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Hypoxia-driven **Pro**state cancer **Gen**omics (**HYPROGEN**)

Illuminating the genomic landscape of hypoxia-driven early metastatic prostate cancer

Chief investigator: Prof Robert G Bristow, MD PhD

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SIGNATURE PAGE

HYPROGEN: HYpoxia-driven PROstate cancer GENomics

This document describes the HYPROGEN study and provides information about procedures for entering patients into it. The protocol should not be used as a guide for the treatment of patients outside of the study.

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined int the ICH Good Clinical Practice guidelines, the Data Protection Act (2018), the Declaration of Helsinki, Ionising Radiation Medical Exposure Regulations (IRMER), the Human Tissue Act (2004), the Research Governance Framework (2005), the Sponsor's SOP, and other regulatory requirement.

The undersigned confirm their agreement to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor.

The undersigned also confirm that the findings of the study will be made publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

Every care was taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to all Investigators in the study as required.

For and on behalf of the Study Sponsor:

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Chief Investigator:

Signature: DocuSigned by: AE0CB9AB53D4436... Date: ...../...../..... 28-Oct-2022

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1 ABBREVIATIONS

AE	Adverse event
APR	Annual progress report
AR	Adverse reaction
CEP	Clinical and Experimental Pharmacology Group
CRUK MI	Cancer Research UK Manchester Institute
CT	Computed tomography
CTC	Circulating tumour cells
ctDNA	Circulating tumour deoxyribonucleic acid
CTIMP	Clinical Trial of an Investigational Medicinal Product
DNA	Deoxyribonucleic acid
ECOG	Eastern central oncology group performance status
EDTA	Ethylenediaminetetraacetic acid
EMT	epithelial-mesenchymal-transition
EpCAM	Epithelial cell adhesion molecule
GCP	Good clinical practice
GLUT1	Glucose Transporter 1
HRA	Health Research Authority
IPU	Interventional Procedures Unit
MCCBS	Manchester Centre for Cancer Biomarker Sciences
MCRC	Manchester Cancer Research Centre
mpMRI	multiparametric magnet resonance imaging
mRNA	messenger ribonucleic acid
NAB	Nucleic Acid Biomarkers group
PCa	Prostate cancer
PIMO	Pimonidazole hydrochloride
PIS	Patient information sheet
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homolog
REC	Research Ethics Committee
RNA	Ribonucleic acid
RNAsep	Ribonucleic acid sequencing
ROI	Region of interest
SAE	Serious adverse event
SOAR	Sponsor oversight activity report
SOC	Standard of care
SOP	Standard operating procedure
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TOG	Translational Oncogenomics Group
TRUS	Trans-rectal ultrasound
USM	Urgent Safety Measure
WES	Whole-exome sequencing

2 STUDY SUMMARY

Study Title	Illuminating the genomic landscape of hypoxia-driven early metastatic and localised prostate cancer
Acronym	HYPROGEN
Study Design	Prospective, non-randomised, exploratory biopsy and imaging biomarker study
Target Patient Population	*Arm 1 - De novo*, treatment-naïve metastatic prostate cancer Arm 2 – De novo, treatment- naïve localised prostate cancer planned for radical prostatectomy
Planned Sample Size	Arm 1 – 30 Arm 2 - 30
Planned Study Period	03 / 09 / 2021 – 03 / 09/ 2023
Research Question/Aim(s)	Assess the differential genomic aberrations and gene expressional alterations in hormone-naïve primary PCa and paired skeletal metastases.
	Determine whether co-existent intra-prostatic hypoxia, EMT and genomic instability is a co-factor in driving early spread.
	Identification of co-existence of hypoxia in primary and metastatic tumour sites to identify co-existent hypoxia in primary PCa and paired bone metastases.
	Assess the prevalence and origin of CTCs in early, aggressive (oligo-) metastatic disease.
	Validation of MRI imaging biomarkers for detection of hypoxia subregions within primary PCa
Arm 1	
Inclusion criteria	Male patients aged 18 years and older
	Histologically proven adenocarcinoma of the prostate (≥cT2) OR Highly suspected metastatic prostate cancer
	PSA value of ≥ 20 ng/mL
	Multiple lesions (≥ 5) suspicious of metastatic spread on routine imaging procedures with at least one amenable* to biopsy (cohort A) OR oligometastatic bone disease (≥1 to ≤ 4) at routine bone scan with at least one lesion amenable* to biopsy (cohort B) *e.g. safely to biopsy and expectably providing sufficient tissue yield

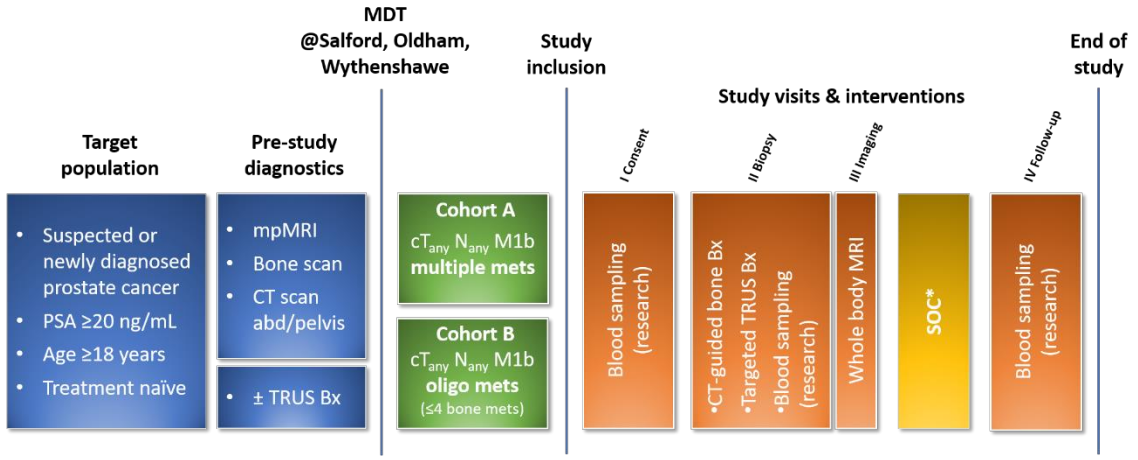
	World Health Organisation (WHO) performance status 0 to 2 with no deterioration over the previous 2 weeks and minimum life expectancy of 12 months
	No prior local and/or systemic treatment for localised prostate cancer
	Willing to donate cancer tissue samples for research purposes (bone metastasis and primary tumour)
Exclusion criteria	Involvement in the planning and/or conduct of the study (applies to staff at the study site)
	Previous enrolment in the HYPROGEN study
	As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g. uncompensated respiratory, cardiac, hepatic or renal disease)
	Evidence of any other significant clinical disorder or laboratory finding that made it undesirable for the patient to participate in the study
	Any investigational agents or study drugs from a previous clinical study within 30 days of the first tissue collection
	Prior treatment of localized prostate cancer including radiotherapy and/or androgen-deprivation therapy
	Judgment by the investigator that the patient should not participate in the study if the patient is unlikely to comply with study procedures, restrictions and requirements
	Contra-indications to MRI (incl. pacemakers etc.)
	Bone metastases in difficult to reach areas or areas which might be at risk for pathological fracture post biopsy as judged by biopsying radiologist / chief investigator
	Increased risk of bleeding as a result of biopsy
	History of bleeding disorders or thrombocytopenia (platelets <100/nL)
	Concomitant treatment with anticoagulant therapy, e.g. warfarin/low molecular weight heparin or Anti-Xa-inhibitors and other NOACs, if temporary cessation medically not justifiable
	Current urinary tract infection (UTI) or prostatitis
Arm 2	
Inclusion criteria	Male patients aged 18 years and older cT_2-T_3 / cN_0-N_1 / cM_0 Any Group Grade (GG) 2-5: this includes Gleason scores 3+4, 4+3, 4+4, 4+5, 5+3, 5+4, 5+5. Any PSA

