



Plasma Analysis for Response Assessment and to Direct the manaGement of Metastatic prostate cancer

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Please note: This study protocol must not be applied to patients outside the PARADIGM study. Cancer Research UK & UCL Cancer Trials Centre (UCL CTC) can only ensure that approved trial investigators are provided with amendments to the protocol.

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1. PROTOCOL SUMMARY

1.1. Summary of Trial Design

Title:	Plasma Analysis for Response Assessment and to Direct the management of Metastatic prostate cancer
Short Title/acronym:	PARADIGM
Sponsor name & reference:	University College London, UCL/18/0513
Funders & reference:	PARADIGM-A and PARADIGM-D cohorts: - Prostate Cancer UK, reference MA-TR15-007 PARADIGM-E cohort: - Astellas Pharma CTC bloods analysis: - Epic Sciences WB-MRI sub-study: - John Black Foundation Gut Microbiome sub-study - John Black Foundation Other Funding: - Cancer Research UK, C35118/A22744; C65130/A26321 - Medical Research Council, MR/P002072/1
Clinicaltrials.gov no:	NCT04067713
Design:	A prospective, observational, biomarker-focused, translational platform, cohort study in newly diagnosed polymetastatic prostate cancer patients starting long-term systemic therapy. There are 3 cohorts: PARADIGM-D: Docetaxel PARADIGM-A: Androgen receptor signalling inhibitor (ARSI) – Abiraterone, Apalutamide or Enzalutamide PARADIGM-E: Enzalutamide PARADIGM-E is a separate cohort that will commence after completion of recruitment to PARADIGM-A, and will serve as a clinical validation of PARADIGM-A.
Target accrual:	~170 men to have 130 men evaluable for the major endpoints in each cohort PARADIGM-E (enzalutamide) cohort: ~85 men to have 65 men evaluable for the major endpoints
Inclusion criteria:	1. Able and willing to provide written informed consent 2. Aged 18 or over 3. Polymetastatic disease defined as one of the following: ≥5 bone metastases ≥1 unequivocal visceral metastasis 4. Eastern Cooperative Oncology Group (ECOG) Performance status 0 to 2 5. No medical contra-indications to an Androgen receptor signalling inhibitor (ARSI) or docetaxel (with or without prednisolone).

	6. Patients should be either of the following: i. Planned to start long-term Luteinizing hormone Releasing Hormone (LHRH) suppression, or ii. Have started long-term LHRH antagonist within the last 14 weeks, or iii. Have started LHRH agonist within the last 16 weeks. When antiandrogens (e.g. bicalutamide) are used in combination with an LHRH agonist to prevent flare, patients have to have started within 18 weeks of starting antiandrogens. 7. Patients should be planned for addition of an ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A), or enzalutamide (PARADIGM-E), or docetaxel (PARADIGM-D) within 14 weeks after start of LHRH antagonist (16 weeks if LHRH agonist is started without anti-androgen) or 18 weeks from start of anti-androgen with a target of 6 cycles (docetaxel) or continuation until progression (ARSI). 8. No concomitant medical conditions likely to reduce life expectancy. 9. Patient agrees to be followed up in the recruiting centre and to having sequential plasma samples collected as per the study protocol.
Exclusion criteria:	1. Concurrent or planned for (i.e. prior to development of castration resistance), treatment with oestrogen, radiotherapy or surgery to the primary tumour. <i>(N.B Patients may also be eligible if randomised to any of the following experimental drugs; PARPi, PD-1/PD-L1, AKTi, PSMA-lutetium, if given in combination with SOC (i.e. ADT + docetaxel or ARSI) treatment in an open-label clinical trial, or to the placebo arm in an open-label clinical trial following discussion and approval of the CI/delegate. Patients can participate in other observational studies.)</i> 2. Prior systemic therapy for prostate cancer other than for LHRHa +/- anti-androgen (started within the time limits defined in inclusion criterion 7). 3. Metastatic brain disease or leptomeningeal disease. 4. Any surgery planned prior to Cycle 4 Day 1 (C4D1) 5. Other current malignancy or malignancy diagnosed or relapsed within the past 5 years (other than non-melanomatous skin cancer, stage 0 melanoma in situ and non-muscle invasive bladder cancer). 6. Patients who consent to the whole-body magnetic resonance imaging (WB-MRI) translational sub-study should have no contraindications to MRI as per local guidelines.
Primary objective:	PARADIGM- A and - D: To determine whether the detection of plasma tumour DNA (ptDNA) after two or three cycles of ARSI or docetaxel added after start of ADT is associated with a worse clinical outcome in newly diagnosed metastatic prostate cancer. PARADIGM-E: To confirm and validate that the detection of ptDNA after two or three cycles of enzalutamide after the start of ADT is associated with a worse clinical outcome in newly diagnosed prostate cancer.

