Trial Title: Investigation of novel molecular imaging techniques for precision surgery and genomic characterisation of high-risk prostate cancer using EMI-137

Short title: Prostate **MO**lecular **T**argeting to **E**nhance surgery using EMI-137 (ProMOTE-EMI-137)

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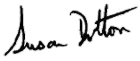
Statistician Signature: 

Conflict of Interest Statement

No potential conflicts of interest

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation, and members of the Research Ethics Committee, unless authorised to do so.

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KEY TRIAL CONTACTS

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Committees	A Trial Steering Committee (TSC) and an independent Data and Safety Monitoring Committee (DSMC) will be set up. The details will be written in the relevant charters.

SYNOPSIS

Trial Title	Investigation of novel molecular imaging techniques for precision surgery and genomic characterisation of high-risk prostate cancer using EMI-137
Internal ref. no. (or short title)	**Pro**state **MO**lecular **T**argeting to **E**nhance surgery using EMI-137 (ProMOTE-EMI-137)
Trial duration	12 months (Stage 1) 90 months (Stage 2; 30 months recruitment plus 60 months follow up)
Clinical Phase	Phase I (Stage 1) Phase II (Stage 2)
Trial methodology	Open label, single centre, dose optimisation (Stage 1) Open label, single centre, randomised controlled (Stage 2)
Trial Design	Evaluation study of molecular imaging techniques in high-risk prostate cancer (PC) patients using deep red/near-infrared (NIR) fluorescence imaging with a fluorescent imaging molecule during robot-assisted laparoscopic radical prostatectomy (RARP) to define extra-capsular and nodal involvement and optimise the extent of surgical resection This will happen in two stages: 1. **Stage 1**: Optimisation of dose administration, pre-operative interval and equipment in up to 20 patients with high-risk disease, using up to four combinations of two EMI-137 doses and two time intervals between administration of the compound and the surgery. 2. **Stage 2**: Prospective validation with optimized dose and time interval to evaluate the benefit of peri-operative molecularly-targeted imaging by comparing two matched groups of patients with high-risk PC both receiving conventional robot-assisted laparoscopic radical prostatectomy (RARP), one with molecular imaging and one without (control group). In order to remove bias in patient selection we will use randomisation to allocate patients to these two groups.
Trial Participants	We will recruit patients with histologically proven high-risk non-metastatic localized or locally advanced (cT3) PC with any of the following risk criteria: ▪ Risk 1: Serum PSA 10-20ng/ml and Gleason 4+3 or greater ▪ Risk 2: Serum PSA ≥20 ng/ml ▪ Risk 3: Grade group 4 or 5 ▪ Risk 4: Clinical T3
Planned Sample Size	Stage 1: Up to 20 patients Stage 2: 100 patients, randomised equally between the IMP and the control arm
Treatment duration	Single infusion before surgery
Follow up duration	For Stage 1: approximately six weeks

	For Stage 2: approximately six weeks for primary outcome, five years for secondary outcomes Note that for both stages routine clinical follow-up will be according to local practice	
Planned recruitment period	Stage 1: approximately 12 months Stage 2: approximately 30 months	
Study Sponsor	University of Oxford	
EudraCT number	2017-003026-32	
Investigational medicinal product (IMP)	Cy-5 labelled EMI-137 peptide targeted against c-Met receptor (EMI-137)	
Formulation, Dose, Route of Administration	24mg EMI-137 in 5ml reconstituted solution will be infused intravenously. Up to twenty patients with high-risk disease will be evaluated. The following dose and intervals will be used in up to five patients for each combination): 1. 130μg/kg injected intravenously 2h before surgery 2. 130μg/kg injected intravenously 4h before surgery If signal saturation is acceptable at 130μg/kg 2h dose and time, the dose will be considered optimized. If not the same dose will be evaluated with a 4h time interval. If the dose /time is optimized as part of test combination 1 or 2, test combinations 3 and 4 will not be tested. However, If equipment is oversaturated at 130μg/kg, the dose will be reduced by 50-75% (to between 32.5μg/kg and 65μg/kg), and the below test combinations will be evaluated: 3. 32.5 - 65μg/kg injected intravenously 2h before surgery 4. 32.5 - 65μg/kg injected intravenously 4h before surgery The decision to change the dose/time combination will be driven by data from the most recent patients tested. The decision to progress to Stage 2, and the dose and the timing of EMI-137 administration during Stage 2 will be based on the results obtained in Stage 1.	
	Objectives	**Outcome Measures**
Stage 1 **Primary Objective:** **Secondary Objective:**	Determine the optimal dose and timing of IMP administration Safety of a single dose of IMP administration	Visibility of lesions at surgery including auto-fluorescence Adverse Events assessment
Stage 2 **Primary Objective:** **Secondary Objectives:**	Improvement in positive surgical margin rates 1. Biochemical relapse	Histopathological assessment 1. Failure of PSA to drop to <0.1ng/ml with residual disease

	2. Time to administration of salvage therapy such as external beam irradiation and/or androgen suppression 3. Longer-term disease-specific and overall mortality 4. Safety of a single dose of IMP administration	2. Recorded time between intervention and the administration of salvage therapy during follow up routine NHS care in the clinic 3. Calculated disease-specific and overall mortality 4. Adverse Events assessment
Inclusion criteria	Men with histologically proven high-risk non-metastatic localized or locally advanced (cT3) PC: ▪ Risk 1: Serum PSA 10-20ng/ml and Gleason 4+3 or greater ▪ Risk 2: Serum PSA ≥20 ng/ml ▪ Risk 3: Grade group 4 or 5 ▪ Risk 4: Clinical T3 • Eligible for robot-assisted laparoscopic radical prostatectomy by local standard of care • An understanding of the English language sufficient to understand written and verbal information about the trial and its consent process • Participant is willing and able to give informed consent for participation in the study. • Aged 18 years or above.	
Exclusion criteria	The patient may not enter the study if ANY of the following apply: • Unfit for radical surgery as assessed by Consultant Anaesthetist • History of any cancer, except non-melanoma skin cancer • Men who have had androgen suppression/hormone treatment within the previous 12 months for their PC • Men who have had previous High Intensity Focussed Ultrasound (HIFU), cryosurgery, thermal or microwave therapy to the prostate. • Men who have undergone a Transurethral Resection of the Prostate (TURP) for symptomatic lower urinary tract symptoms within six months. These patients may be included within the trial if deferred from consenting and screening until at least six months following the TURP. • Presence of metal implants/stents in the urethra • Men with renal impairment with a Glomerular Filtration Rate (GFR) of <60ml/min (unable to tolerate Gadolinium dynamic contrast enhanced Magnetic Resonance Imaging (MRI)) • Previous adverse reaction to the IMP compounds or fluorescent agents • Abnormal liver function tests observed at screening • Abnormal ECG observed at screening	

	• Unable to provide informed consent to participate in the trial as judged by the attending clinician

ABBREVIATIONS

AE	Adverse event
AR	Adverse reaction
CI	Chief Investigator
CRC	Colorectal Cancer
CRF	Case Report Form
CT	Computerised Tomography
CTIMP	Clinical trial of an Investigational Medicinal Product
CTU	Clinical Trials unit
CTRG	Clinical Trials and Research Governance
DSMC	Data and Safety Monitoring Committee
DMP	Data Management Plan
DSUR	Development Safety Update Report
ePLND	extended Pelvic Lymph Node Dissection
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GMP	Good Manufacturing Practice
HIFU	High Intensity Focussed Ultrasound
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICG	IndoCyanine Green
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LED	Light-Emitting Diode
MHRA	Medicines and Healthcare products Regulatory Agency
mpMRI	Multi-Parametric Magnetic Resonance Imaging
MRI	Magnetic Resonance Imaging

NHS	National Health Service
NIR	Near Infra-Red
NVB	Neurovascular Bundle
OCTRU	Oxford Clinical Trials Research Unit
OUH	Oxford University Hospitals
PC	Prostate Cancer
PET	Positron Emission Tomography
PI	Principal Investigator
PIL	Patient Information Leaflet
PPI	Patient and Public Involvement
PSA	Prostate Specific Antigen
PSMA	Prostate specific membrane antigen
QP	Qualified Person
R&D	Research & Development (NHS Trust Department)
RARP	Robot-assisted Laparoscopic Radical Prostatectomy
REC	Research Ethics Committee
RP	Radical Prostatectomy
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SITU	Surgical Intervention Trials Unit
SDV	Source Data Verification
SmPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
TURP	Transurethral Resection of the Prostate
UKCRC	United Kingdom Clinical Research Collaboration

1. BACKGROUND AND RATIONALE

Prostate cancer (PC) is the second most common cause of male cancer deaths in the UK (13%) with 11,287 men who died from the disease in 2014[1]. Whilst randomised screening trials show 21-44% reduction in PC mortality by 11-14 years, many patients diagnosed through Prostate-Specific Antigen (PSA) testing will not suffer clinical consequences during their life time[2, 3]. A substantial proportion harbour lethal disease, requiring careful evaluation and radical treatment. Current management aims to identify high-risk patients amenable to curative treatment including radical prostatectomy (RP). The outcome of surgery for locally advanced PC can be improved by accurate staging with appropriate resection. Imaging is used routinely in the form of MRI, but sensitivity and specificity are limited in defining the presence of pT3 disease, with under-staging as well as over-staging being a significant issue leading to inappropriate surgical resection and treatment planning[4, 5]. Over-staging leads to unnecessary excision of the neurovascular bundles (NVB) with adverse consequences on functional outcomes, particularly related to erectile dysfunction. Recognising these high-risk patients is therefore a critically important unmet clinical need.

During surgery it is often difficult to distinguish tumours confined to the prostate from those extending through the capsule (pT3). We have recently shown that approximately 30% of men diagnosed with clinically localised PC receiving RP demonstrate disease outside the gland, and of those up to 45% have positive surgical margins, with the risk of treatment failure and poor outcomes[6]. In the Scandinavian SPCG-4 study, men with extra-capsular tumour had a risk of death from PC seven-fold higher than patients with organ-confined disease[7]. Further studies demonstrated that the prognosis is worse when surgical margins are positive[8]. Attempts were made to improve patient outcomes with neo-adjuvant androgen suppression prior to surgery, but this had no long-term benefits, and the approach is no longer used[9-12].

There is accumulating evidence that performing RP in high-risk clinical and operable locally advanced disease (cT3) improves outcomes compared with other treatments, but more than half these patients relapse over time, requiring salvage therapies such as external beam irradiation and androgen suppression[13-15]. These failures reflect a number of limitations in management of the disease, including under-staging and the presence of occult lymph-node involvement. In addition, poor understanding of the genetic diversity of the disease limits our ability to stratify patients into risk categories to guide effective therapy[16, 17]. Recent work exploring the genomic landscape in prostate cancer showed extensive genetic heterogeneity that may underlie the ubiquitous behaviour of the disease. The complete genomes of 75 prostate cancers have been reported, together with hundreds of exomes[18-23]. Those studies show that the spectrum of lesions in PC is diverse, and that alterations in specific pathways are recurrent. The most common abnormalities include fusions involving members of the *E-Twenty Six (ETS)* transcription family, loss of the *Phosphatase and Tensin Homolog (PTEN)* tumour suppressor gene, amplification of the androgen receptor gene and amplification of the *MYC* oncogene[24]. Linking these changes to clinically significant outcomes is particularly challenging in PC, since it has a long natural history, necessitating long follow-up on large cohorts. Furthermore, tumour multifocality complicates the discrimination and understanding of initiating versus more advanced lesions. This hinders risk stratification and appropriate management. Small-size studies have identified rare variants and PC specific pathways[20, 25]. However, our understanding of the heterogeneity of the tumour and more distant lesions and its biological relationship with tumour invasiveness and lymph nodes or distant metastasis remains limited.