	Histologically proven adenocarcinoma of the prostate
	Undergoing radical prostatectomy as primary treatment for localised prostate cancer
	World Health Organisation (WHO) performance status 0 to 2 with no deterioration over the previous 2 weeks and minimum life expectancy of 12 months
	No prior local and/or systemic treatment for localised prostate cancer
	Willing to donate cancer tissue samples for research purposes (any metastasis and primary tumour)
Exclusion criteria	Involvement in the planning and/or conduct of the study (applies to staff at the study site)
	As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g. uncompensated respiratory, cardiac, hepatic or renal disease)
	Any investigational agents or study drugs from a previous clinical study within 30 days of the first tissue collection
	Prior treatment of localized prostate cancer including radiotherapy and/or androgen-deprivation therapy
	Judgment by the investigator that the patient should not participate in the study if the patient is unlikely to comply with study procedures, restrictions and requirements
	Contra-indications to MRI (incl. pacemakers etc.)

3 FUNDING

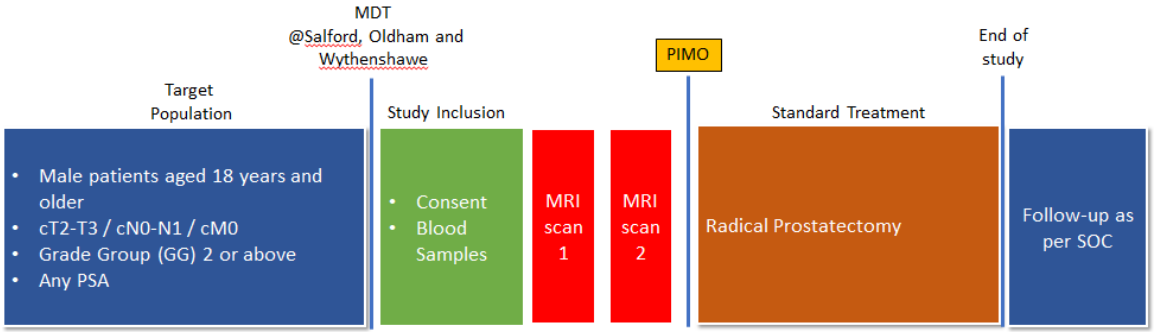
FUNDER(S)	FINANCIAL AND NON FINANCIALSUPPORT GIVEN
FASTMAN Centre of Excellence (PCUK) , CRUK MI – Bristow Funds	Financial coverage for whole study conduct

4 STUDY SCHEMEArm 1



*SOC, standard of care: Androgen deprivation ± Abiraterone Acetate or Docetaxel or Enzalutamide ± radiotherapy to the prostate; check study availability, i.e. STAMPEDE, etc.

Arm 2



5 STUDY INTERVENTIONS

Arm 1

Visit	Consent	Biopsy	Imaging	Follow-up
History taking	▼			
Physical exam	▼			▼

TRUS		▼		
- Prostate biopsy		▼		
CT scan ROI		▼		
- Bone biopsy		▼		
wb MRI			▼	
Blood sampling (routine)	▼			
Blood sampling (research)	▼	▼		▼ (patients 11-30 only)

CT, computed tomography; wb MRI, whole body magnet-resonance imaging; ROI, region of interest; TRUS, transrectal ultrasound

Arm 2

Visit	Consent	1st MRI scan	2nd MRI scan	Procedure
History taking (standard of care)	▼			
Physical exam (standard of care) Diagnostic and staging tests (Standard of care)	▼			
Radical Prostatectomy (standard of care)				▼
MRI scan		▼	▼	
Blood sampling (standard of care)	▼			
Blood sampling (research)	▼			

6 BACKGROUND

6.1 Background

Metastatic prostate cancer

Prostate cancer is the most common malignancy in men and the second commonest cause of cancer-related deaths in Western countries. Around 47,700 patients will be diagnosed with prostate cancer (PCa) in the UK each year [1]. About 15% present with primary M1 disease, which remains incurable despite remarkable improvements of treatment over the last decade. Interestingly, about 60% of M1 patients show solely metastasis to the bone sparing lymph nodes and any other organs. However, there is a paucity of data explaining both, early metastatic spread and the preference of prostate cancers to metastasise to the bones.

Oligometastatic disease

Oligometastatic solid tumours refers to a stage with a limited number of metastases and is perceived to be a distinct subtype of malignancy where more aggressive or at least more effective treatment holds promise to sustainably eradicate all lesions and thus to be curative [2]. For PCa, oligometastatic disease has not yet been adopted as an own subtype of disease, but it can be defined as the presence of ≤ 4 non-locoregional metastases. Recently, the STAMPEDE study reported a remarkable survival improvement by the addition of radiotherapy of the prostate to androgen-deprivation as standard of care in patients with a maximum of 4 bone metastases as identified by routine bone imaging, which is why we decided to apply a cut-off of ≤ 4 bone metastases on routine bone scintigraphy to define a PCa as being oligometastatic [3]. Consequently, novel targeted, biomarker-driven treatment approaches may ultimately help to improve treatment success, particularly in oligometastatic PCa.

Tumour hypoxia

The tumour microenvironment has a strong influence on tumour growth and progression, which renders it a potential target for novel treatment approaches. For instance, it is characterised by dynamic gradients of oxygen diffusion and consumption leading to sub-regions of hypoxia in about half of all solid tumours. Tumour adaptation to imbalanced oxygen supply and demand is associated with elevated genomic instability, resistance to chemotherapy and radiotherapy, immune dampening, altered metabolism, cell death resistance, development of tumour stem cell protective niches, increased mobility and invasiveness though epithelial-mesenchymal-transition (EMT) of tumour cells and increased proclivity for distant metastasis, such as bone metastases, among others [4-6]. Consequently, cancer patients with hypoxic tumours, including PCa, have a dismal prognosis irrespective of the applied treatment modality [7]. Given its central role in tumour aggressiveness and progression and treatment resistance, tumour hypoxia might

well be considered the best validated target that has yet to be successfully exploited in oncology [8].

6.2 Rationale

Ongoing Phase III studies are testing whether oligometastatic disease can be eradicated with local ablation in addition to best systemic care; but a mechanistic basis for further personalised intensified treatment in the systemic arms, e.g. by adding anti-hypoxia compounds for the most hypoxic tumours, is lacking. As hypoxia is scarce in normal, vital tissues, targeting this characteristic of aggressive PCa will most likely primarily kill cancer cells and augment the therapeutic ratio for patients with both, oligometastatic and widespread metastatic disease.

6.3 Theoretical Framework

As described in the background section, tumour hypoxia is a driver of tumour aggressiveness conferring a poor prognosis. Hypoxia can select for cancer cells that are apoptosis-deficient, contain *TP53* mutations, and have increased genomic instability leading to a mutator phenotype [7]. Importantly, the Bristow lab previously reported that the co-presence of tumour hypoxia (based on mRNA signatures or needle electrode measurements) and genomic instability synergistically portend rapid relapse after primary treatment for PCa, supporting the concept that a hostile tumour microenvironment may select for or drive adaptation of a distinctive genomic profile and rapid failure due to occult metastases [9]. In line with this, a study from the West group showed that a prostate-specific hypoxia-associated 28-gene RNA expression signature portends poor prognosis [10]. Therefore, we aim to comprehensively assess the impact of a hypoxic primary tumour on the oxygenation of its bone metastases and the resulting genomic and gene expressional aberrations supporting the development of early metastatic spread to the bone.