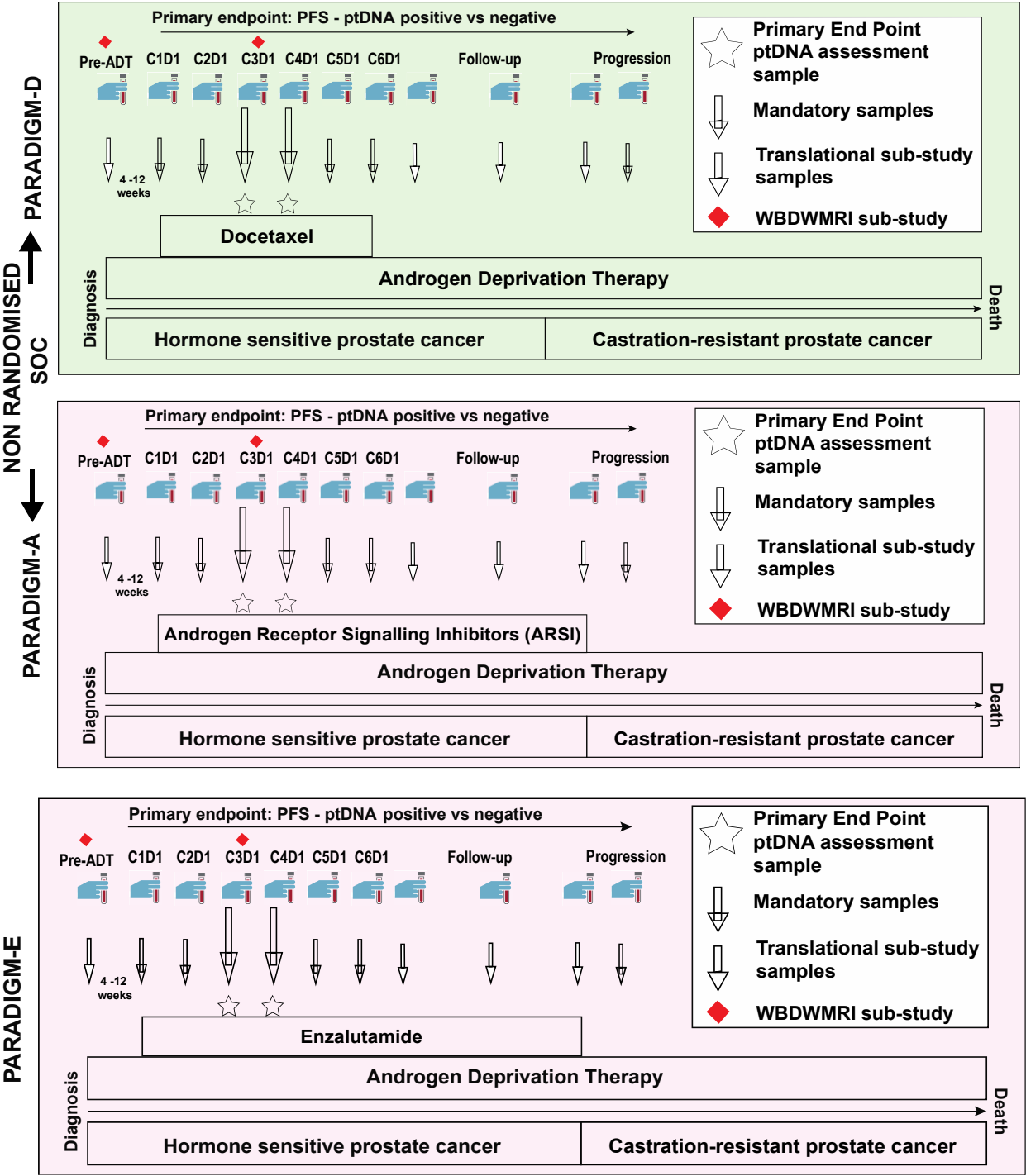
	PARADIGM-E is a separate cohort that will commence after completion of recruitment to PARADIGM A.
Secondary objectives:	All cohorts: 1. To compare ptDNA classification at C2D1 and C5D1 with C3D1 or C4D1. 2. To determine whether the detection of ptDNA after five to sixteen weeks of starting ADT and prior to starting ARSI or docetaxel is associated with a worse clinical outcome. 3. To determine the association between clinical outcome and prostate specific antigen (PSA) level (<0.2, 0.2-4, >4ng/dl) after five to sixteen weeks of starting ADT and prior to starting ARSI or docetaxel, prior to treatment at every cycle and at 7 months after start of ADT. 4. To assess whether ptDNA detection is a better predictor of clinical outcome than PSA level (as assessed in objective 3) after five to sixteen weeks of starting ADT and prior to treatment at every cycle. 5. To compare associations with clinical outcome for the change in ptDNA detection and PSA level (as assessed in objective 3) prior to start of ARSI or docetaxel and at C3D1 or C4D1. 6. To evaluate whether ptDNA fraction prior to LHRHa (stratified by no anti-androgen versus 2-3 weeks anti-androgen) associates with Progression Free Survival (PFS) and Overall Survival (OS).
Exploratory objectives	All cohorts: 1. To develop and test a composite model incorporating different biomarkers for assessing response. 2. To describe ptDNA dynamics and compare to PSA kinetics.
Translational Research	All cohorts: Translational research will be performed on sub-sets of patients from whom the required evaluations are made. It is expected that not all patients will be able to participate in all the translational studies but as a fundamental aspect of PARADIGM, as many patients as possible should be included in these assessments. 1. Predictors of response to systemic treatment • To identify molecular signatures in plasma and tumour that associate with PFS or OS with ARSI or docetaxel. • To identify a molecular signature in pre-ADT plasma or tumour that associates with plasma androgen receptor (AR) aberrant status at progression to castration-resistant disease. 2. Tracking of plasma DNA or extracellular vesicles dynamics • To determine whether detection of ptDNA precedes PFS (or OS) events. • To characterise resistant clones