In the absence of data from randomised controlled studies, recent work used a large cohort of European patients to construct a pre-treatment model to predict pathological stage and lymph-node involvement in high-risk patients, demonstrating that up to 82% are at risk of lymph-node involvement, and over a third have seminal vesicle invasion[16]. Therefore, extended pelvic lymphadenectomy has been advocated for high-risk disease, but the procedure is time-consuming, requires advanced surgical skills, can cause substantial morbidity, and often represents over-treatment[26], particularly as the disease is frequently

over-staged and heterogeneous[27]. There is also a lack of consensus on the definition of high-risk PC. In general this includes unfavourable prognostic parameters including serum PSA levels above 10ng/ml, Gleason score (at least dominant single grade 4), high volume and clinically locally advanced disease[28].

Several attempts were made to visualize prostate margins and pelvic lymph-nodes per-operatively using a variety of techniques, including Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), which are used frequently but have substantial staging limitations. Other techniques such as Positron Emission Tomography (PET), magnetic nanoparticles and fluorescence are under investigation, but none have been adopted for regular use during surgery[29-31]. One of the limitations of fluorescence and PET is their lack of tissue specificity. A recent publication highlights this limitation using Indocyanine Green (ICG)[32, 33]. Another significant drawback is that fluorescence is usually imaged separately from white-light definition of the anatomy to determine the area of interest. This can be imprecise and cumbersome.

We developed cutting-edge real-time optical imaging based on multiple, solid-state sources and a single specialised high sensitivity imager (described in Section 6). The system is uniquely sensitive, providing fluorescence images in near real-time, simultaneously with conventional white light reflectance, by selectively preventing the fluorescence excitation light from reaching the imager. This complements the recent development of a fluorescently labelled compound that can specifically bind to the surface of PC cells. This places us in a unique position to test these imaging techniques in patients with high-risk PC, to determine their value in improving staging to guide 'precision surgery'.

1.1. **IMP: EMI-137**

EMI-137 is a water-soluble 26-amino acid cyclic peptide labelled with a fluorescent cyanine dye which emits deep red/NIR light when excited with red light (peak absorption at approximately 660 nm). It has a low-nanomolar dissociation constant (~2 nM), i.e., high affinity towards human c-Met.

c-Met is a receptor tyrosine kinase that is activated upon binding to its natural ligand hepatocyte growth factor. This binding activates downstream signalling pathways that are important for organ development and cancer progression. Although c-Met plays role in normal physiology, it has been implicated in the development of multiple types of cancer. Overexpression and hyperactivation of c-Met has been reported in various cancer types [36-43].

c-Met is expressed in normal prostate tissue. However several studies have shown that the level of c-Met on the surface of prostate cancer cells is higher than that on normal prostate cells. It has been reported that up to 78.6% of prostate tumours express c-Met on their surface [44-47]. Thus c-Met represents an attractive target to visualize prostate cancer cells in vivo. Because EMI-137 binds to c-Met with high affinity, it represents a promising compound to be administered to patients undergoing radical prostatectomy with the aim of visualizing cancerous cells during surgery.

1.1.1 In vitro validation of EMI-137 use for the detection of prostate cancer cells

c-Met expression was measured in four prostate cancer cell lines PC3, PC3-EGFP, ARCaPE and ARCaPM using western blot, with β-actin as loading control. This showed that prostate cancer cells express readily-detectable levels of c-Met (Figure 1A). mRNA expression of c-Met (MET) in prostate cancer cell lines was mined from the MSKCC prostate cancer expression dataset through cBioPortal (Figure 1B) [48].

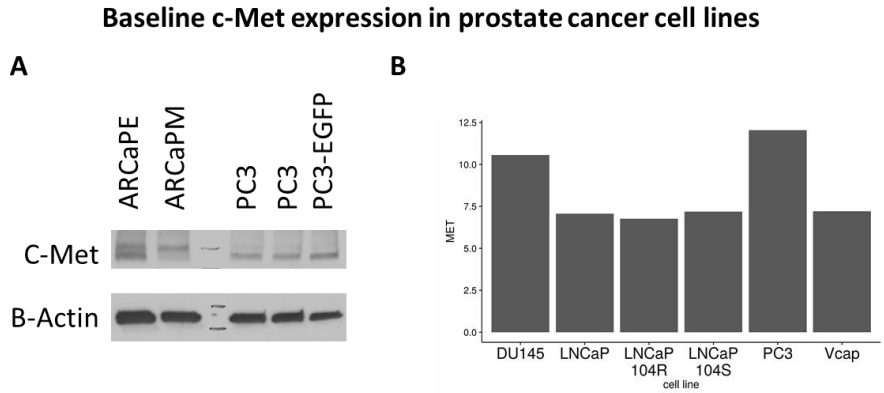

Figure 1: Protein (A) and mRNA (B) expression of c-Met in prostate cancer cell lines

1.1.2 Pre-clinical (mouse) model - validation of EMI-137 use for the detection of human prostate cancer cells

We showed that EMI-137 accumulates specifically in the c-Met positive prostate tumour xenografts in mice. PC3 prostate tumour cells were inoculated into nude mice to grow subcutaneous tumours. Two to three weeks later mice were injected with EMI-137 or the unconjugated Cy5* dye at varying concentrations (1.6µg, 3.2µg, 4.8µg per 20g), and were imaged three hours post-dye administration. Figure 2 shows accumulation of EMI-137 specifically within tumour xenografts, while there was effective clearance of dye from the surrounding tissues. The signal to background ratio was significantly higher for EMI-137 compound, compared to Cy5*, demonstrating c-Met-specific binding in the tumour. This confirms that the tumours can be selectively visualized using EMI-137.

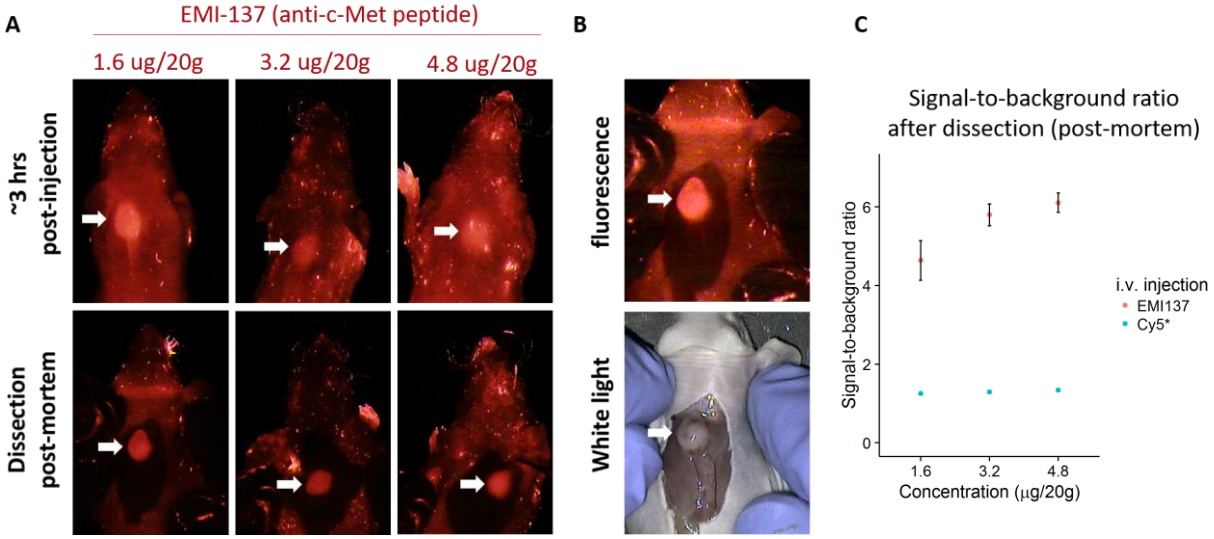

Figure 2: EMI-137 accumulates specifically in prostate tumour xenografts.

(A): Images obtained three hours post-injection, and post-mortem. White arrows show tumours. (B): Tumours visualised in fluorescent and white light. (C): Fluorescence signal to background ratio post-mortem. Data shown are mean fluorescence values from three mice (EMI-137) with error bars showing standard error of mean. Data shown for Cy5*-injected mice are from one animal each.

1.1.3 Pre-clinical safety data.

The available preclinical data indicate sufficient safety margins to support the safety and tolerability of EMI-137 Injection in the clinic up to a maximum dose of 0.36 mg/kg bw (see Investigator Brochure). No acute or delayed toxic effects of EMI-137 Injection have been observed in male and female rats up to

four weeks after a single injection of up to 50 times this maximum clinical dose scaled for body surface area. No toxic effects were detected in the Cynomolgus monkey up to four weeks after 14 days of repeat dosing with a daily dose of up to 15 times this maximum clinical dose scaled for body surface area. In the conscious Cynomolgus monkey, no biologically relevant effects were detected on the cardiovascular system (blood pressure, heart rate, and electrocardiogram) for up to 24 hours after doses up to 43 times the maximum clinical dose scaled for body surface area. Therefore, no pharmacological effects are expected following administration of EMI-137 Injection to human subjects. Based on consistent observations in the pre-clinical efficacy and safety pharmacology studies, it is expected that patients receiving EMI-137 Injection may experience discoloured urine for 24 hours or more after injection.

1.1.4 Clinical experience with EMI-137

EMI-137 is manufactured by Edinburgh Molecular Imaging Ltd (EMI). At present, it does not have a Marketing Authorization in any country. It has been previously investigated as part of a phase I trial involving 20 healthy volunteers and 15 subjects with high suspicion of colorectal cancer (more details provided in the Investigator's Brochure (IB) and the Investigational Medicinal Product Dossier (IMPD) for EMI-137).

Prior to being released into the ProMOTE—EMI-137 trial, EMI-137 IMP will be fully analysed and Qualified Person (QP) released by EMI in accordance with Directive 2001/20/EC, Good Manufacturing Practice (GMP), Good Distribution Practice and all applicable laws and regulations.

1.2. THE OXFORD NEAR-INFRARED IMAGER: AN INNOVATIVE FLUORESCENCE OPTICAL SYSTEM

Prof. Borivoj Vojnovic and his team have developed, through the CR-UK/EPSRC Oxford Cancer Imaging Centre, a novel NIR fluorescence imaging approach. This approach uses a single high-sensitivity optical imager to capture inherently spatially registered images during both visible reflectance and red/NIR fluorescence imaging, visible reflectance imaging being used for essential guidance purposes.