6.4 Research Hypotheses

1. Hypoxia drives genomic instability in primary prostate cancers.
2. Co-existent intra-prostatic hypoxia and genomic instability is a co-factor in driving early metastatic spread to the bone.
3. Hypoxia drives migration and invasion increasing the prevalence of circulating tumour cells (CTCs) disseminating from the primary tumour to form oligo metastases
4. Hypoxia is present in skeletal metastases themselves.

5. Primary prostate cancer and early bone metastases have a distinct genomic and transcriptomic profile related to hypoxia.
6. CTC genomes more strongly correlate with hypoxic regions of primary tumour.
7. MRI hypoxia sequences are able to detect hypoxic sub-regions within prostate cancer.

7 RESEARCH AIMS

Assess the differential genomic aberrations and gene expressional alterations in hormone-naïve primary PCa and paired skeletal metastases.
Whole exome sequencing (WES) analysis will be performed and the results of the paired samples of the study patients will be compared in silico to ~500 patients with pT1-3 N0M0 disease from the CPC-GENE bioinformatics portal as a control set [11]. Moreover, to assess the gene expression profile of primary tumour and metastases, the whole RNA will be sequenced (RNAseq).

Determine whether co-existent intra-prostatic hypoxia, EMT and genomic instability is a co-factor in driving early spread
Hypoxic areas of primary tumour identified through either pimonidazole or other hypoxia labelling method such as GLUT1 immunohistochemical staining (if available, otherwise archival FFPE tissue from primary diagnostic biopsy) and metastatic tumour tissue will be micro-dissected and analysed by WES, RNAseq, and a targeted mRNA analysis to detect the Manchester 28-gene expression signature predicting for tumour hypoxia. Data pertaining to the co-occurrence in regions identified as hypoxic using immunohistochemical stains (such as pimonidazole or GLUT1), Copy Number Alterations, Single Nucleotide Variants and whole RNA expression profiles as well as the Manchester hypoxia 28-gene RNA signature will be compared.

Identification of co-existence of hypoxia in primary and metastatic tumour sites
Results of immunohistochemically identified hypoxic regions (through PIMO-staining or alternate staining method such as GLUT1) and the 28-gene RNA expression of the samples will be compared for each patient to identify co-existent hypoxia in primary PCa and paired bone metastases.

Assess the prevalence and origin of CTCs in early, aggressive (oligo-) metastatic disease

To increase sensitivity of detection marker-independent platforms will be used. CTCs will be isolated and molecularly profiled to determine whether they originate from primary tumour and/or from metastases. Moreover, CTC genomes will be mapped to primary/metastatic tumour regions identified as having high or low levels of hypoxia (through pimonidazole or other staining method) to determine if hypoxia (and hypoxia associated EMT) drives tumour cell dissemination.

8 STUDY PROCEDURES & METHODOLOGY

8.1 Study Design

This is a prospective, non-randomised, single-centre, exploratory biomarker study. In Arm 1 this involves targeted tumour tissue sampling in men with either newly diagnosed, untreated metastatic prostate cancerinvolving patients with either high (cohort A; widespread metastatic disease) or low, oligometastatic (cohort B; ≤ 4 bone metastases) burden of disease. In Arm 2, targeted tumour tissue samples derived from prostate cancers biobanked as per standard MCRC biobanking protocol in 30 men undergoing a radical prostatectomy as part of their standard care.

In Arm 1, to minimize the interventional burden for the patients, patients will be amenable to have a combined diagnostic and research biopsy of the prostate in one session after routine imaging for diagnostic work-up comprising of a CT scan of abdomen and pelvis, bone scan and multi-parameteric MRI of the prostate. Patients, who already had a diagnostic TRUS biopsy will be asked to provide research tissue samples during a second TRUS biopsy as part of the HYPROGEN study interventions. Patients declining a second prostate biopsy will be eligible and will undergo the bone biopsy only. Patient identification will be done through the respective multidisciplinary tumour (MDT) boards and patient referral pathways at Salford Royal, Oldham Royal and Wythenshawe Hospital as further outlined in section 9.1.

To yield highest possible biopsy accuracy and tissue amount and maximum procedure safety, prostate biopsy will be a TRUS-guided targeted transperineal biopsy and bone biopsy will be CT-guided using an automated drill. Moreover, to identify the imaging modality with the highest accuracy in detecting bone-metastatic prostate cancer at first presentation, an additional whole-body MRI will be applied to patients on the study. Study participation will not affect the routine care for this stage of disease, which will start immediately after the biopsy samples have been acquired.

In Arm 2 tumour tissue samples will be acquired from prostatectomy specimens that patients are undergoing as part of there routine standard of care.

8.2 Study Visits and End of Study

Arm 1 Once a patient has been identified at a referring urology centre at Oldham, Salford or Wythenshawe to meet the HYPROGEN Arm 1 study inclusion criteria, he will be informed about this trial by the local care team and provided with the participant information leaflet. The patient will be asked for permission to be contacted by the HYPROGEN study staff at The Christie NHS Foundation Trust to evaluate his interest in study participation. If a patient is willing to participate, he will then be invited to an outpatient research clinic to recapitulate potential risks and benefits of study participation (consent visit).

If a patient agrees to participate, the informed consent form will be signed and the optional non-IMP pimonidazole will be prescribed by the consenting research team member. The hypoxia marker agent pimonidazole will be dispensed by The Christie Research Pharmacy, and the participant will be asked to take it at home the day before the biopsy visit.

Within a further 7 working days from consent to the study, study participants will receive a CT-guided bone biopsy and a TRUS-guided transperineal biopsy of their prostate gland as an outpatient. During the following 10-15 working days, patients will undergo a whole-body MRI scan at the Radiology Department of The Christie.

If the patient refuses the pimonidazole, forgets to take it, or if it is not available, the patient can still participate in the study and their samples will be stained for hypoxia post-biopsy.

After the biopsy, patients will be referred back to their consultant urologists at Oldham, Salford or Wythenshawe for standard routine care or discussion of additional treatment options, i.e. inclusion in the STAMPEDE study, which almost all patients eligible for the HYPROGEN study will be eligible for. The treatment as per standard of care in accordance with the recommendation of the MDT discussion will commence on the day after the biopsy visit.

Within the 3-6 months following the biopsy, the patients will be invited back to the Christie to provide a follow-up research blood sample. The study ends after the follow-up visit of the last participant.

Arm 2

In patients identified as eligible for Arm 2 of the Hyprogen study, the study will be discussed with eligible patients by their referring consultant and provided with the

participant information leaflet. The patient will be asked for permission to be contacted by the PROXIES study staff at The Christie NHS Foundation Trust to evaluate his interest in study participation. If a patient is willing to participate, he will then be invited to The Christie outpatient research clinic to recapitulate potential risks and benefits of study participation (consent visit). If a patient agrees to participate, the informed consent form will be signed and the non-IMP pimonidazole will be prescribed.

The hypoxia marker agent pimonidazole will be dispensed by The Christie Research Pharmacy, and the participant will be asked to take it at home the day before planned radical prostatectomy.

The study ends after the last patient has undergone a radical prostatectomy.