	- To evaluate whether patients who progress with AR gain at the development of metastatic castration resistant prostate cancer (mCRPC) have a shorter time to PFS (on ADT) and OS. 3. Circulating tumour cell (CTC) dynamics at initiation of ADT - To evaluate whether CTC count pre-ADT and after starting ADT associates with shorter PFS and OS. - To identify CTC molecular features prior to and after starting ADT that associate with PFS or OS with ARSI or docetaxel. 4. Interrogation of peripheral immune changes secondary to initiation of ADT and following addition of docetaxel or ARSI - To determine changes in Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) (CD11b+CD33+CD15+ cells) and monocytic myeloid-derived suppressor cells (M-MDSCs) and immune cells ((Natural killer (NK) cells, T-lymphocytes, CD4+ T-lymphocytes, CD8+ T-lymphocytes and B-lymphocytes)) from peripheral blood leukocyte samples after exposure to ADT and subsequently ADT with ARSI or docetaxel. - To determine dynamic changes in IL-23 and other cytokines in plasma after exposure to ADT and subsequently ADT with ARSI or docetaxel. - To evaluate whether patients with rising PMN-MDSCs, M-MDSCs or cytokines post ADT and during ADT with ARSI or docetaxel have a shorter progression free survival (PFS) and radiological progression free survival (rPFS) and OS. - To define the peripheral blood immune profile and correlate with archival tumour tissue and PFS and OS. - To correlate peripheral immune changes pre and post ADT with genomic changes in circulation. To determine T cell receptor (TCR) repertoire changes pre and post ADT and correlate this to PFS and OS. 5. WBMRI derived imaging biomarkers as a surrogate of response (only at select sites and for patients with no contra-indication to MRI) - To determine utility of WBMRI derived quantitative imaging biomarkers in predicting and assessing early response to ARSI and docetaxel as determined by PFS and OS and association with plasma tumour markers both at baseline and changes on treatment. - To identify WB imaging biomarkers (at baseline and changes during treatment) which may associate with increased risk of developing mCRPC and may be incorporated into biomarker composite models of response. 6. Evaluating changes in the gut microbiome with institution of testosterone suppression and development of castration-resistance - To explore the constituent and genomic changes in the gut microbiome at minimal exposure to ADT, after response to ADT with enzalutamide and at progression
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	To test for associations and investigate the role of the gut microbiome in progression to CRPC in patients treated with ADT and enzalutamide
Major Endpoints	PFS and OS for PARADIGM-A and PARADIGM-D and PFS for PARADIGM-E. PARADIGM-A, PARADIGM-D and PARADIGM-E will be reported as separate cohorts. PARADIGM-E will be reported after completion of recruitment to PARADIGM-A, and will serve as a clinical validation of PARADIGM-A. PFS is defined as the interval from start of ARSI or docetaxel to disease failure as determined by at least one or more of these factors: - Symptomatic or asymptomatic new or unequivocal progression of prior distant metastases confirmed by imaging - Symptomatic progression of cancer in the prostate confirmed by imaging - Serum PSA progression in PARADIGM-D - Death from any cause OS is defined as time from start of ARSI or docetaxel with ADT to death from any cause.
Secondary Endpoint	Prostate Cancer Specific Survival (PCSS) defined as time from start of ARSI or docetaxel with ADT to death from prostate cancer.
Treatment Summary (Standard of care)	All patients will receive SOC treatment for metastatic prostate cancer that must include ADT with an LHRHa and addition of ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A) or enzalutamide only (PARADIGM-E), or docetaxel (PARADIGM-D). Prednisolone in combination with abiraterone can be used as per local guidelines. There will be no randomisation and treatment selection will be based on patient and physician choice, funding availability and local guidelines. Men will be recruited to two cohorts concurrently: - ADT + docetaxel, PARADIGM-D - ADT + ARSI, PARADIGM-A Following completion of recruitment to PARADIGM-A, men will then be recruited to: - ADT + Enzalutamide, PARADIGM-E or - ADT + docetaxel, PARADIGM-D (if still recruiting) Men must have plasma collected at C3D1 and/or C4D1 of their allocated treatment and additional plasma samples at C1D1, C2D1 C5D1 and C6D1. Additional samples will be collected for the pre-ADT sub-set of 150 patients as per standard of care (once every 3 months), including at treatment failure/progression, until initiation of the next line of treatment. Patients will be followed up for at least 36 months and up to 120 months (from the first patient registered) for PFS and OS and subsequent treatments will be recorded.

	All patients will be asked to consent to the collection of their archival diagnostic blocks immediately after consent.
Number of sites:	20 (including 2 pre-ADT only sites)
Country:	UK
Duration of recruitment:	Approximately 18 months (PARADIGM-D and PARADIGM-A) Approximately 24 months (PARADIGM-E)
Duration of follow up:	All cohorts: Frequency of visits as per standard of care and duration is up to 120 months after the first patient registered, or until patient meets all required study endpoint events.
Definition of end of study:	For regulatory purposes the end of study will be 120 months after the first patient has been registered, or once all patients have died (whichever is sooner). This timeline also incorporates completion of analyses of samples for the study. If the event rate to report study endpoints is reached prior to this timepoint, and analyses of samples is also completed, then the end of study will be declared earlier. (See section 13: Statistics).

1.2. Study Summary



1.3. Funding

Prostate Cancer UK is the main funder of the study (PARADIGM-A and PARADIGM-D cohorts) and is supporting the central coordination of the study through the UCL CTC (MA-TR15-007). Research A costs will be reimbursed to sites as per the finance section of site agreement.

Cancer Research UK (grant numbers: C35118/A22744; C65130/A26321) supports some of the translational research.

Astellas is the funder of patients recruited into the PARADIGM-E cohort and is supporting central coordination of the study through the UCL CTC.

Epic Sciences, Inc. is providing support for the collection, shipment and analysis of the Circulating Tumour Cells (CTCs).

WB-MRI sub-study and Gut Microbiome sub- study are being funded by Jon Black Foundation.

An MRC Clinical Research Fellowship (MR/P002072/1) supported Anuradha Jayaram during the study design and set-up.

2. INTRODUCTION

2.1. Management of de novo metastatic prostate cancer

In the UK, prostate cancer is the most common cancer in men, with about 1 in 8 men diagnosed with prostate cancer at some point of their lives, equivalent to approximately 47,000 men diagnosed every year. Between 2014-2016, there were approximately 11,500 prostate cancer deaths annually in the UK. Up to a third of prostate cancer deaths in the UK arise in men with metastatic disease at diagnosis: de novo metastatic (M1) prostate cancer is a lethal disease and major health care burden.

Until 2015, long term ADT alone was the SOC for patients with newly-diagnosed metastatic prostate cancer, with a median time to castration resistance of approximately 13 months¹. Recently, randomised controlled trials have demonstrated a survival advantage and prolonged PFS for addition to ADT of systemic treatment with either docetaxel with or without prednisolone/prednisone (Doc)²⁻⁶, or more recently, abiraterone acetate with prednisolone/prednisone (AAP)^{7,8}, enzalutamide (ENZ)⁹ and apalutamide (APA)¹⁰. However, despite significant tumour responses in many patients, the majority progress to CRPC that is lethal and leads to significant suffering. There is an urgent need to improve the management of men with de novo metastatic disease. This will require accurate treatment selection, early detection of relapse and deep interrogation of treatment resistance.

2.1.1. Docetaxel

The benefit of the addition of Doc to long-term ADT in patients with metastatic hormone sensitive prostate cancer (mHSPC) was demonstrated in two studies: CHAARTED and STAMPEDE. In both studies, docetaxel is administered every 3 weeks for a total of 6 cycles. In the CHAARTED study, the median PFS was 20.2 months in the Doc plus ADT arm versus 11.7 months with ADT alone (Hazard ratio (HR) 0.61; 95%CI: 0.51-0.72; p<0.001)⁶. Long term survival analysis after a median follow-up of 53.7 months, demonstrated the median OS was 57.6 months for the chemo-hormonal therapy arm versus 47.2 months for ADT alone (HR 0.72; 95%CI, 0.59-0.89; p=0.0018). For patients with high-volume disease defined as the presence of visceral metastases or ≥4 bone lesions with ≥1 beyond the vertebral bodies and pelvis, the median OS was 51.2 months with chemo-hormonal therapy versus 34.4 months with ADT alone (HR, 0.63; 95%CI, 0.50-0.79; p<0.001). The time to CRPC was 19.4 months in the combination arm versus 11.7 months in the ADT alone arm (HR 0.61; 95%CI, 0.52-0.73; p < 0.001). For high-volume disease, the median time to CRPC was 14.9 months for the combination arm versus 8.6 months for the ADT alone arm (HR 0.58; 95%CI, 0.47-0.71; p < 0.001).