Although NIR imaging is available on the da Vinci Si®, the Firefly system cannot operate at the excitation/emission wavelengths associated with Cy5*, nor does not have the required sensitivity or simultaneous colour guidance imaging [51, 52]. Our unique, lower-cost technology is based around:

1. a semiconductor illumination source providing >>10 mW/cm^2 excitation power-density on 15-25 cm^2 fields; and

2. a white light-emitting solid-state source to provide guiding illumination. These are combined in a customised optical arrangement coupled to the laparoscope light-guide (Figure 3).

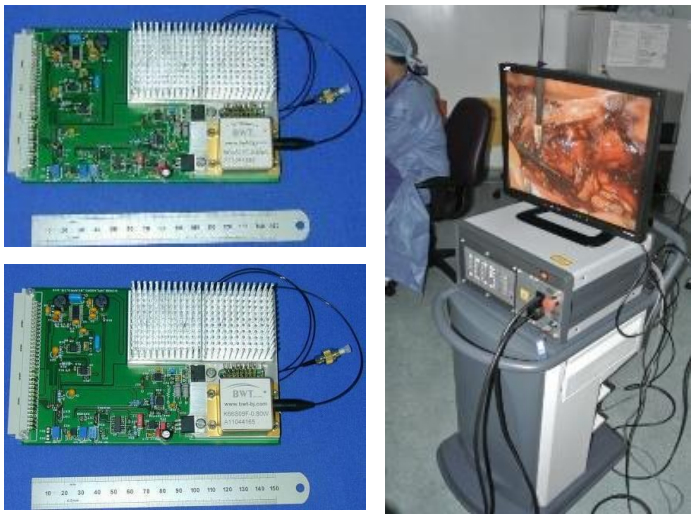

Figure 3: The Oxford Near Infrared Imager.

Top left: one of the in-house developed pigtailed fibre NIR sources used in the system electronics shown on the right. Bottom left: NIR-visible imaging head attached to a 10 mm rigid Hopkins-lens type laparoscope. Right: The Oxford NIR Imager deployed in an operating theatre at the Oxford University Hospital (OUH), Oxford.

The uniqueness of this technique is provided through:

1. the use of one or more narrow-band, very deep rejection filters (>3 x 10^6 attenuation) to eliminate excitation light, leaving both fluorescence emission and visible light components for capture by the imager
2. automated white-light intensity modulation to provide appropriate display dynamic range, as camera sensitivity and/or integration times are varied during fluorescence imaging
3. the use of broadband imaging optics to eliminate focus shift across the full 400-900 nm spectral range, and
4. absence of any mechanical or electromechanical components which could reduce system reliability. This technology can be used at multiple wavelengths if required.

The technology has been validated in the clinic during sentinel lymph-node dissection in gynaecological cancers, using ICG (a non-specific dye) to delineate the lymphatic system [53]. Our Oxford NIR Imager is currently used at OUH, Oxford during both open and keyhole (laparoscopic) surgical procedures (Figures 3 and 4).

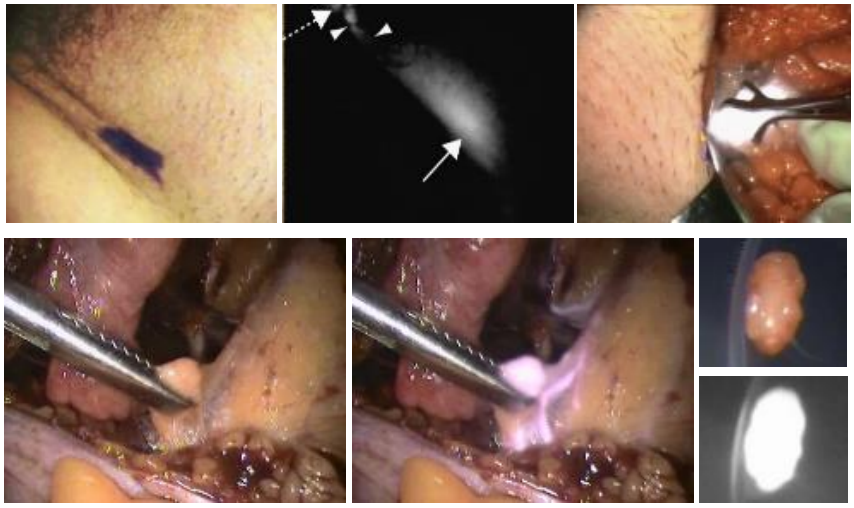

Figure 4: Use of Oxford NIR Imager for open and laparoscopic surgical procedures.

Top panels: visible light (left), percutaneous fluorescence (middle) and combined white light and fluorescence imaging (right) of a lymph node during vulval cancer surgery; Bottom panels: white light (left) and simultaneous white light and fluorescence (middle) imaging during an endometrial cancer laparoscopic surgery. The lower right images show an ex-vivo imaged node imaged under white light and fluorescence illumination (Imaging was performed in collaboration with A. Ahmed, P Pathyraja (Nuffield Department of Obstetrics and Gynaecology, OUH, Oxford) and D. Volpi (Department of Oncology).

2. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure
Stage 1: Primary Objective Determine the optimal dose and timing of IMP administration	Visibility of lesions at surgery including auto-fluorescence	During surgery and after histopathological and genetic assessment of any selective fluorescent tissue biopsies collected
Stage 1: Secondary Objective Safety of a single dose of IMP administration	Adverse Events assessment	Patients will be monitored for AEs starting from consent and until discharge from hospital. All AEs will be recorded until patient's 6 week appointment
Stage 2:Primary Objective Improvement in positive surgical margin rates	Histopathological assessment	After histopathological and genetic assessment of any selective fluorescent tissue biopsies collected.
Stage 2: Secondary Objective		
1. Biochemical relapse	1. Failure of PSA to drop to <0.1ng/ml with residual disease	1. PSA blood test at approximately six weeks after surgery (part of routine care, only access to the test result required)
2. Time to administration of salvage therapy such as external beam irradiation and/or androgen suppression	2. Recorded time between intervention and the administration of salvage therapy during follow up routine NHS care in the clinic	2. Documentation of the time of salvage therapy
3. Longer-term disease-specific and overall mortality	3. Calculated disease-specific and overall mortality	3. Documentation of prostate cancer-specific deaths and all deaths
4. Safety of a single dose of IMP administration	4. Adverse Events assessment	4. Patients will be monitored for AEs starting from consent and until discharge from hospital. All AEs will be recorded until patient's 6 week appointment

3. TRIAL DESIGN

The trial is open labelled and will consist of two stages. Stage 1 will be a non-randomised dose optimisation study and will recruit up to 20 patients in order to optimise the dose and timing of IMP administration. Stage 2 will recruit 100 patients who will be randomised equally to either standard RARP, or to fluorescence image-guided RARP using the optical agent, according to the optimal dose and timing of IMP administration established in Stage 1.

The trial will be conducted by the Surgical Intervention Trials Unit (SITU) at the UKCRC-registered Oxford Clinical Trials Research Unit (OCTRU). HRA, ethical and regulatory approvals will be in place prior to patient enrolment. The protocol was fully discussed, developed and agreed with the trials unit team prior to this submission.

3.1. Justification for the proposed doses

The 130μg/kg dose has been safely used previously in colorectal cancer patients in a Phase 1/2a clinical study (GE-137-001).

3.2. Stage 1: Optimisation of dose administration, pre-operative interval and equipment

3.2.1. IMP - EMI-137

Up to twenty patients with high-risk disease will be evaluated. The following dose and intervals will be used in up to five patients for each combination):

1. 130μg/kg injected intravenously 2h before surgery
2. 130μg/kg injected intravenously 4h before surgery

If signal saturation is acceptable at 130μg/kg 2h dose and time, the dose will be considered optimized. If not the same dose will be evaluated with a 4h time interval. If the dose /time is optimized as part of test combination 1 or 2, test combinations 3 and 4 will not be tested. However, If equipment is oversaturated at 130μg/kg, the dose will be reduced by 50-75% (to between 32.5μg/kg and 65μg/kg), and the below test combinations will be evaluated:

3. 32.5 - 65μg/kg injected intravenously 2h before surgery
4. 32.5 - 65μg/kg injected intravenously 4h before surgery

We will adapt the dose and time interval between injection and surgery on a patient-by-patient basis according to the following observations:

- Level of fluorescence observed
- Saturation outside the prostate
- Histopathological assessment of resected tissue from all available previous patients
- Safety concerns raised following drug administration in all previous participants regardless of dose.

If there is oversaturation, and/or safety concerns the investigators will either reduce the IMP dose, increase the time interval between IMP administration and surgery, or both. If there is no visible fluorescence and no safety concerns, the investigators will increase the dose. If neither of these occur the dose will be repeated in up to 5 participants. Optical equipment will be adjusted as necessary to improve the images. If there is visible fluorescence, no oversaturation, and no safety concerns using a specific dose and timing combination, and the TMG are satisfied that the dose and timing have been

optimised, no further combinations will be tested and the study will progress to stage 2. Figure 5 shows the details of the approach.

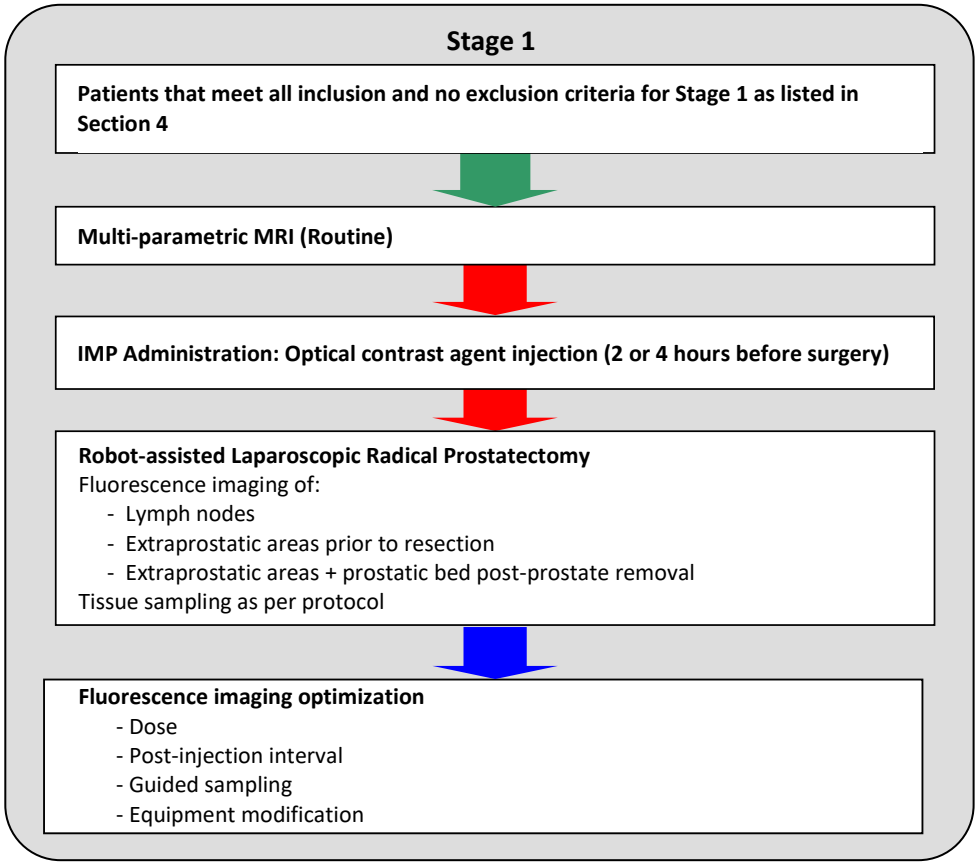

Figure 5: Details of Stage 1 of the trial: dose and timing optimization stage (n=≤20 patients).