8.2.1 Pimonidazole Hydrochloride

For the purposes of this study patients will be asked to ingest an oral formulation of pimonidazole hydrochloride (HCl) (Oral Hypoxyprobe^{TM}-1). Pimonidazole HCl is a marker for hypoxia in tumour tissue when ingested as an encapsulated solid. Following oral administration, pimonidazole distributes throughout the body where it covalently binds to normal and tumour tissues that have regions of low oxygen concentrations (pO_2 of ≤ 10 mmHg at 37°C). The tissue binding can be visualised by immunohistochemistry / light microscopy. Pimonidazole HCl has been issued by the US Food and Drug Administration as Investigational New Drug status as a diagnostic for human tissue hypoxia and a number of studies in the US, Canada and Europe use encapsulated pimonidazole HCl to study of human tumour hypoxia *in vivo*. Pimonidazole HCl is given orally as capsules at a dosage of 0.5 g/m^2 body surface area. For oral administration, pimonidazole HCl is supplied in the form of capsules containing 200 mg (coloured white) and 300 mg (coloured orange brown) of the substance, admixed with lactose excipient in the case of 200 mg capsules. The colouring is supposed to prevent errors in the choice of capsules. A clinical investigation begins with a calculation of the total amount of pimonidazole HCl needed to achieve a dosage of 0.5 g/m^2. For choosing the correct number of capsules, the total amount of pimonidazole HCl in milligrams is rounded to the nearest whole number according to the investigator's brochure (see following table).

Total amount of pimonidazole HCl	200 mg capsules	300 mg capsules	Total No. of capsules
600 mg	0	2	2
700 mg	2	1	3
800 mg	1	2	3
900 mg	0	3	3
1000 mg	2	2	4

1100 mg	1	3	4
1200 mg	0	4	4

The dose limiting toxicity for pimonidazole hydrochloride in humans was identified, when pimonidazole was evaluated as a radiosensitising agent for solid malignancies. It was central nervous system toxicity [11, 12]. Importantly, oral administration of 0.5 g/m^2 has been well tolerated without any signs of toxicity.

Following ingestion, pimonidazole HCl is distributed to all tissues in the body including the brain but forms adducts with proteins only in those cells that have oxygen concentrations, e.g. a pO_2 of less than 10 mm Hg at 37°C. In addition to tumours, a number of normal tissues such as liver, kidney and skin possess cells at, or below, a pO_2 of 10 mmHg. These normal tissues will also bind pimonidazole. Pimonidazole HCl is primarily eliminated via urinary excretion mostly within 72 hours of ingestion, a small amount appears in the faeces or gets eliminated by tissue metabolism.

The capsules are to be taken within 8-16 hours (optimal timepoint 12 hours) before the planned first biopsy within Arm 1 and before radical prostatectomy for patients in Arm 2. If the patient refuses the pimonidazole, forgets to take it, or if it is not available, the patient can still participate in the study and their samples will be stained for hypoxia post-biopsy.

8.3 Patient Benefit and Overall Risk

This is a prospective, translational, exploratory biomarker study, which is why there will be no direct benefit derived from the data obtained for the individual PCa patient participating in this study. However, this study may ideally lead to the identification of tumour hypoxia and its related aberrations as a therapeutic target in (oligo-)metastatic PCa in the near future. Results of the HYPROGEN study will not affect treatment according to the current standard of care including - for patients in Arm 1 - androgen deprivation with or without more intensified treatment including docetaxel, abiraterone acetate plus prednisone and/or irradiation of the prostate.

Within Arm 1 Patients who are enrolled onto this study will need to make four additional outpatient visits to the hospital for the duration of the study (consent, biopsy, imaging, and follow up visit).

Patients entering this study will undergo additional imaging with a whole-body MRI as well as additional biopsies of tissues affected by their cancer at two body areas as well as additional blood sampling for research purposes. The tissue biopsy sites include the prostatic gland and the bones. There may be side effects associated with these biopsies.

The additional whole-body MRI scan will help to further define the sensitivity of routine bone scanning and MRI scanning to detect a metastatic state.

Biopsies of the prostate are part of normal regular care performed on all patients with *de novo* metastatic PCa. As for routine diagnostic purposes, prostate biopsies will be done though the perineum guided by trans-rectal ultrasound (TRUS) targeting lesions, which may have already been identified in the pre-study diagnostic multiparametric MR scan of the prostate (if available). Preferably, routine diagnostics and research sampling will be undertaken during the same biopsy procedure. However, if a diagnostic TRUS biopsy of the prostate has been performed before, the research biopsy will require an additional intervention surplus the bone biopsies and the blood sampling, which are not part of routine diagnostics and thus cannot be seen as standard of care. If a second prostate biopsy solely for research purposes is denied by the patient, only a bone biopsy and a blood sample will be taken. Importantly, the techniques of image-guided biopsy taking are routinely done for the diagnosis and management of patients with different types of cancers and thus safely applicable. As part of the HYPROGEN study, the bone biopsies will be used to histologically confirm the diagnosis of metastatic disease in a radiographically bone metastatic stage.

Most side effects associated with the medical procedures in this study are expected to be mild to moderate and to disappear after a short time. The main risks related to the HYPROGEN study interventions comprise the following:

Pain or Discomfort

Biopsies involve needle tests of deeper tissues and can cause pain or discomfort both at the time of the biopsy and also for up to several days after the biopsy. Local anaesthetics injection will be given for both the prostate biopsies as well as the bone biopsies to numb the respective site as good as possible to reduce the possible discomfort. The local anaesthetic injection itself may cause some temporary pain or burning sensation until the onset of numbness, and as the anaesthetics wear off pain may re-appear. Simple systemic analgesics such as paracetamol may be advised if pain occurs as a result of the biopsies.

Bleeding or Bruising

Any needle test can be associated with bleeding or bruising. Blood clotting will be assessed during the screening process and patients will not proceed to the biopsy visit if they appear to be at higher bleeding risk than normal. Anticoagulant treatment will be halted if medically justifiable. Biopsies close to higher risk areas such as large blood vessels will be avoided. However, despite these precautions, rarely the bleeding from biopsies can cause significant blood loss or risk to life.

Infections

Patients are vulnerable to infection while having a biopsy or blood sample taken. Minor infections can become life-threatening. Symptoms of infection include fever, shivering, sweats, sore throat, diarrhoea, dysuria, haematuria, cough or breathlessness. Patients will be asked to measure the core body temperature on the days after the biopsy visit and to report any febrile temperatures or signs of infection immediately to the study staff or via The Christie Hotline under 0161 446 3658, which is open 24/7.

All patients undergoing a transperineal prostate biopsy will receive antibiotic prophylaxis as per local policy for prophylactic antibiotic treatment for prostate biopsies.

Arm 2

This is a prospective, translational, exploratory biomarker study, and it is unlikely to directly benefit the participants of this study. However, the results of this study may provide benefit for prostate cancer patients in the future.

Participants will take an oral pimonidazole tablet prior to their radical prostatectomy as part of the study. Oral pimonidazole at a dose of 0.5 g/m^2 has been well tolerated without any signs of toxicity.

Participants in Arm 2 agreeing to additional imaging will undergo 2 additional MRI scans during which they will receive intravenous gadolinium contrast and be asked to breath 100% oxygen through an oxygen mask.

8.4 CT-guided Bone Biopsy (Arm 1)

A CT-guided biopsy of a bone metastasis that is deemed to be easy to biopsy and in an area without major risk for pathological fracture or bleeding will be taken during the biopsy visit. Patients will receive routine local anaesthetic of the region to be biopsied followed by thorough disinfection of the biopsy site with antiseptic wipes. Patients will be asked to fast on the day of the procedure and to have an intravenous cannula inserted to allow the use of medication causing minimal sedation (for example midazolam and/or fentanyl) during the procedure if required to alleviate discomfort or pain. For biopsy sampling the Arrow® OnControl® Powered Bone Access System (Teleflex, USA) will be used. In patients with greater than one bone metastasis assessed as amenable to bone biopsy, biopsies will be obtained from a maximum of two sites of bone metastases. A maximum of three specimens from each biopsy site will be taken. Samples will be examined on site by a histopathologist to warrant collection of high-quality material and advise the sampling as needed. Maximum duration of procedure will be 45-60 min.

Following the bone biopsy, the patient will be observed in the Radiology Department to be assessed for any excessive bleeding before transferring.