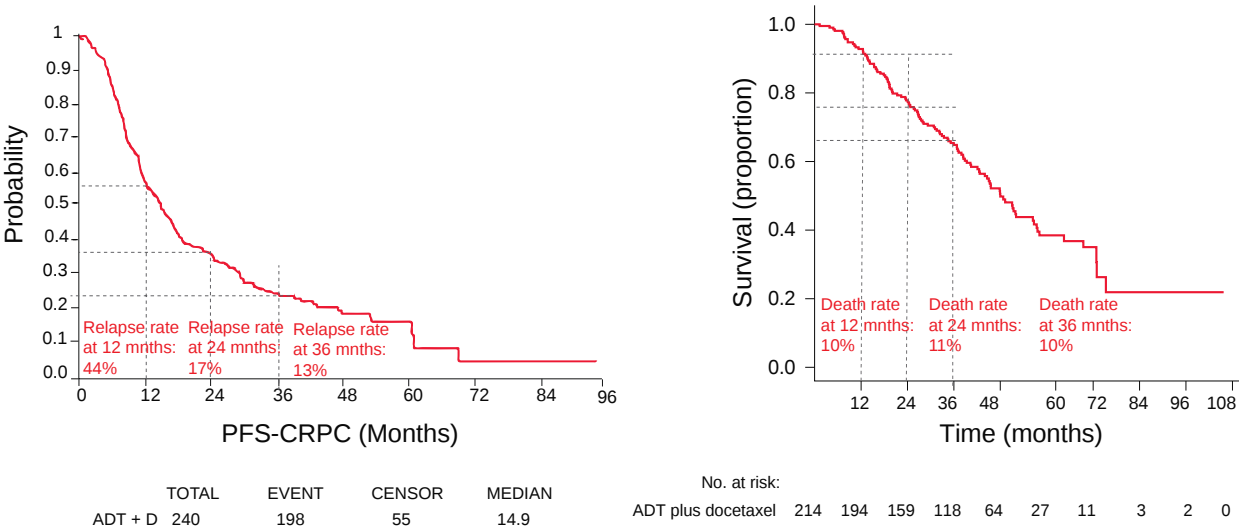


Figure 1: PFS and OS with ADT and docetaxel in de novo high-volume M1 prostate cancer in the CHAARTED trial with relapse rate estimates at 12 months, 12-24 months and 24-36 months

For M1 patients randomised in the STAMPEDE trial, after a median follow-up of 43 months (Interquartile range (IQR) 30–60), the median survival with ADT alone was 45 months (IQR 23–91, 5-year survival 39%) and 60 months (IQR 27–103, 5-year survival of 50%) for patients who received ADT with docetaxel (HR 0.76, 95%CI 0.62-0.92; $p=0.005$). The proportion of patients who developed CRPC was 81% for ADT alone versus 70% for ADT with docetaxel (HR 0.61, 95% CI 0.53-0.70; $p=0.413 \times 10^{-13}$). A post-hoc analysis of the OS and PFS in high-volume M1 is planned but has not been presented yet.

2.1.2. Androgen receptor signalling inhibitors

2.1.3. Abiraterone Acetate

The benefit of the addition of AAP to ADT is supported by both the LATITUDE⁷ and STAMPEDE studies. The LATITUDE study recruited only high-risk mHSPC patient defined as at least 2 of 3 high-risk criteria: Gleason score ≥ 8 , presence of ≥ 3 lesions on bone scan or presence of measurable visceral disease. After a median follow-up of 30.4 months, patients receiving AAP and ADT had a longer rPFS of 33 months versus 14.8 months for patients receiving ADT alone (HR=0.47; 95% CI, 0.39-0.55; $p<0.001$) and a longer OS (not reached vs. 34.7 months; HR=0.61; 95% CI, 0.51-0.76; $p<0.001$)⁷.

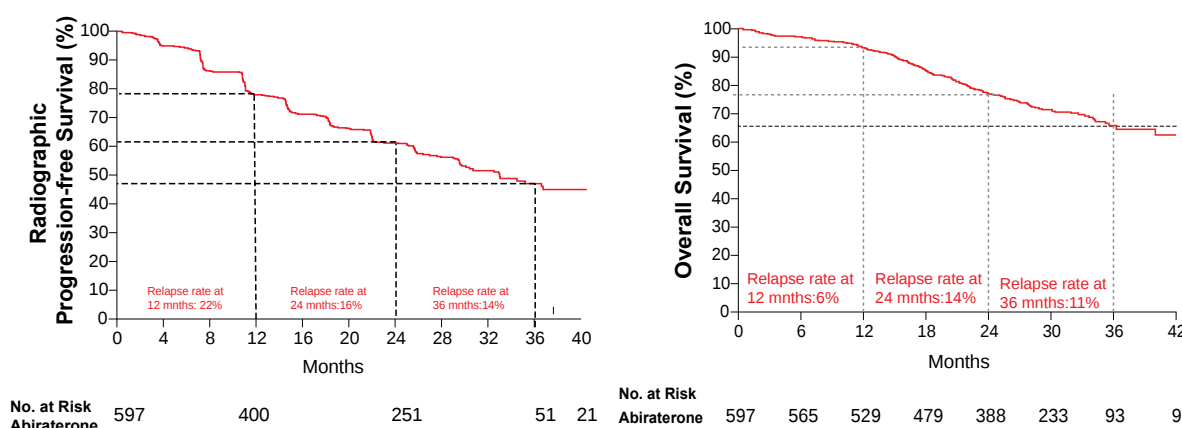


Figure 2: rPFS and OS for high-risk mHSPC patients in the LATITUDE trial with relapse rate estimates at 12 months, 12-24 months and 24-36 months

In the STAMPEDE trial, after a median follow-up of 40 months, the OS benefit in the high-risk mHSPC patient population was similarly significant (not reached versus 34 months, HR 0.54, 95%CI 0.41-0.70; $p<0.001$)⁸. The STAMPEDE trial did not measure rPFS but reported failure-free survival (as for time to CRPC above). This was 30 months versus 7.9 months in the high-risk M1 group, (HR 0.31; 95%CI 0.25-0.39; $p<0.001$). Abiraterone was administered with prednisolone every 4 weeks till progression in both LATITUDE and STAMPEDE.