3.3. Decision to proceed to Stage 2

If no fluorescence is visible or histopathological assessment of biopsies demonstrates that cancerous tissue was not highlighted and/or healthy tissue is highlighted by the compound using any dose and timing combination during stage 1, the study will not progress to stage 2.

3.3.1. Stage 2: Validation

Following fluorescence imaging optimization, prospective validation with optimized doses and intervals will be conducted and compared with standard RARP. We will evaluate the benefit of peri-operative molecularly-targeted imaging by comparing two matched groups of patients with high-risk PC both receiving standard RARP, one with molecular imaging (intervention group) and one without (control group).

In order to remove bias in patient selection, we will use randomisation to allocate patients to these two groups (Figure 6). Randomisation will be stratified by age, PSA level and Gleason scoring. The same recruitment criteria will be used for high-risk PC as used in the Stage 1. One hundred men will be randomly allocated to receive either 'molecular imaging' with fluorescence-image-guided RARP, or standard RARP, following informed consent. The procedure for image-guided RARP will be finalised based on the results obtained during Stage 1.

The primary and secondary end points are summarised in Section 2.

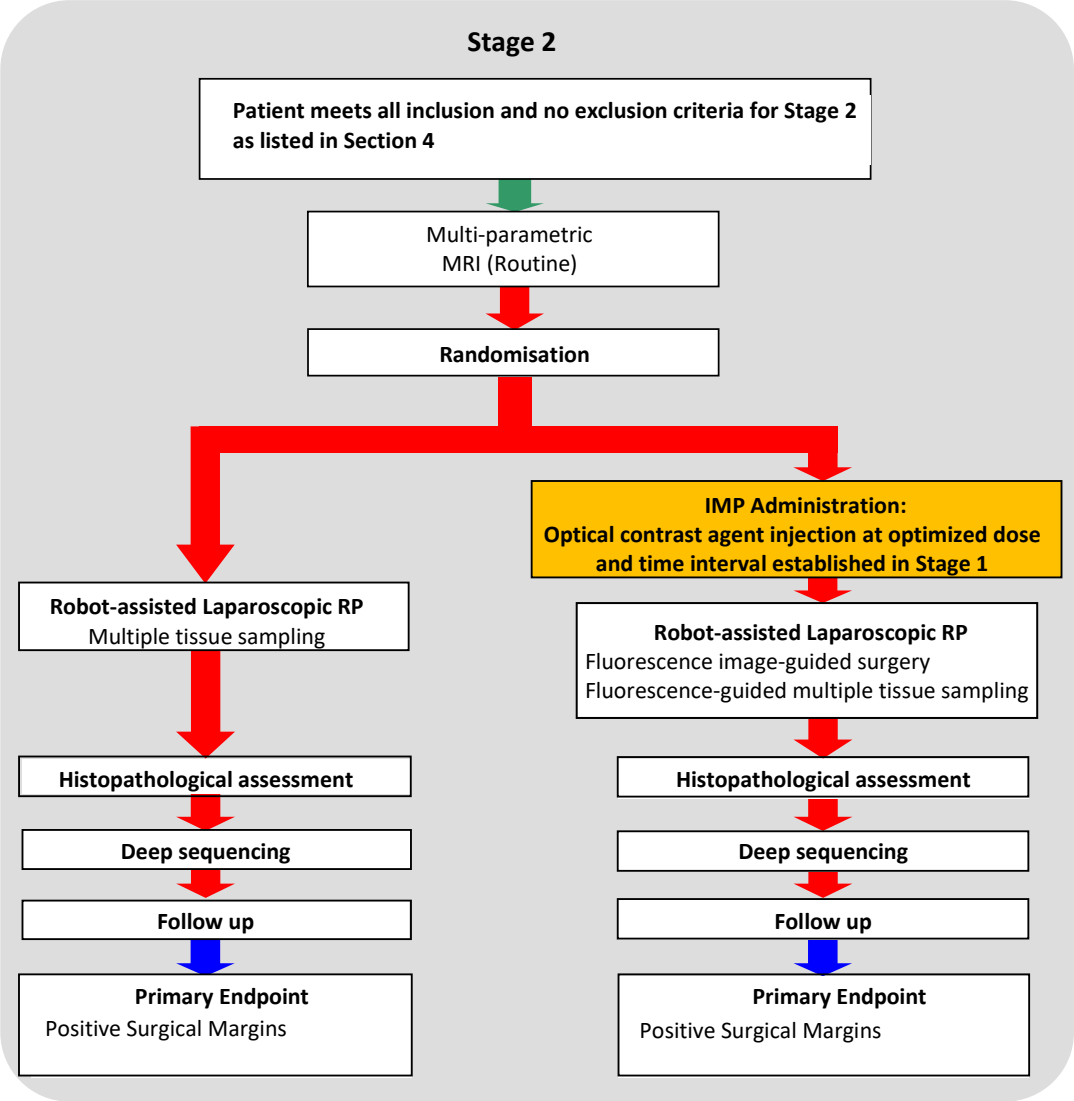

Figure 6: Flowchart of Stage 2, randomised study.

One hundred patients will be randomised to either standard RARP, or to fluorescence image-guided RARP using the optical agent.

4. PARTICIPANT IDENTIFICATION

4.1. Trial Participants

Patients will be diagnosed and recruited through Urology clinics at Oxford.

4.2. Inclusion Criteria

Men with histologically proven high-risk non-metastatic localized or locally advanced (cT3) PC with any of the following risk criteria:

Risk 1: Serum PSA 10-20ng/ml and Gleason 4+3 or greater
Risk 2: Serum PSA ≥20 ng/ml
Risk 3: Grade group 4 or 5

Risk 4: Clinical T3

- Eligible for prostatectomy by local standard of care
- An understanding of the English language sufficient to understand written and verbal information about the trial and its consent process
- Participant is willing and able to give informed consent for participation in the study
- Aged 18 years or above

4.3. **Exclusion Criteria**

The participant may not enter the study if ANY of the following apply:

- Unfit for radical surgery as assessed by Consultant Anaesthetist
- History of any cancer, except non-melanoma skin cancer
- Men who have had androgen suppression/hormone treatment within the previous 12 months for their prostate cancer
- Men who have had previous HIFU, cryosurgery, thermal or microwave therapy to the prostate.
- Men who have undergone a Transurethral Resection of the Prostate (TURP) for symptomatic lower urinary tract symptoms within six months. These patients may be included within the trial if deferred from consenting and screening until at least six months following the TURP.
- Presence of metal implants/stents in the urethra
- Men with renal impairment with a GFR of <60ml/min (unable to tolerate Gadolinium dynamic contrast enhanced MRI)
- Previous adverse reaction to the IMP compounds or fluorescent agents
- Abnormal liver function tests observed at screening
- Abnormal ECG observed at screening
- Unable to provide informed consent to participate in the trial as judged by the attending clinician

5. TRIAL PROCEDURES

The schedule of the study procedures is shown in Appendix A.

5.1. **Screening and Eligibility Assessment**

Patients will undergo routine NHS assessment as part of standard of care (serum PSA levels, assessment of biopsy results, Gleason staging and multi-parametric MRI results including presence of local t-stage disease and notes) and the patient's clinical care team will access the results to establish eligibility. Consented patients will undergo additional assessments: physical examination, vital signs, ECG, liver function test and safety laboratory tests (Hemogram and biochemistry). They will also be asked to provide a blood sample for a fresh PSA test.

5.2. **Recruitment**

In Stages 1 and 2, patients will be identified as potential participants for the trial through weekly MDT Uro-oncological clinics at Oxford.

Patients who select surgery as their preferred treatment option as part of routine care will receive full information about the ProMOTE trial by a member of the clinical team or consultant urologist, once they have agreed to be approached by a member of the research team they will be invited to participate by a

research nurse from the Surgical Research Team. They will be given the Patient Information Leaflet (PIL) which will detail no less than: the exact nature of the study, what it will involve for the patient; the implications and constraints of the protocol and the known side effects and any risks involved in taking part. They will have the opportunity to thoroughly discuss the trial with the consultant urologist and the dedicated study nurse.

5.3. Informed Consent

Informed consent will be obtained by the investigators or by the Research Nurse who will countersign and date the consent form. Patients who agree to be recruited will personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Prospective participants will be allowed as much time as wished to consider the information provided and, if necessary, to consult with their general practitioner (GP) or other independent parties so as to make an informed decision about their participation in the study. Another meeting with the investigator will be scheduled if the patient needs more time to decide.

Written informed consent is considered given once the latest approved version of the ICF has been signed and dated by the patient and by the Research Nurse who presented and obtained the written informed consent. The person who obtains the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator (CI). Before the ICF is signed by both parties, the Research Nurse is obliged to explain clearly that the patient is free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.
A copy of the signed ICF will be given to the participant. The original signed ICF will be retained in the Investigator Site File (ISF) and a copy kept in the patient's medical notes.

5.4. Randomisation, blinding and code-breaking

This is an open labelled trial, so no blinding or code-breaking will be performed.

In Stage 1, all patients will receive the intervention, so no randomisation will be performed at this stage. In Stage 2, eligible patients will be randomised using the web based secure randomisation system provided by the Oxford Clinical Trials Research Unit (OCTRU). The web-based system will have a back-up telephone service in place. Randomisation will be stratified by age, PSA level, and Gleason Score.

5.5. Baseline Visit

In Stage 1 and Stage 2, pre-operative clinico-pathological data will be entered in the study electronic database including clinical and pathological stage, Gleason grade, serum PSA, pre-biopsy imaging results (mpMRI, PET-CT) and biopsy results (all routine NHS assessments).

5.6. IMP Administration Visit (Day of Surgery)

5.6.1. Administration of the IMP

All patients in Stage 1, and the 50 patients in Stage 2 that are randomised to fluorescence image-guided RARP, will receive the IMP by intravenous infusion.

In Stage 1, the dose of the IMP and the timing of the administration before surgery will be either 2 or 4 hours before surgery according to the "Optimisation of dose administration, pre-operative interval and equipment" scheme identified in Section 3.1.

In Stage 2, the dose of the IMP and the timing of administration before surgery will be the optimised parameters from Stage 1.