8.5 TRUS-guided Targeted Transperineal Prostate Biopsy (Arm 1)

Transperineal Prostate Biopsy will be performed following standard clinical practice of local department. This will include pre-operative oral analgesia and prophylactic antibiotic treatment according to local hospital policy for transperineal prostate biopsies.

Patients will be placed in lithotomy position andthe scrotum will be carefully taped out of the way. As per local hospital practice the procedure may - at the discretion of the performing surgeon - involve application of topical ointment (ie 2% diltiazem) to relax the anal sphincter, insertion of a transurethral catheter for the procedure and/or the use of a local lidocaine-containing gel (Instillagel) inserted into the patients back passage and applied to the anus to alleviate discomfort related to the ultrasound probe. The perineal skin will be cleaned with an antiseptic solution. An ultrasound probe is then passed into the back passage, which will remain there for the duration of the procedure to visualise the prostatic gland to guide perineal sampling. In order to numb the perineum and prostate as much as possible, a local anaesthetic will then be injected underneath the skin and into the deeper tissue layers guided by ultrasound. Systematic and targeted core biopsies from the prostate will be acquired.In addition to core biopsies required for standard of care diagnostic purpose, lesions seen on a mpMRI (if available) will be targeted to allow a minimum of 4 core biopsies from any detected lesion as well as at least 2 core biopsies from uninvolved prostate tissue.Otherwise cognitive biopsies guided by ultrasound will be obtained. If a second prostate lesion is detected, core biopsies will also be acquired from this lesion. In total, in addition to any core biopsies required as standard of care a maximum number of 8 to 12 additional prostate core biopsies will be taken for research purposes. Maximum duration of procedure will be 60 minutes.

8.6 Radical Prostatectomy (Arm 2)

Radical Prostatectomy will be performed according to standard of care robotic approach used at The Christie Hospital and as relayed to the patient by the attending urologic surgeon. The side effects of the surgery are the ones reported in the literature and the latest participant information leaflet provided prior patient consent (e.g. risk of erection disfunction, incontinence, etc.).

The current study will not imply any change of the current surgical procedure as standard of care conducted routinely at the Christie Hospital. It will not increase the incidence of known potential co-morbidities already described in general for a radical prostatectomy nor instructions to the patient by the attending urologic surgeon.

8.7 Blood Sampling

For patients in Arm 1 - A blood sample for research purposes will be withdrawn at the each of the following visits:

(i) Baseline visit (pre- pimonidazole)

- 2 x 10mL *Streck cell-free DNA blood collection tubes*® for circulating tumour cell (CTC) collection and circulating tumour DNA (ctDNA) extraction.
- 1-2 x 3.5mL blood samples collected into EDTA tubes for germ line DNA extraction and processing to PBMC for banking and future profiling of immune cell populations
- 1 x 10mL serum tubes for future, biobank related research projects, ie lipidomics, metabolics and microRNA analysis

(ii) Biopsy visit (pre-biopsy)

- 2 x 10mL *Streck cell-free DNA blood collection tubes*® for circulating tumour cell (CTC) collection and circulating tumour DNA (ctDNA) extraction.
- 1 x 10mL serum tubes for future, biobank-related research projects, ie lipidomics, metabolics and microRNA analysis

(iii) Follow up visit (within 6 months post-treatment, for patients 11-30)

- 2 x 10mL *Streck cell-free DNA blood collection tubes*® for circulating tumour cell (CTC) collection and circulating tumour DNA (ctDNA) extraction.
- 1-2 x 3.5mL blood collected into EDTA tubes for germline DNA extraction and processing to PBMC for banking and future analysis of immune cell populations
- 1 x 10mL serum tubes for future, biobank related research projects, ie lipidomics, metabolics and microRNA analysis

For Patients in Arm 2

A blood sample (maximum 20ml) will be taken for standard of care blood tests prior to prostatectomy including Full Blood Count, Renal Function and PSA. At the same time these standard of care bloods are taken, additional bloods - a maximum of 30ml – will be taken for research purposes as required for the following downstream analysis:

- Germ line DNA extraction and optional processing to PBMC for banking and future profiling of immune cell populations
- Biobank related research projects, ie lipidomics, metabolics and microRNA analysis

8.8 Whole-body MRI (Arm 1)

For patients within Arm 1, Whole-body MR imaging (wbMRI) will be performed once, before or after the biopsy study visit, depending on available examination slots in the Department of Radiology at The Christie NHS Foundation Trust. wbMRI images will allow comparison of the numbers of bone metastases detected by routine bone scan and wbMRI for sensitivity assessment of both techniques for oligometastatic disease.

MR Sequences acquired following Christie Hospital Whole Body MRI Image Acquisition Guidelines. The wbMRI scan is estimated to last around 70 minutes.

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8.9 Prostate MRI scans (Arm 2)

Patients within Arm 2 will be offered the option to undergo additional MR imaging of the pelvis in addition to any standard of care imaging acquired. In patients who agree to undergo additional scans, MRI scans will be performed on 2 occasions prior to the radical prostatectomy. MRI scans will be acquired on either the MR sim diagnostic scanner, on the MR Linac scanner or on both within The Christie NHS Foundation Trust.

The MR sequences obtained will include sequences for oxygen-enhanced MR, IVIM, DCE and BOLD and aim to detect hypoxic sub-regions of tumour that can be validated against pimonidazole staining regions following prostatectomy. Patients will be asked to will breathe 21% followed by 100% oxygen gas through an oxygen mask during the scan. They will receive a gadolinium-based contrast agent through an intravenous cannula. Scan time will not exceed 60 minutes per scan. Any patients not agreeing to undergo additional MRI scans are still able to be enrolled into Arm 2 of the trial.

8.10 Tissue Processing and Storage

The PrestoCHILL freezing device (Milestone Medical, Menarini Diagnostics, UK) will be used in order to access tumour content and select appropriate material for obtaining the highest quality DNA and RNA, retaining also an appropriate amount for FFPE evaluation and studies. Samples will be collected and stored at the Manchester Cancer Research Centre Biobank. Serum samples will be centrifuged and frozen at -20°C at the MCRC biobank. The other blood samples will be taken to the biobank at room temperature, *Streck* tubes and EDTA tubes will then be send to the CRUK Manchester Institute Cancer Biomarker Centre (CBC) (Prof Caroline Dive).

Prostate specimens will be routinely sectioned with a cryotome and sections for diagnostic routine H/E histopathology as well as additional sections for pimonidazole or alternative hypoxia staining method (such as GLUT1 immunohistochemical staining) and nucleic acid extraction for subsequent genomic/transcriptomic analysis cut. Bone specimens will be similarly frozen and analysed but cut on the cryotome using special *cryo tungsten carbide blades*. Tissue cryo-preservation, sectioning and processing will be carried out by the Histopathology Department at The Christie NHS Foundation Trust and Cancer Research UK Manchester Institute (CRUK MI).

8.11 Hypoxia Assessment and Analysis of Tumour Genome and Transcriptome

Fresh-frozen tumour samples will be analysed by both conventional histopathology and comprehensive assessment of the tumour genome and functional gene expression profile. 4-5µm sections will be taken for immunohistochemistry, 10 µm sections will be taken for nucleic acid extraction. A consultant histopathologist (Dr Pedro Oliveira, The Christie NHS Foundation Trust) will (i) confirm the diagnosis of prostate cancer and will allow for proper diagnostic work-up including grading according to the Gleason score (H/E staining) and (ii) allow assessment of the presence of intratumoural hypoxia as detected by immunohistochemical staining for pimonidazole. If the patient did not receive pimonidazole pre-biopsy then an alternative immunohistochemical stain such as GLUT1 will identify hypoxic areas. Hypoxic areas will be marked to guide microdissection for subsequent nucleic acid extraction from the respective slides.