Enzalutamide and Apalutamide

The benefit of the addition of Enzalutamide or Apalutamide to ADT is reported in two studies: the ENZAMET⁹ and TITAN¹⁰ study respectively. Both Enzalutamide (ENZ) and Apalutamide (APA) are second generation antiandrogens prescribed every 4 weeks till progression in these two studies. The ENZAMET study, recruited men with mHSPC and randomised patients to either ENZ or first-generation antiandrogens with ADT. Unlike the LATITUDE study, men with either high (defined as the presence of visceral metastases or at least four bone lesions with at least one lesion located beyond the vertebral bodies and pelvis) or low volume metastatic disease and prior docetaxel (maximum of 2 cycles) were eligible for study. After a median follow-up of 34 months, patients receiving ENZ and ADT had significantly less death events versus the antiandrogen group (HR 0.67; 95% CI 0.52-0.86, $p=0.002$). While the median OS was not yet estimable, Kaplan–Meier estimates of OS at 3 years was 80% in the ENZ group and 72% in the antiandrogen group. Patients receiving ENZ also had a longer PSA PFS (HR, 0.39; 95% CI $P<0.001$) and clinical PFS (HR hazard ratio, 0.40; 95% CI, 0.33 to 0.49; $P<0.001$). In the ENZAMET study, there was

no difference in OS or clinical PFS in patients with high volume disease receiving either enzalutamide or placebo (HR 0.8).

The TITAN study randomised patients to APA and ADT to placebo and ADT. Unlike the ENZAMET study, patients who had previously received a maximum of 6 cycles of docetaxel prior to randomisation were eligible for enrolment. At the first interim analysis, with a median of 22.7 months of follow-up, patients treated with APA had a significantly better rPFS than placebo (HR 0.48; 95% CI, 0.39 to 0.60; $P < 0.001$). OS at 24 months was also greater with APA than with placebo (HR 0.67; 95% CI, 0.51 to 0.89; $P = 0.005$). In a subgroup analyses, patients with high volume metastatic disease treated with APA, had longer rPFS (median 23.7 months versus 14.9 months; HR 0.71; 95% CI, 0.43 to 1.18) and OS (median NE versus 26.6, HR 0.99; 95% CI, 0.55 to 1.77).

2.2. Plasma DNA analysis

Plasma DNA can be extracted from the non-cellular blood compartment from all individuals. It is fragmented to an average length of 140 to 170 base pairs (bp) and is very amenable to next-generation sequencing (NGS). In cancer patients, a fraction of plasma DNA is tumour in origin ranging from $<1\%$ to $>90\%$ of total plasma DNA, with an association observed with increasing tumour or metastatic volume. PtDNA is usually present as only a few thousand amplifiable copies per millilitre of blood^{11-13 14-16 17}. Studies have demonstrated that the half-life of ptDNA is around 2 hours, and its release could therefore be a very sensitive indicator of tumour behaviour¹⁸. This approach has been primarily tested for detection of minimal residual disease (MRD) after primary treatment or for tracking disease response in metastatic disease¹¹.

The utility of ptDNA to quantify MRD and predict post-operative relapse has been studied in lung cancer. One of the main goals of a study published by Abbosh et al was to examine the capability of detecting MRD and the tumour subclones that drive relapse using patient-personalised ptDNA. The authors collected pre- and post-operative ptDNA for a sub-group of 24 patients, and patients were followed-up for every 3 to 6 months and up to 31 months for relapse. Of the 14 patients that were confirmed with relapse, 13 were ptDNA positive defined as at least 2 single nucleotide variants (SNVs) detected, and the median time between ptDNA detection and relapse confirmation was 70 days. Conversely, 9 out of 10 patients who are ptDNA negative lived disease free within the follow-up period. The remaining one patient that had ptDNA detected prior to adjuvant chemotherapy, but remained ptDNA negative after the treatment, was free of relapse 688 days post-surgery. Despite the relatively small sample size, the results of the study demonstrate the utility of circulating tumour DNA (ctDNA) for predicting post-operative relapse of non-small cell lung cancer with both a sensitivity and specificity above 90%¹⁹.

Similarly, in colorectal cancer, the detection of ptDNA after resection of stage II colon cancer has demonstrated utility in detecting recurrence. In a study of 230 patients with resected stage II colon cancer, in patients not treated with adjuvant chemotherapy, ptDNA was detected postoperatively in 14 of 178 (7.9%) patients, 11 (79%) of whom had recurred at a median follow-up of 27 months; recurrence occurred in only 16 (9.8 %) of 164 patients with negative ctDNA (HR 18; 95% CI 7.9-40; $p < 0.001$). In patients treated with chemotherapy, the presence of ptDNA after completion of chemotherapy was also associated with an inferior recurrence-free survival (HR 11; 95% CI 1.8-68; $p = 0.001$). This study also evaluated the sensitivity of serial ptDNA analysis during the follow up period to predict subsequent radiologic recurrence. ptDNA was more frequently positive in 23 out of 27 patients than carcinoembryonic antigen (CEA) elevation at the time of radiologic recurrence (85% versus 41%); $p=0.002$) The time between ctDNA detection and radiologic recurrence (median, 167 days; IQR, 81 to 279 days) was significantly longer than the time between CEA elevation and radiologic recurrence (median, 61 days; IQR, 0 to 207 days; $p = 0.04$).