The dispensed dose will be infused under the supervision of qualified clinical staff or designee. The patient will be monitored for Adverse Events (AEs) beginning with the IMP infusion and until patient's last visit at the six-week appointment. Concomitant medications will be recorded at baseline visit and during follow-up visits until the six-week appointment.

Patients will be asked to provide urine samples before and after IMP administration to study the kinetics of EMI-137 clearance by measuring fluorescence levels in urine.

5.6.2. Fluorescence image-guided surgery

In all patients in Stage 1 and in those randomised to the intervention arm of Stage 2, fluorescent areas will be sampled for histopathological and genetic analysis.

Prior to surgery, patients will undergo systemic administration of the IMP. During surgery we will deploy an additional robot-mounted laparoscope inserted through one of the 12mm ports, separate from the four robot-assisting ports used to insert instruments. This will provide near real-time fluorescence images simultaneously with image-guidance afforded by white-light reflectance to identify tumour lying outside the prostate. Following patient positioning, routine insertion of the laparoscopic ports and docking of the robot, fluorescence imaging will be applied to check for any areas of fluorescence along the lymph-node chains from the obturator fossa to the common iliac vessels. An extended pelvic lymph node dissection (ePLND) will be performed. Fluorescent areas will be sampled separately for histopathological examination and genetic analysis. Standard RARP will be performed, with nerve-sparing procedure as indicated. Fluorescence imaging will be used at several stages during the procedure (Figure 7) and captured images will be stored as part of the trial data. Fluorescence will be detected and recorded:

1. Prior to the bladder being taken down to visualise the pelvic lymph-nodes
2. After ePLND (*in vivo* with additional sampling if necessary, and *ex vivo* to compare and sample fluorescence if different)
3. Immediately after the bladder is taken down
4. After dissection of the prostate and NVB if appropriate and prior to transection of the urethra, to identify any fluorescing areas outside the boundary of the prostate, including the NVB, apex and bladder-neck
5. Following transection of the urethra and removal of the prostate en-bloc with seminal vesicles, and thorough washout, further higher sensitivity fluorescent imaging will be applied to identify any remaining fluorescing tissue which will be sampled separately

The study team will record details of fluorescence visibility in the imaging worksheet.

Injection of the imaging agent (IMP) at separate visit 2 or 4 hours before surgery

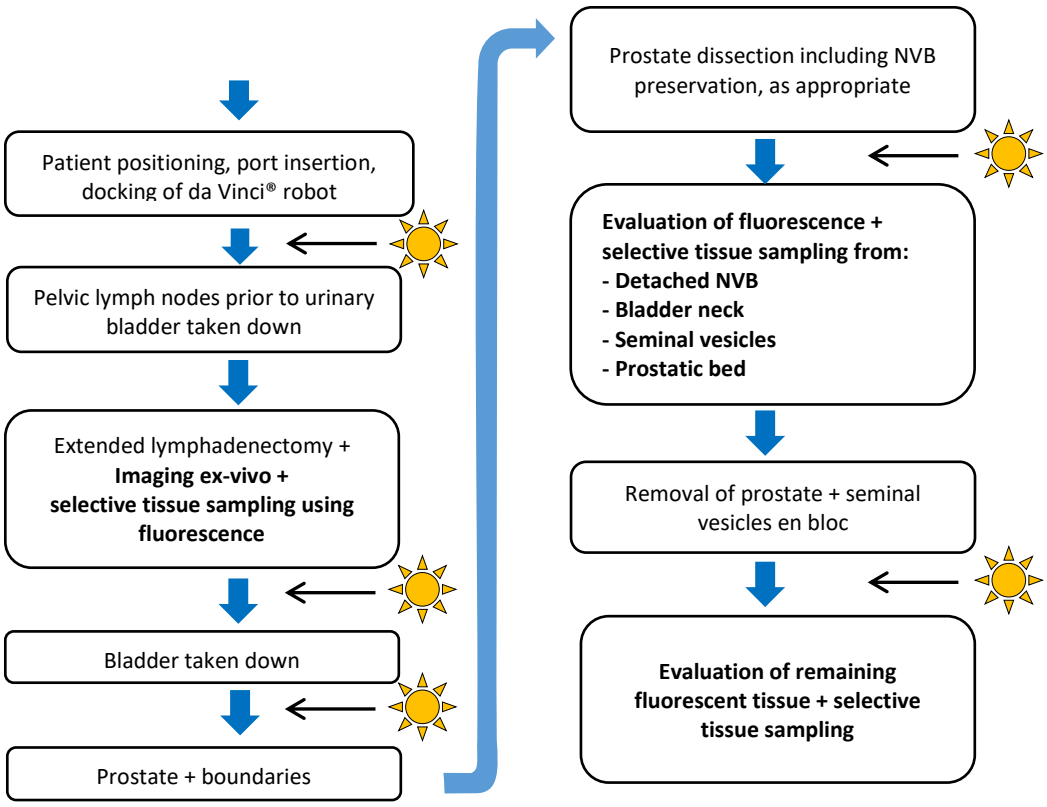

Figure 7: Outline of the surgical procedure.

The 'sun' symbol indicates the use of fluorescence imaging. Text in bold applies only to patients in Stage 1 or those randomised to fluorescence image-guided RARP in Stage 2.

Following surgery, patients will stay in hospital for one night or longer as required clinically, as per routine care.

While the patient remains in hospital the research team will collect urine samples from catheter at several time-points to study the clearance of EMI-137 in urine by measuring fluorescence levels.

5.7 Subsequent Visits

The schedule of subsequent patient visits for patients in Stage 1 and in both arms of Stage 2 will be the same as routine local practice:

1. Patients will stay in hospital for one night after surgery or longer as required clinically as per standard of care. All AEs occurring after consent and all concomitant medications will be entered into the trial electronic database. Urine samples will be collected from catheter by the research team before patients are discharged from hospital. After discharge, patients will be asked to collect urine samples from catheter twice a day (morning and evening) until their catheter removal visit at 10-14 days after surgery.
2. Patients will come back into clinic for routine removal of catheter 10-14 days after surgery. All AEs occurring since the last visit and all new concomitant medications will be entered into the trial electronic database. Patients will bring the urine samples which they have been collecting since discharge from hospital.
3. Patients will attend a clinic approximately six weeks post-surgery as per routine NHS care. They will have had a routine PSA test prior to their appointment, the result of which will be entered into

the trial electronic database. All AEs occurring since the last visit and all new concomitant medications will be entered into the trial electronic database. Patients will undergo additional safety assessments: physical examination, vital signs, ECG and safety laboratory tests (Hemogram and biochemistry). Patients will be asked to provide urine sample.

4. The information obtained from histopathological assessment of samples will be entered into the trial clinical database when it becomes available.
5. Patients will continue to attend the clinic regularly according to routine clinical practice. The study research nurse will review patient's medical notes annually for five years after surgery and will enter the following routinely available clinical information (as it becomes available) into the trial electronic database: PSA test results, further imaging results if available, evidence of disease progression, the need for salvage therapy, disease-specific and all-cause mortality.

Symptom-directed physical examination will be performed at any moment during the study if clinically indicated by the study investigators.

5.8 Sample Handling

Tissue sampling

All patients' routine diagnostic pathology samples (which will be stored in a pathology diagnostic archive) may be accessed for use in this research. In all patients in Stage 1, and in patient randomised to fluorescence image-guided RARP in Stage 2, tissue samples will be taken at different stages during surgery as described in Section 5.6. In addition to primary, treatment-naive intra-capsular cancer and normal tissue, we will sample extracapsular tumour spread, local lymph nodes and NVBs. Tissue sampling will be performed according to Standard Operating Procedures (SOPs). Collected samples will be linked before being stored in anonymous form. Tissue samples will be used for histopathological and genetic analysis according to SOPs and may be used in other ethically approved research in the future.

5.9 Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason. All participants will continue to be followed up as per routine NHS standard of care. The investigator may replace subjects who withdraw from the study in Stage 1, but not after patients have been randomised in Stage 2.

Withdrawn participants will be asked whether the data acquired up to the point of withdrawal can be retained. The reason for withdrawal will be recorded in the participant's medical records and in the Care Report Form (CRF). If the participant is withdrawn due to an AE, the investigator will arrange for follow-up visits until the AE has resolved or stabilised.

5.10 Definition of End of Trial

Stage 1 will end after the final patient has undergone surgery, attended their six week follow up visit, all data have been collected and all queries resolved. Stage 2 cannot start until the Stage 1 data has been analysed and peri-operative NIR visibility of histopathologically confirmed PC lesions has been demonstrated in a minimum of 50% of patients.

If the study progresses to Stage 2, the end of the trial will be after the five year follow-up data have been collected and all queries resolved.

The trial will be stopped prematurely:

- If mandated by the Ethics Committee;
- Following recommendations from the Trial Steering Committee (TSC);
- If review of safety data in Stage 2 suggests one arm has significant disadvantages to the extent that patient safety is endangered

- If there is evidence that an alternative compound is superior to EMI-137 IMP for peri-operative visualisation of PC cells

6. THE OXFORD NEAR INFRARED IMAGER FLUORESCENCE-GUIDED SURGERY DEVICE

This is a clinical study involving a non-CE marked device for use exclusively within the University of Oxford Hospitals NHS Trust. No CE marking or commercialisation of the device is intended.

The Fluorescence-Guided Surgery (FGS) device was developed by the Vojnovic lab (Advanced Technology Development Group) of the Dept. of Oncology, Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, Old Road Campus Research Building, Oxford OX3 7DQ. The device provides high-definition visible and NIR fluorescence imaging during laparoscopic procedures. It is intended to provide surgeons with the ability to perform routine visible light laparoscopic procedures and in addition, to provide NIR imaging of fluorescence markers with the aim of assessing tumour margins. The latter is achieved as a result of systemic or local injection of a fluorescently-labelled contrast agent. This agent could be a small molecule, a peptide, an antibody, a minibody or other moieties amenable to NIR dye conjugation.

The FGS device consists of the following components:

- A clinically approved 10 mm diameter Storz laparoscope, capable of transmitting both visible and NIR light/images.
- A camera head designed to be mounted on the laparoscope eyepiece, containing appropriate filters, lenses etc. coupled to a sensor capable of imaging a wide range of wavelengths.
- A laparoscopic illuminator and image processor that provides both visible and NIR illumination to the surgical laparoscope via a flexible light guide cable, and acquires, processes, displays and stores simultaneous real-time visible and NIR fluorescence images.
- A flexible light guide cable that is compatible with standard Storz-type laparoscopes.
- A foot-operated switch for enabling/disabling fluorescence modes.
- A cart/stand that houses accessories when not in use, the laparoscopic illuminator and image processor, and a range of video converters/drivers appropriate to coupling images to a range of theatre monitors and to a surgical robot.
- A medical grade high-definition monitor

The laparoscopic illuminator generates controlled intensities of white light in the wavelength range of 430-650 nm, deep red fluorescence excitation light at a wavelength of 660 nm and NIR fluorescence excitation light at 779 nm. The deep red fluorescence excitation light is appropriate for excitation of Cy5 and Cy5-like (Cy5*, Cy5.5) fluorophores while the 779 nm wavelength is appropriate for IRdye800 and similar fluorophores.