Further analysis of the tumour DNA, RNA and functional transcriptome will include whole exome sequencing (WES), which may be carried out by a third party as appropriate, with all necessary agreements in place.

8.12 Assessment of circulating tumour cells (CTCs) and circulating tumour DNA (ctDNA)

In collaboration with the CRUK Manchester Institute Cancer Biomarker Centre (CBC) (Prof Caroline Dive) blood samples will be used to assess the prevalence and origin of CTCs and ctDNA. Previous studies have demonstrated that the presence of 5 or more CTCs at baseline, as determined using the CellSearch platform, are a prognostic factor for overall survival in metastatic prostate cancer [14, 15]. Although the CellSearch assay is the only FDA-approved method for CTC detection in prostate cancer, the fact that the system relies on CTCs expressing EpCAM antigen for capture means that CellSearch compares unfavourably with marker-independent platforms for CTC capture in this disease [16]. The CRUK MI CBC have extensive experience of CTC analysis in a variety of clinical settings, and have developed a number of platforms and methods for CTC

capture and analysis. In addition to the CellSearch platform, in routine use in the CBC are microfluidics-based, marker independent CTC enrichment systems (Parsortix, ClearCellFX), and also slide-based “no cell is lost” capture systems (RareCyte, High-Definition Single Cell Analysis, HD-SCA). These marker-independent platforms are amenable to the study of CTCs in prostate cancer. For this study CTCs will be enriched using the Parosortix platform and enumerated and characterised using bespoke assays developed by CBC. Characterisation will include staining of captured cells for known CTC and exclusion markers, plus if required bespoke staining assays can be developed for markers relevant to prostate cancer, e.g. AR, PTEN. Additionally, single CTCs can be isolated for genomic analysis to complement analysis of the tumour genome, and analysis of ctDNA. CTC genomes will then be compared to primary and metastatic lesions to determine their origin(s). Moreover, it will be assessed if CTC genomes are enriched for hypoxic regions (based on pimonidazole labelling or an alternative hypoxia biomarker such as GLUT1).

Profiling of ctDNA is a key aspect of the work of the Nucleic Acid Biomarkers (NAB) team, and which complements the CTC analysis outlined above. The NAB team has developed methods for the isolation of ctDNA from patient samples, and also analytical approaches including gene copy number variation (CNV), targeted sequencing using droplet-digital PCR and selected gene panels, and discovery approaches such as whole exome sequencing (WES). There are a number of examples where analysis of ctDNA from patient blood samples has confirmed and extended detections of mutations in primary tumours and metastases in prostate cancer (e.g. [17, 18]). Analysis of patient ctDNA is particularly amenable to the stated aims of this study, specifically assessing the genomic aberrations in hormone-naïve primary prostate cancer, and determining genomic instability driving early spread. Certain genomic aberrations have been documented as prognostic in metastatic prostate cancer, including androgen receptor point mutations and splice variants [19-21], PTEN loss which is associated with decreased AR activity [22], and mutations in DNA repair genes [23]. A panel of these genes for targeted sequence analysis based on ctDNA will be applied, consistent with the biopsy-driven analysis described earlier.

8.13 Storage and use of tissue and blood sample left-overs

Patients participating in this study also consent that any unused samples at the end of this study will be gifted to the Manchester Cancer Research Centre (MCRC) Biobank for use in future research projects. The MCRC Biobank makes samples available to all kinds of researchers, including pharmaceutical companies for all kinds of cancer research and genetic studies. Access to Biobank samples is strictly regulated and the samples will only ever be released for high quality cancer research projects approved by

the MCRC Biobank Access Committee. Researchers using samples will only ever receive anonymised data and personal identifiable information will be kept strictly confidential.

9. PATIENT SELECTION AND RECRUITMENT

9.1 Patient Identification

Patients eligible for study inclusion will be identified from their medical records after routine staging procedures for newly diagnosed prostate cancer during the respective multidisciplinary team meeting (MDTs) or patient referral pathway and then be informed of this study by their local clinical care team within the respective urology clinics at the University Hospital of South Manchester NHS Foundation Trust in Wythenshawe (Urology consultant: Prof V Ramani), The Royal Oldham Hospital (Urology consultant: Mr J Oates), and The Salford Royal NHS Foundation Trust (Urology consultants: Prof N Clarke, Mr Satish Maddineni).

The local clinical care team provides eligible patients with the participant information leaflet and requests the patient to allow the study staff at The Christie NHS Foundation Trust to contact the patient to evaluate his interest in study participation. If a patient is willing to participate in the HYPROGEN study an appointment at The Christie outpatient research clinic for another, detailed recapitulation of the studies' aims and potential risks and benefits of study participation will be scheduled for within the next 5 working days (consent visit). Patients that are either not suitable for inclusion or whom do not wish to participate will continue along the routine care pathway.

9.2 Sample Size

A total number of 60 patients will be recruited into this pilot study, 30 patients with metastatic disease in Arm 1 and 30 patients with localised prostate cancer in Arm 2. Within Arm 1, 15 patients with a high disease burden identified as a number of bone metastases ≥ 5 with or without visceral metastases (cohort A) and 15 with a low burden of bone metastases defined as ≤ 4 bone metastases and no signs of visceral disease (cohort B) detected on routine bone scan imaging. As this is a feasibility study, no power calculation was applied for patient number estimation.

9.3 Patient Selection Criteria

All patients must meet all of the inclusion criteria and none of the exclusion criteria for this study at the time of registration. Under no circumstances can there be exceptions to this rule.

9.4 Inclusion Criteria

Arm 1

No	Inclusion Criteria Patients are eligible only if they meet all of the following criteria
1	Male patients aged 18 years and older
2	Histologically proven adenocarcinoma of the prostate (≥ cT2) OR Highly suspected metastatic prostate cancer
3	PSA value of ≥ 20 ng/mL
4	Multiple lesions (≥ 5) suspicious of metastatic spread on routine imaging procedures with at least one amenable* to biopsy (cohort A) OR oligometastatic bone disease (≥1 to ≤ 4) at routine bone scan with at least one lesion amenable* to biopsy (cohort B) *e.g. safely to biopsy and expectably providing sufficient tissue yield
5	World Health Organisation (WHO) performance status 0 to 2 with no deterioration over the previous 2 weeks and minimum life expectancy of 12 months
6	No prior local and/or systemic treatment for localised prostate cancer
7	Willing to donate cancer tissue samples for research purposes (bone metastasis and primary tumour)

Arm 2

No	Inclusion Criteria Patients are eligible only if they meet all of the following criteria
1	Male patients aged 18 years and older cT_2-T_3 / cN_0-N_1 / cM_0 Any Group Grade (GG) 2-5: this includes Gleason scores 3+4, 4+3, 4+4, 4+5, 5+3, 5+4, 5+5. Any PSA
2	Histologically proven adenocarcinoma of the prostate
3	Planned radical prostatectomy as primary treatment for localised prostate cancer
4	World Health Organisation (WHO) performance status 0 to 2 with no deterioration over the previous 2 weeks and minimum life expectancy of 12 months
5	No prior local and/or systemic treatment for localised prostate cancer
6	Willing to donate cancer tissue samples for research purposes (any metastasis and primary tumour)