In a prospective cohort of 55 early breast cancer patients receiving neoadjuvant chemotherapy, detection of ptDNA in plasma after completion of apparently curative treatment—either at a single postsurgical

time point or with serial follow-up plasma samples—predicted metastatic relapse with high accuracy. Patients with detectable ptDNA in a single post treatment sample had a median disease-free survival (DFS) of 6.5 months (HR 25.1; 95% CI 4.08-130.5; log-rank $p < 0.0001$). Detection of ctDNA in serial samples was predictive of early relapse [disease-free survival: median of 13.6 months (ptDNA detected) versus median not reached (ptDNA not detected); HR, 12.0; 95% CI 3.36-43.07], with a C-index of 0.75. Detection of ptDNA by mutation tracking was a significant predictor of early relapse in a multivariable model. Mutation tracking in serial samples increased sensitivity for the prediction of relapse, with a median lead time of 7.9 months over clinical relapse.

PtDNA analyses in mCRPC have shown a strong association with clinical outcome and clinico-pathological variables²⁰⁻²⁴. In mCRPC, a phase II study of cabazitaxel versus abiraterone or enzalutamide in poor prognosis mCRPC presented at European Society of Medical Oncology (ESMO) 2018, demonstrated that notably no patients with undetectable ptDNA had died in the study. Patients with ptDNA percent of 30-100 had a HR for progression of 4.2 (95%CI 2.04-8.68, $p < 0.001$). ptDNA change on therapy was also highly prognostic - ptDNA increase while on therapy had a HR of 6.24 (95%CI 2.09-16.63, $p = 0.001$) for OS. Additionally, on treatment change of ptDNA was prognostic. Patients with an increase in ptDNA fraction between baseline and end of cycle 4 had a shorter PFS (HR 4.26; 95% CI 1.76-10.32, $p < 0.001$) as well as shorter OS²⁵. Preliminary data from analysis of plasma DNA collected from M1 patients prior to and after starting ADT shows a rapid decline in ptDNA levels with ~20% of patients remaining with detectable ptDNA on treatment.

Studies of sequential plasma samples in mCRPC have identified i) emergence of genomic aberrations harboured by resistant clones several months prior to clinical or radiological progression and ii) drivers of resistance, for example AR mutations in patients treated with abiraterone and prednisolone or BRCA2 reversion mutations in BRCA2 mutant patients treated with PARP inhibitors^{23,26,27,28}. This introduces the opportunity to expand this approach to analysis of patients treated with ADT and docetaxel or abiraterone. Given tumour fraction may be lower than later stage mCRPC, higher sensitivity approaches will be required. Plasma AR copy number (CN) gain is detected in 15% of patients at development of CRPC and associates with worse clinical outcome in mCRPC patients treated with AR targeting agents²⁹. Prostate cancer patients who have plasma AR gain at development of CRPC have a significantly shorter response to ADT (Figure 3), suggesting pre-existence of the AR aberrant clone and introducing the opportunity to detect it prior to development of mCRPC, allowing treatment intensification. These reports are retrospective and potential biases resulting from presenting metastatic burden have not been controlled for.

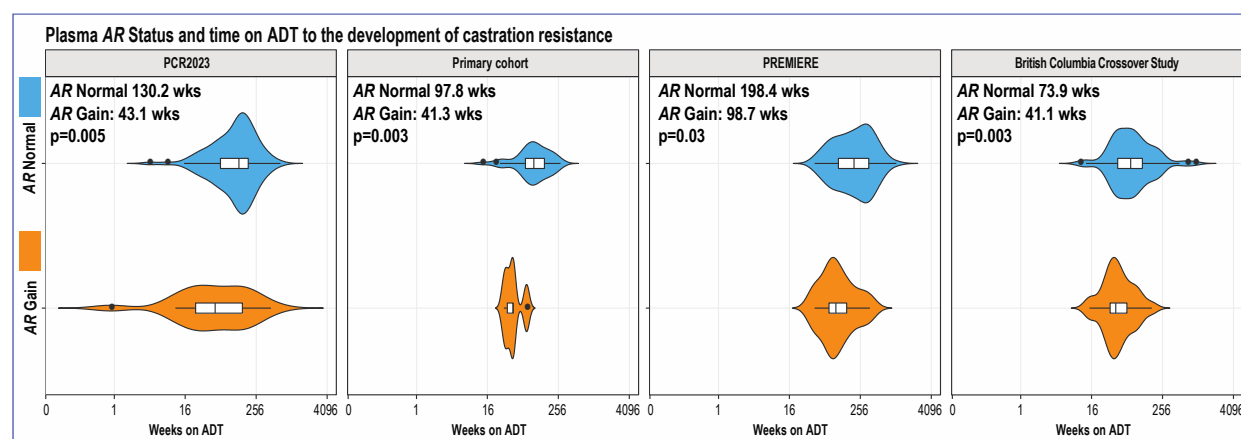


Figure 2 Plasma AR copy number status and time on ADT to the development of castration resistance. X axis (weeks) scaled as log 2

PtDNA detection in early disease requires approaches with high sensitivity and specificity that are best achieved by bespoke optimisation for the tumour type and disease setting. For example, amplicon-based or customized target enrichment using improved biochemistry of random molecular barcoding and optimized, error-correcting analysis on ultra-deep sequencing (i.e. >10,000X) can potentially improve the sensitivity of rare mutation and indel detection³⁰⁻³³. Epigenetic information, such as DNA methylation status, can be extracted from plasma DNA using modified NGS protocols to obtain information additional to the genomic status³⁴. DNA methylation, the addition of methyl group to cytosine, is a modification that occurs at thousands of sites across the genome and is tissue-of-origin and cancer specific. This could be used to improve the sensitivity of assays. Targeted methylation plasma DNA assays have recently demonstrated high sensitivity and tissue specificity and could be implemented in the PARADIGM trial to detect and track circulating prostate tumor DNA^{35,36}.