6.1. Device Safety

6.1.1 Electrical Safety

The bulk of the system (the laparoscopic illuminator and image processor) is constructed within an electrically screened, electromagnetic compatible earthed metal enclosure. All internal electronics are powered by a low voltage (18V dc), derived from an internal medical grade power supply intended to be connected to a 230 V

ac power line, via double-fused switched International Electortechnical Commission (IEC) input power socket. All internal construction corresponds to medical approval standards. An external earthing pin is provided. All other input and auxiliary output connections operate at low voltages. Auxiliary control inputs are optically or magnetically isolated. The device contains several processors that use battery-back-up in case of unexpected brown-outs or black outs.

The camera head is constructed from a locally/internally shielded insulating material and does not present any electrical hazard. Similarly the foot pedal does not present a hazard as it is both earthed and operates at 5V dc.

6.1.2 Optical Safety

The device is capable of generating intense light levels, in common with any other laparoscopy equipment. However, this light output is disabled when the light guide is unplugged, when the camera head lead is unplugged and when the camera head/laparoscope is tilted upwards. Furthermore an 'emergency off' switch is provided on the device front panel. In common with other laparoscopic equipment, the output from the light guide, before it is connected to the laparoscope, could pose a risk. However, although the light output from this light guide is very divergent, users should avoid looking directly at it, prior to connection to the laparoscope.

6.2. **Device Maintenance**

The FGS device does not contain any user-serviceable parts and does not require any preventive inspection or maintenance. It must not be disassembled, modified nor should any attempts be made to repair it. Patient or user injury and/or instrument damage can result if the device is tampered with.

6.3. **Device Storage**

For storage and transport, ensure that the device components are not subjected to mechanical strain to prevent damage to sensitive parts. The FGS device should be stored in a location protected from direct sunlight and with temperature range -10 +55 °C and relative humidity 10% - 85%.

7. INVESTIGATIONAL MEDICINAL PRODUCT (IMP) – EMI-137

Detailed information about the IMP is provided in the IMPD and IB.

7.1. **IMP Description**

EMI-137 Injection is an IMP for optical imaging. The active ingredient is a 26-amino acid cyclic peptide labelled with a fluorescent cyanine dye which emits red light when excited with red light (peak absorption at approximately 660 nm). EMI-137 has high affinity for human c-Met with a dissociation constant in the low nanomolar range (approximately 2 nM).

EMI-137 for Injection is a sterile freeze-dried blue powder supplied in 10ml glass vials. Each vial contains 24mg of EMI-137. EMI-137 Injection is prepared by reconstitution of EMI-137 for Injection with 5ml sterile water for injection. From a microbiological point of view, the reconstituted product EMI-137 should be used immediately.

Stage 1 is a dose-finding stage and doses of EMI-137 will be administered as described in Section 3.1.1. The exact dose and timing of IMP administration during Stage 2 will be decided based on the results obtained in Stage 1.

The IMP will be infused intravenously as described in Section 5.6.1. 'Administration of the IMP'.

7.2. Supply of IMP

EMI-137 is manufactured by the Edinburgh Molecular Imaging Ltd (UK-based company), and will be QP released either by Edinburgh Molecular Imaging Ltd or by the contracted QP. It will be shipped to the OUHT Clinical Trials Pharmacy in batches for storage.

7.3. IMP labelling

The IMP will be labelled by Edinburgh Molecular Imaging Ltd according to Annex 13 of the GMP guidelines.

7.4. Storage of IMP

The freeze-dried product, EMI-137 for Injection, should be refrigerated (2 to 8^{o}C) and protected from light. From a microbiological point of view, the reconstituted product EMI-137 should be used immediately.

7.5. Compliance with Trial Treatment

Participants will receive one dose of IMP administered 2 or 4 hours before surgery. The IMP will be infused under the supervision of qualified clinical staff or designee.

7.6. Accountability of the Trial Treatment

Full accountability records for receipt, use and return/destruction of IMP will be maintained according to OCTRU procedures. A full accountability record will be maintained at the trial site. The product will be administered as a single intravenous infusion under the supervision of qualified clinical staff or designee. Any dose reductions or alterations will be recorded and reported in the trial database.

7.7. Concomitant Medication

There are no contraindicated medications. Data relating to concomitant therapies will be collected at each trial visit and recorded in the CRFs.

7.8. Post-trial Treatment

No IMP will be given outside of the trial.

8. SAFETY REPORTING

8.1. Definitions

Definitions of the various categories of Adverse Events are set out in the table below.

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.

	The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

8.2. **Reportable adverse events**

All AEs (irrespective of their causality) occurring from the time of consent and until 6 weeks following surgery should be recorded on the AE Report Form.

8.3. **Causality**

The relationship of each AE to the IMP will be determined by the investigator (or a delegate who is medically qualified and listed on the site delegation log) according to the following definitions:

Relationship to intervention	Attribution (Causality)	Description
Unrelated	Unrelated	The AE is clearly NOT related to the intervention
	Unlikely	The AE is doubtfully related to the intervention
Related	Possible	The AE may be related to the intervention

	Probable	The AE is likely related to the intervention
	Definite	The AE is clearly related to the intervention

Once the SAE form is submitted to the CTU (Sponsor Representative), there will be a review of the SAE by a Nominated Person and any queries will be sent to the site for clarification. The assessment of causality by the investigator will not be downgraded by the CTU, even if the Nominated Person does not concur with the assessment. Under these circumstances both assessments will be recorded and causality deemed as 'related'.

8.4. **Expectedness**

Assessment of Expectedness of AEs will be performed centrally by the Nominated Person, and determined according to the Reference Safety Information Section of the IB.

8.5. **Procedures for Recording Adverse Events**

All AEs occurring after consent and before the 6 week visit that are observed by the Investigator or reported by the participant, will be recorded on the paper Case Report Form (CRF), whether or not attributed to trial medication. The same CRF will capture the seriousness of the event, so no separate SAE report form will be provided.

Any AE, irrespective of its perceived relationship to the IMP under investigation and/or the surgical procedure, will be captured in the participant's medical records. The relevant data will be recorded in the paper CRF and entered into the trial database following the general procedure for recording of trial data. Patients will be given an AE diary card to record any AEs occurring from consent to their 6 week follow up visit and this will be reviewed at their routine catheter removal and 6 week follow up visits.

The following information will be recorded:

(i) Description/event term
(ii) date of onset and end date
(iii) severity
(iv) assessment of relatedness to IMP
(v) action taken
(vi) seriousness

Follow-up information should be provided as necessary. The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the IMP (as judged by a medically qualified investigator or the nominated person for safety review) will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable. For these cases an Early Termination Visit CRF will be completed.

8.6. **Reporting Procedures for Serious Adverse Events**

All SAEs will be recorded starting from consent and until the six-week visit.

All SAEs must be reported on the trial-specific AE Report Form to SITU/Trial Manager, **within 24 hours** of the site study team becoming aware of the event. A scanned copy of the relevant AE Report Form must be emailed to promote@nds.ox.ac.uk. The Trial Manager will date stamp the form and perform an initial check of the report, request any additional information, and will pass it on to the Nominated Person without delay.

Additional information (follow-up or corrections to the original case) will be detailed on a new AE Report Form.

The Trial Manager will enter data from the paper AE report form directly into the electronic database.

All SAE Reports will be processed by SITU as per the detailed instructions in OCTRU's SOP governing safety reporting for a Clinical Trial of an IMP (CTIMP).

8.7. **SUSAR Reporting**

All SUSARs will be reported by SITU to the relevant Competent Authority (MHRA), the REC, and other parties as applicable. For fatal and life-threatening SUSARs, this will be done no later than seven calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within eight calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

8.8. **Data and Safety Monitoring Committee**

The Data and Safety Monitoring Committee (DSMC) will monitor the safety of the trial. The DSMC will review all SAEs (and AEs if requested) reported for the trial at each meeting, will evaluate the risk of the trial continuing and will take appropriate action where necessary.

8.9. **Development Safety Update Reports (DSUR)**

SITU, on behalf of the CI, will submit DSURs once a year throughout the clinical trial, or on request, to the MHRA, REC, HRA (where required), Host NHS Trust, and Sponsor.

9. STUDY OVERSIGHT

9.1. **Trial Management Group**

The Trial Management Group (TMG) consists of those individuals responsible for the operational management of the trial such as the CI, key members of the scientific and clinical team (scientists, pathologists and research nurses), the Trial Manager, and representatives from OCTRU (statisticians and head of regulatory affairs and quality). The TMG will meet every two months throughout the treatment phase of the trial and every six months throughout the five-year follow up phase of the trial, and will:

- Supervise the conduct and progress of the study, and adherence to the study protocol.
- Assess the safety and efficacy of the interventions during the study.
- Monitor the safety of the participants, and review safety data to look for any emerging trends including increases in severity or frequency of SAEs or SARs (which may require expedited reporting to the MHRA and relevant REC).

- Evaluate the quality of the study data.
- Review relevant information from other sources (e.g. related studies).
- Escalate any issues for concern to the Sponsor, specifically where the issue could compromise patient safety or the integrity of the study or quality of the study data.

9.2. **Trial Steering Committee**

The TSC is an independent body responsible for overall supervision of this study on behalf of the Sponsor (the University of Oxford) and the Funder (Cancer Research UK) in order to ensure that:

- Progress is satisfactory and the study is adhering to its overall objectives as set out in the protocol.
- Patient safety is not being compromised.
- The study is being conducted in accordance with Good Clinical Practice (GCP) and the UK Clinical Trial Regulations.

Decisions about continuation or termination of the study or substantial amendments to the protocol are usually the responsibility of the TSC, and the TSC will provide information and advice to the Sponsor, Funder and TMG in this regard.

Meetings of the TSC will take place annually, or at shorter intervals if required. Representatives of the Sponsor and the Funder will be invited to all TSC meetings. The TSC will adopt a Charter as per OCTRU SOPs.

9.3. **Data and Safety Monitoring Committee**

An independent Data and Safety Monitoring Committee (DSMC) will be established to safeguard the interests of trial participants, potential participants and future patients, to assess the safety and efficacy of the interventions during the trial, and to monitor the overall conduct of the trial, protecting its validity and credibility. The DSMC will adopt a DAMOCLES charter which defines its terms of reference and operation in relation to oversight of the trial. They will not be asked to perform any formal interim analyses of effectiveness. They will, however, see copies of data accrued to date, or summaries of that data by treatment group. They will also consider emerging evidence from other trials or research on the intervention. They may advise the chair of the TSC at any time if, in their view, the trial should be stopped for ethical reasons, including concerns about participant safety. DSMC meetings will be held at least once a year during the recruitment phase of the study and the reports will be forwarded to the TSC. The TSC will ultimately have the final say in stopping the trial early. Full details will be found in the DSMC and TSC Charters.