9.5 Exclusion Criteria

Arm 1

No	Exclusion Criteria Patients will not be entered in the study for any of the following reasons
1	Involvement in the planning and/or conduct of the study (applies to staff at the study site)
2	Previous enrolment in the HYPROGEN study
3	As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g. uncompensated respiratory, cardiac, hepatic or renal disease)
4	Evidence of any other significant clinical disorder or laboratory finding that made it undesirable for the patient to participate in the study
5	Any investigational agents or study drugs from a previous clinical study within 30 days of the first tissue collection
6	Prior treatment of localized prostate cancer including radiotherapy and/or androgen-deprivation therapy
7	Judgment by the investigator that the patient should not participate in the study if the patient is unlikely to comply with study procedures, restrictions and requirements
8	Contra-indications to MRI (incl. pacemakers etc.)
9	Bone metastases in difficult to reach areas or areas which might be at risk for pathological fracture post biopsy as judged by biopsying radiologist / chief investigator
10	Increased risk of bleeding as a result of biopsy
11	History of bleeding disorders or thrombocytopenia (platelets <100/nL)
12	Concomitant treatment with anticoagulant therapy, e.g. warfarin/low molecular weight heparin or Anti-Xa-inhibitors and other NOACs, if temporary cessation medically not justifiable
13	Current urinary tract infection (UTI) or prostatitis

Arm 2

No	Exclusion Criteria Patients will not be entered in the study for any of the following reasons
1	Involvement in the planning and/or conduct of the study (applies to staff at the study site)
2	As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g. uncompensated respiratory, cardiac, hepatic or renal disease)
3	Any investigational agents or study drugs from a previous clinical study within 30 days of the first tissue collection
4	Prior treatment of localized prostate cancer including radiotherapy and/or androgen-deprivation therapy
5	Judgment by the investigator that the patient should not participate in the study if the patient is unlikely to comply with study procedures, restrictions and requirements
6	Contra-indications to MRI (incl. pacemakers etc.)

9.6 Study Payment

Patients will not receive any allowance for study participation including the provision of biopsy tissue or blood samples. However, a reimbursement of up to £40 per research visit may be requested to cover costs incurred for travel to research appointments for those patients within Arm 1 only.

9.7 Informed Consent

A member of the MDT will identify the patient at the MDT and approach the patient for possible inclusion into the study and provide the participant information leaflet either during a routine appointment to discuss the MDT recommendations or by post. All responsible consultant urological surgeons and clinical oncologists are subinvestigators of this study. Informed consent will be obtained appropriate members of the Christie urological research team at The Christie NHS Foundation Trust.

All potential study participants will be given a participant information leaflet prior to informed consent. It should be made clear to the potential trial participant that consent is voluntary and can be withdrawn without giving reasons and without prejudicing their ongoing/future care.

Potential study participants will be given adequate time to consider participation in the study based on the participant information leaflet. If the patient is willing to participate, an appointment will be made at the Christie NHS Foundation Trust within 5 working days, where the informed consent form will be signed. The patients' pimonidazole hydrochloride tablets will be dispensed from the Christie Pharmacy, if they have agreed to it and it is available. A research blood sample will also be taken at this visit.

Consent will be taken by a researcher who is a GCP trained doctor and who has been delegated by the PI to undertake this activity (and this is clearly documented on the delegation log). No study-related activities can be undertaken prior to consent.

The original, signed copy of the patient information sheet and consent form(s) will be retained in the Investigator Site File, with a copy in the patient notes. A copy will also be given to the participant.

9.8 Ineligible and Non-recruited Patients

Patients who consent to participate in the study, but who are later deemed ineligible during the screening process will be logged on the screen-failure log. Lack of inclusion in this study will in no way affect their ongoing/future clinical care.

9.9 Study Registration

Patients who have consented to participate will be assigned a unique ID code by the HYPROGEN study co-ordinator. This ID code will be used throughout their participation in the study. The master list will be held at the Urology Research Teams' office at The Christie NHS Foundation Trust.

9.10 Withdrawal of Participants

If at any time a patient expresses a wish to withdraw consent for ongoing study participation the following procedures will apply:

- Withdrawal of consent will be clearly documented in the study documentation and the study participant's medical record.
- No further clinical data will be collected from the study participant. However, existing clinical data held will be retained and used in the study.
- Samples of tissue and/or blood already collected will be retained for use in the study.
- Sample left-overs after all study-related assessments have been finished will be donated and stored anonymised at the Manchester Cancer Research Centre biobank.
- The study participant's privacy will be respected and preserved.

The investigator may also advise that a patient discontinues study participation before undergoing the biopsies, should any untoward event occur. Reasons for investigator withdrawal include (but are not limited to):

- Adverse events or acute onset of co-morbidities compromising the patient in having biopsies within the specified study criteria.
- Any start of prostate cancer treatment prior to the first biopsy / blood samples being taken.
- Severe non-compliance to this protocol.

10 DATA MANAGEMENT

10.1 Case Report Forms

Data will be collected on paper case report forms (CRFs) and then entered into an electronic database. The database will be located at The Christie NHS Foundation Trust, and only accessible from The Christie Intranet. The database will be password protected and accessible only to those delegated to input or review the data.

10.2 Data Flow

Each participant will be assigned a trial identity code number, allocated for use on CRFs, other trial documents and any electronic spreadsheet. These documents and database will use date of birth (dd/mm/yy) and patient number.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial in accordance with regulatory requirements and for follow-up as required.

Completion of CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log'.

All paper forms shall be filled in using black or blue ballpoint pen. Errors shall be crossed out (but not obliterated by using correction fluid) and the correction inserted, initialled and dated. Corrections should be made legibly and initialled and dated by approved personnel. The reasons for significant changes must be provided.

If any data are not available, omissions should be indicated on the case report forms. The NHS Code of Confidentiality will be followed for this study.

Sensitive data stored on NHS or University computers will be encrypted to applicable NHS standards (AES256). The results of the study may be published in the medical literature, but patient identity will not be revealed.

10.3 Storage of Imaging and Sequencing Data

The data collected as part of the scanning procedures will be stored for fifteen years. The electronic data will be archived on computer systems managed by the Christie NHS Foundation Trust and the University of Manchester, and paper records will be stored in appropriately secure conditions. Although some personal (identifiable) data will be retained for up to five years from the day of the scan, most of the scan data itself will be anonymized shortly after the scan has taken place.

Data from nucleic acid sequencing will be anonymized, encrypted and securely stored by SciCom and for a period of 10 years.

11 ADMINISTRATIVE AND REGULATORY DETAILS

11.1 Study Sponsor

The Christie NHS Foundation Trust will act as the sponsor for this study. Delegated responsibilities will be assigned to the Chief Investigator and HYPROGEN research team to manage the study on behalf of the sponsor.

11.2 Funding Body

Professor Robert G Bristow, PhD (Director of the Manchester Cancer Research Centre, MCRC) using funds from the FASTMAN Centre of Excellence award (Prostate Cancer UK) and CRUK MI Leadership funds.

This study is an academic lead study that has been costed in accordance with DOH guidelines: Attributing the costs of health & social care Research & Development (AcoRD).

The study has been adopted on to the NIHR portfolio providing access to service support costs.

11.3 Peer Review

The study design, protocol and methodology were independently reviewed, discussed and approved by Professor Robert Jones (Clinical Trials Unit Glasgow, Beatson West of Scotland Cancer Centre, Glasgow, UK) on behalf of the National Cancer Research Institute (NCRI) Prostate Clinical Study Group (CSG) and Dr Simon Chowdhury (Medical Oncology Department, Guy's and St. Thomas' NHS Foundation Trust, London, UK).

11.4 Patient and Public Involvement

To provide easily understandable and patient centred study materials, e.g. participant information leaflet and consent form, a prostate cancer patient representative (RM, Stockton-on-Tees, UK) has reviewed and approved these documents and was involved in study design discussion. The patient is completely independent from the sponsor, funder and all investigators of the study.

11.5 Ethical Approval

No patients will be entered onto the study before ethical approval has been confirmed. The study protocol has received the favourable opinion of the HRA Ethics Committee for the study protocol, informed consent forms and other relevant documents.