2.3. Nomenclature and categorisation related to disease metastatic status

The CHAARTED and LATITUDE trials pre-defined an M1 patient population with a more homogeneous worse outcome using poor prognosis variables based on radiologically defined metastatic volume or a composite of radiological and pathological criteria (used for sub-group analysis or patient selection) respectively. The CHAARTED trial used the term high-volume, and the LATITUDE trial used high risk. The LATITUDE trial composite had one of its requirements as Gleason score >8, which was present in ~85% of patients. The radiological criteria differed slightly between the two trials: both recognised visceral disease as a criterion for inclusion but CHAARTED specified a requirement for bone metastases outside the spine or pelvis whilst the LATITUDE trial required ≥ 3 lesions on bone scan. Other trials have used higher numbers of bone metastases detected on bone scan for categorising patients, for e.g. the HORRAD trial sub-group analyses used alternative cut-offs of <5 lesions, 5–15 lesions, >15 lesions on bone scan³⁷. The sub-group analysis of Arm H in the STAMPEDE trial, namely the randomisation of patients to radiotherapy to the primary tumour in combination with ADT, used the CHAARTED definition for its pre-planned primary analysis but plans sub-group analyses using the LATITUDE and HORRAD definitions³⁸. The primary publication of this trial used the term “high burden.” Although approximately 18% of patients show a discordance in classification by LATITUDE or CHAARTED criteria, the OS of the ADT alone arms in the two trials was very similar: 34.4 months in CHAARTED and 34.7 in LATITUDE. Post-hoc analysis of patients randomised to abiraterone in the STAMPEDE trial have shown an equivalent benefit for all patients regardless of volume status, using both the CHAARTED and LATITUDE definitions, suggesting an equivalent treatment effect across all M1 patients. A recent analysis of the STAMPEDE trial showed that high burden (defined as for CHAARTED) derived no benefit from radiotherapy to the primary tumour (prostate) in men with metastatic disease in contrast to men with low volume disease⁷². This supports significant biological and outcome differences in men selected based on radiologically defined disease burden. A post-hoc analysis supports a cut-off of 5 extra-pelvic metastases for distinguishing poly-metastatic disease. The STAMPEDE trial is initiating a new comparison that will enrol men with <6 extra-pelvic metastases in bone and/or lymph nodes to receive treatment to their primary and will be randomised to receive targeted treatment to their metastases. These patients will not be eligible for PARADIGM, and we are therefore using a similar cut-off to select poly-metastatic patients:

- To maximise detection of ctDNA given the technical challenges for its detection in lower volume patients
- The high relapse rate and relatively short survival make improved outcomes in this population an urgent unmet need.

Patient support groups have expressed concern about the use of terms such as “high-risk” or “high-volume,” as they increase the anguish of a man recently conveyed a diagnosis of prostate cancer. Following discussions with a number of patient representatives, we have decided to use the term polymetastatic. This term will also distinguish from “oligo-metastatic” disease that will be increasingly managed differently and more radically than the polymetastatic population.

2.4. Justification for conducting PARADIGM

As both docetaxel and ARSI are effective therapeutic combinations, there are now several distinct standard-of-care treatment options for patients with prostate cancer starting ADT. There is currently very little data to guide clinicians as to which is the more effective treatment; direct, randomised, comparative analysis of the ADT and Doc with the ADT and AAP arm showed no evidence of a difference in overall or prostate cancer-specific survival, nor in other important outcomes such as symptomatic skeletal events³⁹. Moreover, multiple new therapeutic approaches are being considered for evaluation in this setting. Physicians use a maximum of six cycles of docetaxel and continue ARSI until radiographic disease progression. Earlier detection of treatment futility or predictive biomarkers of sensitivity could minimise unnecessary toxicity and maximise efficacy with improved treatment sequencing. A PSA level (<0.2, 0.2-4, >4ng/dl) after 7 months of starting ADT with or without docetaxel is associated with shorter survival (85% vs 73% vs 55%) but is insufficiently sensitive to be used alone to guide treatment⁴⁰ and the 7-month time-point for analysis limits utility for early discontinuation of ineffective treatment. The challenges with using serum PSA have been highlighted in several studies in mCRPC and include the relatively long half-life of serum PSA and due to its exquisite androgen-regulated sensitivity, serum PSA may not be reflective of less androgen-regulated clones⁴¹.

The PARADIGM study major endpoints are PFS and OS. PFS is represented as progression to CRPC and initiation of the next line of treatment. The criteria used by physicians for initiating first-line treatment for mCRPC differ by whether ARSI or docetaxel are used and this is reflected in the definitions of PFS in PARADIGM-A, PARADIGM-D. These findings will be clinically validated in a cohort of patients treated with enzalutamide (PARADIGM-E) which is separately funded by Astellas.

2.5. Immune Profiling in Metastatic Prostate Cancer

The well-established dependency of cancer cells on the tumour microenvironment indicates that the microenvironment might control the emergence of CRPC. Changes in peripheral blood cell fractions of the innate and adaptive immune system have been found in mCRPC when compared to sex-matched healthy volunteers. In mCRPC, it was noted that there was a trend for decreased levels of NK cell ($p=0.09$), lower T-lymphocytes ($p<0.0001$), CD4+ T-lymphocytes ($p<0.0001$), CD8+ T-lymphocytes, as well as a trend for a lower CD4 over CD8 ratio ($p=0.11$), and decreased B-lymphocytes ($p<0.003$). Monocytic myeloid derived suppressor cells (M-MDSC) levels are increased in the peripheral blood of mCRPC patients, with an increase in M-MDSC levels following 1 or 2 lines of hormonal treatment (abiraterone or enzalutamide), mirrored by a decreased Human leukocyte antigen – DR isotype (HLA-DR) expression on mature monocytes. M-MDSCs were found to be associated with a shorter PSA-PFS (HR 1.072 with 95%CI 1.001-1.149, $p=0.047$). PMN-MDSCs is also associated with a lower likelihood of PSA response (OR 0.310 with 95%CI 0.101-0.954, $p=0.001$). M-MDSC and CD14+CD15+ PMN-MDSC were associated with shorter OS (HR 1.064 with 95%CI 1.021-1.109, $p=0.003$ and HR 1.13 with 95%CI 1.018-1.247, $p=0.021$, respectively). Conversely, NK and CD4+ T-cells were associated with a longer OS (HR 0.580 with 95%CI 0.366-0.917, $p=0.02$ and HR 0.962 with 95%CI of 0.932-0.993, $p=0.015$, respectively)⁴⁹.