10. STATISTICS

Full details of the statistical analysis will be detailed in a separate statistical analysis plan (SAP) which will be drafted early in the trial and finalised prior to the primary analysis data lock. The SAP will be written in accordance with the current OCTRU SOPs and will be finalized and agreed by the study statistician, the CI and the TMG. Stata (StataCorp LP) or other appropriate validated statistical software will be used for analysis. A summary of the planned statistical analysis is included here.

10.1. **Description of Statistical Methods**

Descriptive statistics of the patient population will be reported using numbers (with percentages) for binary and categorical variables and means (standard deviations), or medians (with interquartile range) for continuous variables for each cohort of patients in stage 1 and for each randomised arm in stage 2.

The primary and secondary outcome measures for Stage 2 are summarised in section 2.

For the primary outcome during Stage 2, the proportion of patients to have positive surgical margins following histopathological assessment will be reported. Difference in proportions between intervention groups will be analysed using the chi-squared test and the treatment effect will be reported as odds ratios, together with 95% confidence intervals. Additional analyses will be undertaken in a multi-variable framework using logistic regression, adjusting for stratification and important prognostic factors.

If the primary outcome is statistically significant then the imaging modality will be considered promising and further testing in a phase III multi-centre setting will be considered.

Time to biochemical relapse, time to salvage therapy, disease specific survival and overall survival will be analysed using survival analysis techniques including Kaplan Meier plots, log-rank tests and reporting the treatment effects using hazard ratios, with 95% confidence intervals, and multi-variable Cox proportional hazards regression adjusting for stratification and important prognostic variables.

10.2. Number of Participants

In Stage 1, up to 20 patients will be included, up to five in each dose and time optimization group. This number has been chosen to give a reasonable assessment of the endpoints in order to enable the choice of optimal dose and timing for the validation study to be chosen. The investigator may replace patients who withdraw from the study.

In Stage 2, 100 patients will be included (50 per intervention) to provide 80% power and 5% (2-sided) significance to detect a large reduction in positive surgical margin rates (a reduction from 45% to 20%) in patients with high-risk PC. Patients who withdraw from the study may not be replaced in this stage.

10.3. The Level of Statistical Significance

All tests will be undertaken at a 5% 2-sided significance. All comparative results will be presented as treatment effects with 95% confidence intervals and reported in accordance with the CONSORT statement.

10.4. Procedure for Accounting for Missing, Unused, and Spurious Data.

Missing data will be reported and summarised by intervention arm during Stage 2. The distribution of missing data will be explored in order to assess the assumption of data being missing at random. Multiple-imputation will be utilised, if appropriate. Full details will be provided in the SAP.

10.5. Inclusion in Analysis

The intention to treat population includes all patients with data for the appropriate outcome in Stage 1 and all patients randomised in Stage 2, grouped by allocated intervention regardless of treatment received. The per-protocol analysis population includes those patients who complied with the protocol in terms of eligibility, treatment and availability of the outcome measurements. Full details of exclusions will be detailed in the SAP.

Safety data will be reported for all patients who started treatment.

10.6. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Any deviations from the finalised SAP will be described and justified in the protocol and /or in the final report at the end of the study.

11. DATA MANAGEMENT

A Data Management Plan (DMP) will be produced for the trial according to OCTRU SOPs. Monitoring procedures are described in Section 12.

11.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, surgery worksheets, laboratory and pharmacy records, and pathology reports.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the patient will be referred to by the trial number/code, with no personal identifiable information.

11.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11.3. Data Recording and Record Keeping

The trial data (including data for AEs) will be entered onto a validated OpenClinica study database (www.openclinica.com) developed and maintained by OCTRU and which can only be accessed by authorised users via the OpenClinica application. The OpenClinica application resides on a webserver hosted and managed by Oxford University's Medical Services Division IT Services department (http://www.imsu.ox.ac.uk/). The server is on the university's backbone network and is backed up nightly to a secure off-site location. Consent will be obtained from the patients to be able to share information and prior to sharing, data will be anonymised. In addition, any indirect identifiers that may lead to deductive disclosures will be removed to reduce the risk of identification. After closure of the trial and data analyses, the data will be made publicly available at the time of publication. The Trial Master File will be archived for five years from the end of the study.

A patient will be identified solely by a study ID in the CRF – the name and any other identifying details of a participant will not be included in the CRF.

12. QUALITY ASSURANCE PROCEDURES

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and OCTRU SOPs. A risk assessment will be undertaken and a proportionate monitoring plan will be put in place to decide on the extent and nature of any on-site monitoring. Central monitoring of incoming data and operational aspects of the trial will be done by SITU according to a written plan.

An independent DSMC will be established with an independent chair and suitable multi-disciplinary representation. The DSMC will meet annually.

13. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within seven days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial”

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the NHS host organisation within seven calendar days.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

14.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with GCP.

14.3. Approvals

The protocol, informed consent form, PIL and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

14.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor. The DSUR will be reported as per section 8.9.

14.5. Participant Confidentiality

We will record patient’s names and NHS numbers for the long term follow-up using medical notes. The trial staff will ensure that the participants’ anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRFs, where participant initials may be added. All documents will be stored securely and only

accessible by trial staff and authorised personnel. The trial will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be anonymised as soon as it is practical to do so.

14.6. Expenses and Benefits

Participants will not incur any additional expenses as a result of participating in this study.

14.7. Other ethical considerations

In Stage 2 of the study, we will record patient's names and NHS numbers for long term follow-up using medical notes. Only dedicated trial staff will have access to those data. Patients will be informed and asked to consent to this prior to joining the study. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be anonymised as soon as it is practical to do so.

The first IMP dose and timing combination will be tested in up to five patients. If 1) there is no visible fluorescence, or 2) oversaturation and 3) there are no safety concerns, the next dose/timing combination in the protocol will be tested. If no fluorescence is visible or histopathological assessment of biopsies demonstrates that cancerous tissue was not highlighted and/or healthy tissue is highlighted by the compound using any dose and timing combination during stage 1, the study will not progress to stage 2.

Participants won't experience any delay to their surgical treatment or any impact on standard of care due to participation in the study.

The additional biopsies may add around five minutes to the total length of the procedure, but the duration of the surgical procedure will not be increased significantly.

In case of incidental finding of cancer cells in the additional biopsies taken outside of the prostate in patients in Stage 1, and those randomised to fluorescence guided RARP in Stage 2, the clinical team will discuss the results and best course of action with the patient at their routine follow up visit.

Patients in the control arm of Stage 2 of the study will be treated and followed up as per local standard of care, with annual review of medical notes to capture secondary outcomes.

15. FINANCE AND INSURANCE

15.1. Funding

The study is funded by Cancer Research UK as part of a programme award (C1380/A18444).

15.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

16. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by Cancer Research UK. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

Results will be disseminated in the form of national and international presentations at learned societies, and published in abstract and full manuscripts in peer-reviewed journals.

17. REFERENCES

1. http://www.info.cancerresearchuk.org/cancerstats. [Available from: http://www.info.cancerresearchuk.org/cancerstats.
2. Hugosson J, Carlsson S, Aus G, Bergdahl S, Khatami A, Lodding P, et al. Mortality results from the Göteborg randomised population-based prostate-cancer screening trial. The Lancet Oncology. 2010;11(8):725-32.
3. Schröder FH, Hugosson J, Roobol MJ, Tammela TLJ, Ciatto S, Nelen V, et al. Prostate-Cancer Mortality at 11 Years of Follow-up. New England Journal of Medicine. 2012;366(11):981-90.
4. Brajtbord JS, Lavery HJ, Nabizada-Pace F, Senaratne P, Samadi DB. Endorectal magnetic resonance imaging has limited clinical ability to preoperatively predict pT3 prostate cancer. BJU International. 2011;107(9):1419-24.
5. Hoeks CMA, Barentsz JO, Hambrock T, Yakar D, Somford DM, Heijmink SWTPJ, et al. Prostate Cancer: Multiparametric MR Imaging for Detection, Localization, and Staging. Radiology. 2011;261(1):46-66.
6. Catto JW, Robinson MC, Albertsen PC, Goepel JR, Abbod MF, Linkens DA, et al. Suitability of PSA-detected localised prostate cancers for focal therapy: experience from the ProtecT study. Br J Cancer. 2011;105(7):931-7.
7. Bill-Axelson A, Holmberg L, Ruutu M, Garmo H, Stark JR, Busch C, et al. Radical Prostatectomy versus Watchful Waiting in Early Prostate Cancer. New England Journal of Medicine. 2011;364(18):1708-17.
8. Schelin S, Madsen M, Palmqvist E, Mäkelä E, Klintenberg C, Aus G. Long-term follow-up after triple treatment of prostate cancer stage pT3. Scandinavian Journal of Urology and Nephrology. 2009;43(3):186-91.
9. Selli C, Montironi R, Bono A, Pagano F, Zattoni F, Manganelli A, et al. Effects of complete androgen blockade for 12 and 24 weeks on the pathological stage and resection margin status of prostate cancer. Journal of Clinical Pathology. 2002;55(7):508-13.
10. Aus G, Abrahamsson PA, Ahlgren G, Hugosson J, Lundberg S, Schain M, et al. Three-month neoadjuvant hormonal therapy before radical prostatectomy: a 7-year follow-up of a randomized controlled trial. BJU International. 2002;90(6):561-6.
11. Klotz LH, Goldenberg SL, Jewett MAS, Fradet Y, Nam R, Barkin J, et al. Long-term Followup of a Randomized Trial of 0 Versus 3 Months of Neoadjuvant Androgen Ablation Before Radical Prostatectomy. The Journal of Urology. 2003;170(3):791-4.
12. Sorrentino C, Musiani P, Pompa P, Cipollone G, Di Carlo E. Androgen Deprivation Boosts Prostatic Infiltration of Cytotoxic and Regulatory T Lymphocytes and Has No Effect on Disease-Free Survival in Prostate Cancer Patients. Clinical Cancer Research. 2011;17(6):1571-81.
13. Zelefsky MJ, Eastham JA, Cronin AM, Fuks Z, Zhang Z, Yamada Y, et al. Metastasis After Radical Prostatectomy or External Beam Radiotherapy for Patients With Clinically Localized Prostate Cancer: A Comparison of Clinical Cohorts Adjusted for Case Mix. Journal of Clinical Oncology. 2010;28(9):1508-13.
14. Boorjian SA, Karnes RJ, Viterbo R, Rangel LJ, Bergstralh EJ, Horwitz EM, et al. Long-term survival after radical prostatectomy versus external-beam radiotherapy for patients with high-risk prostate cancer. Cancer. 2011;117(13):2883-91.