The study will be conducted in accordance with, but not limited to, the Human Rights Act 1998, the Data Protection Act 2018, Freedom of Information Act 2000 subject to the provisions of sections 41 and 43 thereof, the EU Clinical Trials Directive, the Human Tissue Act 2004, ICH GCP E6 R2 addendum, the Declaration of Helsinki 1996, Ionising Radiation Medical Exposure Regulations (IRMER), and the UK Policy framework for Health and Social Care research as amended from time to time.

Where patients agree to take part in the study, they will be informed of how data are recorded, collected, stored and processed and may be transferred to other countries, in accordance with The General Data Protection Regulation (GDPR) (EU) 2016/679. As the sponsor of the study trial is a non-commercial organisation the legal basis for the handling and processing of data is 'task in the public interest'.

Annual progress reports will be submitted to REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended.

11.6 Confidentiality

All Investigators and site staff involved with the study must comply with the requirements of General Data Protection Regulations 2018 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles.

Mr Wes Dale, Managing Director (Research, Innovation and Education) at The Christie NHS Foundation Trust will act as the custodian of the data collected in this study. Prof Rob Bristow will act as the custodian for the samples collected during the study.

Key principles include:

- The research team will have access to the clinical data for the purpose of this study. Study data may be inspected by individuals from the sponsor or regulatory authorities for monitoring and audit purposes. Informed consent will be obtained to cover this activity.
- Any personal data recorded will be regarded as confidential, and any information which would allow individual patients to be identified will not be released into the public domain.

- Appropriate storage, restricted access and disposal arrangements for patient's personal and clinical details. Patient's notes and study files will be held in a secure storage area with limited access. Computers used to collate data will have limited access measures via usernames and passwords.

11.7 Protocol Compliance

The study protocol must be adhered to at all times. Intentional deviations from the protocol are not permitted under any circumstances.

Accidental protocol deviations must be reported to the Chief Investigator and the sponsor. Recurring deviations will be investigated by the sponsor to deem if it can be classed as a serious breach.

Identified/suspected serious breaches of the study protocol or GCP will be notified to the Operational Director of Research, or Quality Manager via: 0161 918 7572, breaches@christie.nhs.uk.

The R&D Sponsor Team will be notified of other deviations significantly impacting the risk profile of the study or significantly interrupting study services or supplies via: 0161 918 7357, christiesponsoredresearch@christie.nhs.uk.

Minor deviations should be monitored by the research team for seriousness and trends. Sponsor shall maintain oversight of these through periodic sponsor oversight activity reports (SOAR) and review of trial management group minutes.

11.8 Trial Monitoring

A trial monitoring plan will be developed and agreed by the research team and the sponsor. The procedures and anticipated frequency will be based on the trial risk assessment.

11.9 Study Oversight

The safety of this study is being over seen by the Safety Review Committee (SRC) which will be under the chairmanship of the Chief Investigator and will comprise of the co-investigators and study co-ordinator. The committee will meet to review the study conduct over the first five and ten patients enrolled.

A Trial Management Group (TMG) will be established and will include those individuals responsible for the day to day management of the study including the Chief Investigator, Co-investigators and study managers as detailed in the key study contacts section of this protocol. Meetings will take place after inclusion of the fifth and tenth patient and every 3 months thereafter in order to evaluate recruitment and feasibility of the study

interventions and to optimise study procedures by developing protocol amendments, if needed.

Key findings/decisions from these meeting will be notified to the sponsor as soon as possible.

Sponsor shall maintain oversight of the study conduct through

- Reviews of Annual Progress Reports (APR).
- Periodic sponsor oversight activity reports (SOAR) by the research team.
- Reviews of Trial Management Group and Safety Review Committee minutes.

11.10 Protocol Amendments

Any changes in research activity will require an amendment and will be initiated by the Chief Investigator. Proposed changes must be submitted in writing to the sponsor. The amendment will be categorised as substantial or non-substantial.

Any required changes to the documents that supported the original ethical approval will be submitted as an amendment to the appropriate ethical and regulatory authorities by the research team. Substantial amendments will not be implemented until the HRA and REC grant approval.

For any amendments that will affect a participating site's local capability and capacity, the research team will confirm with each participating sites R&D department that local capability and capacity is ongoing.

An Urgent Safety Measure (USM) may be put in place with immediate effect without gaining prior authorisation by the REC in order to protect clinical trial participants from any immediate hazard to their health and safety. USMs must be approved by the sponsor prior to implementation however REC and HRA must be notified by substantial amendment within 3 days of the measures being taken.

11.11 Liability and Indemnity

As the Chief Investigator (Prof RG Bristow) is a member of staff of the University of Manchester, study insurance will be covered under the University's policy for Medical Malpractice Errors and Omissions.

As the sponsor, The Christie NHS Foundation Trust, is an NHS organization, the NHS indemnity scheme will apply in the event of harm to participants arising from the conduct or management of the study.

11.12 Adverse Events and Adverse Reactions

The study does not involve any drug/device treatment (androgen deprivation and/or chemotherapy is given after the end of study as part of clinical routine care or a subsequent clinical trial unrelated to this study) and all procedures performed are part of standard clinical practice. Pimonidazole hydrochloride is used as a hypoxia marker substance without any known side effects at the prescribed dose of 0.5 g/m^2.

Adverse events following the biopsy visit should be monitored as per standard of care by the local urology on call team at the study site where the biopsies have been taken.

Adverse events do not need to be recorded on the CRF unless they affect study biopsy collection or study participation. Any adverse events which do not affect a patients' participation in this study, will not be recorded.

A Serious Adverse Event (SAE) is any untoward medical occurrence in a study patient that:

- Requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect
- Is life threatening
- Results in death

We do not anticipate any serious adverse events. However, any AEs meeting the definition of a Serious Adverse Event (SAE) must be reported to the CI/delegated research staff using the trial specific SAE Report Form immediately, and within 24 hours of observing or learning about the event. As many sections of the SAE form should be completed as possible (with further information being provided as it becomes available).

The CI will be responsible for reviewing the reports for:

- Completeness
- Causal link to the study procedures
- Expectedness

The Sponsor will be notified by the CI/delegated research staff through sae@christie.nhs.uk if an SAE is identified as related to the study and is unexpected (SUSAR). Identified SUSARs will be reported (by the CI/ delegated research staff) to the relevant REC within 15 days of becoming aware of the event, using the HRA Non-CTIMP Safety Report Form.

SAEs will only be collected up to 30 days after the last biopsy sample is taken. All SAEs must be followed-up until resolution and site investigators must provide follow-up SAE reports if the SAE had not resolved at the time the initial report was submitted.

11.13 End-of-Study Notification

The end of study notification will be submitted in writing to the REC within 90 days of the date of the patient inclusion. In the event of early study termination, the notification will be submitted to the REC within 15 days.

11.14 Study Summary Report

The study team is responsible for compiling and submitting the final study report to the sponsor and REC within one year of study closure.

11.15 Archiving

Essential documents are documents that individually and collectively permit evaluation of the conduct of the trial and substantiate the quality of the data collected. Essential documents will be maintained at The Christie NHS Foundation Trust in a way that will facilitate the management of the study including audit. Essential documents, including personal data will be stored for up to 5 years after the end of the study.

At the point where it is decided that the trial documentation is no longer required, the sponsor will authorise the destruction of all essential documents related to the HYPROGEN study.

11.16 Publication Policy

The data arising from the study will belong to the trial sponsor, The Christie NHS Foundation Trust.

The main study results will be published in a peer reviewed journal, on behalf of all co-investigators and collaborators. All presentations and publications relating to the trial must be authorised by sponsor.

Participants will be given the option to request a copy of the study results should they so wish.

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