data and the above

clinical data will be held securely in a file designated for the study in the RLTB folder in the Barts Cancer Centre server. The file will be maintained and updated by the RLTB data administrator and the CRF. The file will be password protected and only be accessible to authorised staff, inclusive of RLTB manager, study PI, RLTB data administrator and the CRF.

A sample receipt form will be completed by the research team upon receipt of sample to the Barts Cancer Institute. The time of sample receipt to research site, the condition it is received and where they are stored will be recorded separately which will be accessible to research team. Location of sample will be recorded in item tracker for traceability purposes as a separate arm under RLTB storage.

The data generated from this study will include immunofluorescence images of CTCs, RNA expression data of the metastasis associated genes in CTCs and follow-up data of clinical and cancer progression associated to PCa patients. These data will also be securely stored in a designated folder in the BCC IT server with access to PI and his research team. The data will be curated throughout its life-cycle (during the study and before the data is published). Each Hyzip (a CTC multiplex gene expression analysis system at Angle) gene expression value will be recorded in the original data format and saved in the above designated folder in BCC server and will be available for data sharing after the publication.

With relevant safeguards applied for intellectual property and publications have been done of the main findings, anonymised data of the findings will be publicly accessible. These research data of the main outcomes, will be included in an excel spread sheet located in the above-mentioned designated folder in the server. This file, with relevant ethical, legal and institutional policies and regulatory permissions, will be made available through a web link published in the scientific journals and/or the BCI Institute website.

The electronic data will be securely stored in the BCC server for 25 years. If required, the Arkivum long-term data archival service, which is used by the institute will be used. The clinical information asset will be registered in our institute IT system using the Data Asset Register Toolkit (DART). Paper records will be archived using the QMUL archive system and stored for 25 years.

Data obtained for each patient and generated through the research project will be handled in accordance to the Data Protection Act 2018 UK and in reference to the General Data Protection Regulation (GDPR). All staff that will be involved with the project that handle data will have relevant training prior to handling and managing the data.

10. Safety procedures if there's any adverse events or deviations from the process

Obtaining blood samples for this study will include the normal associated risks with venepuncture, such as temporary discomfort and bruising. Blood samples for pre-operative preparation and pre-and post-operative PSA monitoring is part of the NHS standard of care for PCa patients, so obtaining samples for this study will not involve any additional risk or burden for patients.

The study findings will not affect patient care in any way. All treatment management decisions for patients will be made by the clinical team unrelated to the study.

11. **Monitoring and auditing**- to briefly describe how any changes will be monitored and recorded and if any auditing will be taking place

    The Sponsor, funding body and/or regulatory bodies may audit the study, study site or central facility.

    The PI will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting, and source data verification to ensure adequate data quality.
    The PI will inform the Sponsor should they have concerns which have arisen from monitoring activities, and/or if there are problems with any oversight or monitoring procedures.

    RLTB will be auditing consent forms on receipt and study auditing will be carried out as per local RLTB policy with prior agreement with the study team.

12. **Study management and oversight for the spin off project and also feedback to RLTB committee**

    The study's management group (SMG), consisting of the CI, PIs/Co-PIs, key collaborators, the CRF and statistician will convene on a monthly basis. The CRF and study manager will update the RLTB steering committee annually of the project progression and any feedback received will be communicated back to the SMG.

    The Study Steering Committee (SSC), which includes a patient member, a consultant urologist (chair), a biomarker study specialist/statistician and a basic research scientist independent from the study will be formed to monitor recruitment and study progress in terms of outcome data collection and statistical assumptions underpinning the study, including confidential review of the event rates.

13. **Finance-who is funding the spinoff study and what is funded**

    The spin-off study is funded by Prostate Cancer UK (PCUK) initially for 5 years and will be renewed with separate application at end of the 1st 5-year period with review from the funding body. The first 5-year PCUK funding will include salary for the CRF (70% FTE to work on this project), postdoctoral research assistant, 55% salary for study manager, 20% salary for data administrator, 40% salary for a statistician (last 3 of the 5 years) as well as consumables. Parsortix CTC isolation systems and Hyzip multiplex CTC gene expression system, are covered by Angle.

14. **Any insurance and indemnity for participants**

    The insurance that Queen Mary University of London has in place provides cover for the design and management of the study as well as "No Fault Compensation" for participants, which provides an indemnity to participants for negligent and non-negligent harm.

15. **Dissemination of research findings**

To ensure the results and impact of our research are widely communicated to their intended beneficiaries, we will disseminate them via:
a) High impact peer-reviewed publications with open access;
b) Scientific conferences and seminars;
c) Communication with the general public (fundraisers and schools);
d) Press releases (our previous researches were successfully communicated in TV/newspapers/online media);
e) Summaries of study progress and clinical relevance as feedback to participating patients;
f) Interaction with the relevant healthcare authorities, such as MHRA, to translate the research findings into clinical use.