15. Hsu C-Y, Joniau S, Oyen R, Roskams T, Van Poppel H. Outcome of Surgery for Clinical Unilateral T3a Prostate Cancer: A Single-Institution Experience. European Urology. 2007;51(1):121-9.
16. Joniau S, Spahn M, Briganti A, Gandaglia G, Tombal B, Tosco L, et al. Pretreatment Tables Predicting Pathologic Stage of Locally Advanced Prostate Cancer. European Urology. 2014.
17. Haffner M, Mosbruger T, Esopi D, Fedor H, Heaphy C, Walker D, et al. Tracking the clonal origin of lethal prostate cancer. The Journal of Clinical Inevstigation. 2013;123(11):4918-22.
18. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat J-P, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet. 2012;44(6):685-9.
19. Grasso CS, Wu Y-M, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012;487(7406):239-43.
20. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature. 2011;470(7333):214-20.
21. Lindberg J, Klevebring D, Liu W, Neiman M, Xu J, Wiklund P, et al. Exome Sequencing of Prostate Cancer Supports the Hypothesis of Independent Tumour Origins. European Urology. 2013;63(2):347-53.
22. Baca Sylvan C, Prandi D, Lawrence Michael S, Mosquera Juan M, Romanel A, Drier Y, et al. Punctuated Evolution of Prostate Cancer Genomes. Cell. 2013;153(3):666-77.
23. Weischenfeldt J, Simon R, Feuerbach L, Schlangen K, Weichenhan D, Minner S, et al. Integrative Genomic Analyses Reveal an Androgen-Driven Somatic Alteration Landscape in Early-Onset Prostate Cancer. Cancer Cell. 2013;23(2):159-70.
24. Barbieri CE, Tomlins SA. The prostate cancer genome: Perspectives and potential. Urologic Oncology: Seminars and Original Investigations. 2014;32(1):53.e15-53.e22.
25. Teles Alves I, Hartjes T, McClellan E, Hiltemann S, Bottcher R, Dits N, et al. Next-generation sequencing reveals novel rare fusion events with functional implication in prostate cancer. Oncogene. 2014.
26. Sagalovich D, Calaway A, Srivastava A, Sooriakumaran P, Tewari AK. Assessment of required nodal yield in a high risk cohort undergoing extended pelvic lymphadenectomy in robotic-assisted radical prostatectomy and its impact on functional outcomes. BJU International. 2013;111(1):85-94.
27. Briganti A, Blute ML, Eastham JH, Graefen M, Heidenreich A, Karnes JR, et al. Pelvic Lymph Node Dissection in Prostate Cancer. European Urology. 2009;55(6):1251-65.
28. Bastian PJ, Boorjian SA, Bossi A, Briganti A, Heidenreich A, Freedland SJ, et al. High-Risk Prostate Cancer: From Definition to Contemporary Management. European Urology. 2012;61(6):1096-106.
29. Brouwer OR, Buckle T, Bunschoten A, Kuil J, Vahrmeijer AL, Wendler T, et al. Image navigation as a means to expand the boundaries of fluorescence-guided surgery Physics in Medicine and Biology. 2012;57(10):3123-36.
30. van der Poel HG, Buckle T, Brouwer OR, Valdés Olmos RA, van Leeuwen FWB. Intraoperative Laparoscopic Fluorescence Guidance to the Sentinel Lymph Node in Prostate Cancer Patients: Clinical Proof of Concept of an Integrated Functional Imaging Approach Using a Multimodal Tracer. European Urology. 2011;60(4):826-33.
31. Fukuhara H, Inoue K, Satake H, Tamura K, Karashima T, Yamasaki I, et al. Photodynamic diagnosis of positive margin during radical prostatectomy: Preliminary experience with 5-aminolevulinic acid. International Journal of Urology. 2011;18(8):585-91.
32. Manny TB, Patel M, Hemal AK. Fluorescence-enhanced Robotic Radical Prostatectomy Using Real-time Lymphangiography and Tissue Marking with Percutaneous Injection of Unconjugated Indocyanine Green: The Initial Clinical Experience in 50 Patients. European Urology. 2014;65(6):1162-8.
33. Tax C, Rovers MM. Re: Ted B. Manny, Manish Patel, Ashok K. Hemal. Fluorescence-enhanced Robotic Radical Prostatectomy Using Real-time Lymphangiography and Tissue Marking with Percutaneous Injection of Unconjugated Indocyanine Green: The Initial Clinical Experience in 50 Patients. Eur Urol 2014;65:1162–8. European Urology. 2014;66(1):e15-e6.
34. Schülke N, Varlamova OA, Donovan GP, Ma D, Gardner JP, Morrissey DM, et al. The homodimer of prostate-specific membrane antigen is a functional target for cancer therapy. Proceedings of the National Academy of Sciences. 2003;100(22):12590-5.

35. Mhawech-Fauceglia P, Zhang S, Terracciano L, Sauter G, Chadhuri A, Herrmann FR, et al. Prostate-specific membrane antigen (PSMA) protein expression in normal and neoplastic tissues and its sensitivity and specificity in prostate adenocarcinoma: an immunohistochemical study using mutiple tumour tissue microarray technique. Histopathology. 2007;50(4):472-83.
36. Miller CT, Lin L, Casper AM, Lim J, Thomas DG, Orringer MB, et al. Genomic amplification of MET with boundaries within fragile site FRA7G and upregulation of MET pathways in esophageal adenocarcinoma. Oncogene. 2006;25(3):409-18.
37. Hara T, Ooi A, Kobayashi M, Mai M, Yanagihara K, Nakanishi I. Amplification of c-myc, K-sam, and c-met in gastric cancers: detection by fluorescence in situ hybridization. Lab Invest. 1998;78(9):1143-53.
38. Kuniyasu H, Yasui W, Kitadai Y, Yokozaki H, Ito H, Tahara E. Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun. 1992;189(1):227-32.
39. Houldsworth J, Cordon-Cardo C, Ladanyi M, Kelsen DP, Chaganti RS. Gene amplification in gastric and esophageal adenocarcinomas. Cancer Res. 1990;50(19):6417-22.
40. Tong CY, Hui AB, Yin XL, Pang JC, Zhu XL, Poon WS, et al. Detection of oncogene amplifications in medulloblastomas by comparative genomic hybridization and array-based comparative genomic hybridization. J Neurosurg. 2004;100(2 Suppl Pediatrics):187-93.
41. Di Renzo MF, Olivero M, Serini G, Orlandi F, Pilotti S, Belfiore A, et al. Overexpression of the c-MET/HGF receptor in human thyroid carcinomas derived from the follicular epithelium. J Endocrinol Invest. 1995;18(2):134-9.
42. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A. 2007;104(52):20932-7.
43. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316(5827):1039-43.
44. Jacobsen F, Ashtiani SN, Tennstedt P, Heinzer H, Simon R, Sauter G, et al. High c-MET expression is frequent but not associated with early PSA recurrence in prostate cancer. Exp Ther Med. 2013;5(1):102-6.
45. Hurle RA, Davies G, Parr C, Mason MD, Jenkins SA, Kynaston HG, et al. Hepatocyte growth factor/scatter factor and prostate cancer: a review. Histol Histopathol. 2005;20(4):1339-49.
46. Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LW. c-met proto-oncogene expression in benign and malignant human prostate tissues. J Urol. 1995;154(1):293-8.
47. Watanabe M, Fukutome K, Kato H, Murata M, Kawamura J, Shiraishi T, et al. Progression-linked overexpression of c-Met in prostatic intraepithelial neoplasia and latent as well as clinical prostate cancers. Cancer Lett. 1999;141(1-2):173-8.
48. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18(1):11-22.
49. Barber PR, Tullis IDC, Pierce GP, Newman RG, Prentice J, Rowley MI, et al. The Gray Institute 'open' high-content, fluorescence lifetime microscopes. Journal of Microscopy. 2013;251(2):154-67.
50. Ghosh A, Wang X, Klein E, Heston WDW. Novel Role of Prostate-Specific Membrane Antigen in Suppressing Prostate Cancer Invasiveness. Cancer Research. 2005;65(3):727-31.
51. Hassan M, Kerdok A, Engel A, Gersch K, Smith JM. Near infrared fluorescence imaging with ICG in TECAB surgery using the da Vinci Si surgical system in a canine model. J Card Surg. 2012;27(2):158-62.
52. Tobis S, Knopf J, Silvers C, Yao J, Rashid H, Wu G, et al. Near infrared fluorescence imaging with robotic assisted laparoscopic partial nephrectomy: initial clinical experience for renal cortical tumors. J Urol. 2011;186(1):47-52.
53. Volpi D TI, Laios A, Pathiraja PNJ, Haldar K, Ahmed AA, Vojnovic B. A novel multiwavelength fluorescence image-guided surgery imaging system. Proceedings of SPIE.8395(Advanced Biomedical and Clinical Diagnostic Systems XII, 89350G).

18. APPENDIX A: SCHEDULE OF PROCEDURES

PROCEDURES	Visits					Follow-up
	1 Screening and eligibility	2 Baseline visit	3 Surgery	4 Routine Follow up visit 1	5 Routine Follow up visit 2	6 Data Collection only
TIME			**Day 0**	**Day 10-14**	**Approx. 6 weeks after surgery**	**Annually for 5 years after surgery (Stage 2 only)**
Patient Information Sheet	T					
Eligibility assessment	T					
Informed consent	T					
Liver function test and PSA test	T					
Demographics		T/R				
Medical history		T/R				
Baseline data*		T /R				
Randomisation **(Stage 2 only)**		T (Stage 2 only)				
IMP administration (injection)			T			
Urine sample to study IMP level			T	T	T	
Fluorescence image-guided RARP**			T			
Standard RARP**			R			
Catheter removal				R		
Recording of routine PSA result					T	T
Safety assessments (physical exam, vital signs, ECG, hemogram, biochemistry)	T				T	
Adverse events†	T	T	T	T	T	
Concomitant medications		T	T	T	T	
Stage 2 only: Review of medical records#						T (Stage 2 only)

R = Routine procedure
T= Trial specific procedure
T/R= Done as part of routine procedure but access needed as part of the trial data collection
***** Clinical and pathological stage, Gleason grade, serum PSA, pre-biopsy imaging results (mpMRI, PET-CT) and biopsy results

**Only patients in Stage 1, or those randomised to fluorescence image-guided RARP will undergo fluorescence image-guided RARP, patients in Stage 2 randomised to standard RARP will not undergo fluorescence image-guided RARP.
† Patients will be monitored for AEs in clinic from time of consent until discharge from hospital following surgery. Patients will receive a diary card to record any other AEs which following discharge from hospital and up until 6 weeks after surgery.
#PSA results, further imaging results, evidence of disease progression, need for salvage therapy, disease-specific and all-cause mortality

19. AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
2	3.0		Kate Belaya	1. Added PSA test after consent. 2. Added collection of urine samples before and after IMP administration, during hospital stay, between hospital discharge and catheter removal visit, and at 6 week visit. 3. Made the protocol GDPR compliant 4. Added clarification regarding the dosing decision. 5. Updated Sponsor contact details
1	2.0	28 May 2018	Kate Belaya	Added revisions requested by MHRA: 1. Added physical examination, assessment of vital signs, ECG, liver function test and safety laboratory tests (Hemogram and biochemistry) at eligibility assessment and at 6 week appointment. 2. Extended period of AE collection to start at consent and until the 6-week appointment. 3. Amended eligibility criteria to exclude patients with abnormal liver function test and abnormal ECG. 4. Changed exclusion criteria from GFR<35ml/min to GFR<60ml/min. 5. Added exclusion criteria to exclude previous adverse reaction to IMP compounds or fluorescent agents.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee and/or MHRA.