Analysis of biopsies from patients with CRPC compared with castration-sensitive prostate cancer (CSPC) revealed that CRPCs had an enrichment of PMN-MDSC (CD11b⁺CD33⁺CD15⁺ cells) but not CD11b⁺CD15⁻ which were localized in close proximity to Epithelial Cell Adhesion Molecule (EpCAM) + epithelial tumour cells. In mouse models of CRPC, PMN-MDSC infiltration is linked to AR activation, conferring castration resistance. With castration, PMN-MDSCs number increased over time, paralleling the emergence of CRPC. While PMN-MDSCs increased in castrated tumours, the frequency of tumour associated macrophages (TAMs) were decreased. It has been noted that PMN-MDSCs represented the major subset of immune cells that increased in Pten-null tumours upon castration. Additionally, mouse models of PTEN null prostate tumours are heavily infiltrated by a population of infiltrating CD11b⁺Gr-1⁺ myeloid cells. These cells protect a fraction of proliferating tumour cells from senescence and therefore maintaining tumour growth. It has been suggested that MDSCs may drive chemo-resistance in human prostate cancer.

In the adjuvant setting, it was observed that prostate cancer patients having tumours infiltrated by CD33fl myeloid cells, relapsed after docetaxel treatment⁴³.

Tumour-infiltrating MDSCs secrete IL23, which induce transcription of AR target genes, confer resistance to androgen deprivation and promote prostate cancer cell proliferation and survival⁴⁴. In CRPC patients IL23 is expressed in PMN-MDSCs from biopsies and plasma levels of IL-23 was substantially higher than in patients with CSPC. Plasma IL-23 levels statistically correlated with tumour-infiltrating PMN-MDSC counts (EpCAM-CD11b+CD33+CD15+ cells) but not with other myeloid cell population counts (CD11b+CD15- cells).

MDSC blockage has recently been shown to revert docetaxel chemo-resistance in a mouse model of prostate cancer, thus suggesting that combinatorial approaches aimed to affect MDSC trafficking or functionality should be taken in consideration. Indeed, docetaxel-induced senescence and efficacy was increased in Pten-null prostate tumours when the percentage of tumour was reduced by treating the mice with an antagonist of CXC chemokine receptor 2 (CXCR2). Therapeutically, docetaxel-induced senescence and efficacy were higher in PTEN null tumours when the percentage of tumour-infiltrating CD11b⁺Gr-1⁺ myeloid cells was reduced using a CXCR2 antagonist⁴³.

Some studies have suggested that ADT with a Gonadotropin-releasing hormone (GnRH) analogue 1) can induce an expansion of the naïve T-cell compartment with continued thymic output; 2) may decrease the T-cell activation threshold; and 3) may elicit changes and adaptive responses by 1 month after beginning therapy with changes persisting over the course of therapy. This may augment the desired effects prostate cancer-directed immunotherapies by increasing the pool of naïve T-cells which could respond to the immunotherapy, by enhancing T-cell responsiveness, and these effects may persist over the course of androgen deprivation. Patients treated with ADT develop persistent changes in adaptive immune responses. In particular some patients developed a continued expansion of naïve T-cells through thymic output demonstrated by an increase in the CD4⁺ naïve T-cell (CCR7⁺, CD45RO⁻) and Recent Thymic Emigrants (RTE) (CD31⁺, CD45RO⁻) population. This suggests the pool of T-cells with greater TCR diversity may be present after ADT. In addition, T-cells in the periphery of treated patients proliferated more robustly to TCR and co-receptor stimulation, and IgG responses developed to proteins of the prostate which could be detected in the sera by one month after beginning ADT⁴⁵. A number of studies have suggested that whole-blood gene profiling could identify gene- expression signatures that stratify patients with castration-resistant prostate cancer into distinct prognostic groups^{46,47}.

2.6. Circulating tumour cells in Metastatic Prostate Cancer

Several studies in mCRPC have shown CTC count to be strongly prognostic. Molecular analyses have suggested additional utility. The detection of AR-V7 in CTCs is associated with worse outcome with AR targeting therapies. AR-V7 is a splice variant that encodes a truncated AR protein that lacks the C-terminal ligand-binding domain but retains the transactivating N-terminal domain. Although the resulting truncated proteins are unable to bind ligand, they are constitutively active as transcription factors and capable of promoting activation of target genes. The association between the detection of AR-V7 messenger RNA (mRNA) in an enriched (selected) fraction of CTCs, poor PSA responses, and shorter radiographic progression-free survival times after treatment with AR targeting therapy was first reported in 2014^{48,49}. Follow-up studies with the same assay showed not only a negative association with OS for patients positive for AR-V7 who were treated with AR targeting therapy but also that PSA response and survival with taxane-based therapy were not affected by AR-V7 status.

Use of the mRNA determinant as a blood-based biomarker has limitations such as stability of the blood sample, which varies as a function of the collection tube used and the time to sample processing, and in the case of a transcription factor such as AR-V7, an inability to discern if the coded protein is actually localized in the nucleus of cells where it functions to drive tumour growth. The Epic Sciences platform has developed a protein-based assay that can discern the presence and cellular localization of AR-V7

protein in CTCs. This approach deposits all nucleated cells from a patient's blood sample onto proprietary, positively charged slides and uses fluorescent scanners to image each cell and identify CTCs. The approach enables a high sensitivity of CTC detection with minimization of cell loss or damage⁵⁰⁻⁵³, as well as protein biomarker assessment on individual CTCs. Higher PSA response rates, longer radiographic progression-free survival times, and better OS were observed among patients with detectable nuclear-localised AR-V7– positive CTCs who received taxanes, relative to those who received AR targeting therapy^{50,54}. Additional value appears to arise in mCRPC from including molecular features in prognostic algorithms, including CTC nuclear AR-V7 expression, features of intra-patient inter-cellular heterogeneity and indices of genomic instability^{50,52}. Utilizing digital pathology features on individual CTCs enables defining phenotypically distinct cell types. This enables heterogeneity to be quantified on the basis of the diversity of cell types in individual patient samples using the Shannon index. Low CTC phenotypic heterogeneity was associated with better OS in patients treated with AR targeting therapy, whereas high heterogeneity was associated with better OS in patients treated with taxane chemotherapy. Limited data for CTC analysis exists for de novo polymetastatic prostate cancer but capture and analysis of single CTC may provide additional information to tissue biopsy and plasma DNA analysis.

Micro-fluidic based and very sensitive CTC capture platforms combined with PSA/ Prostate specific membrane antigen (PSMA) dual immunophenotyping detected CTCs in patients with newly-diagnosed metastatic prostate cancer. AR activity was predominantly positive among the patients with detectable CTCs, with the vast majority of CTCs showing an “AR-on” phenotype (PSA⁺/ (PSMA)⁻). The initiation of ADT resulted in a change from an “AR-on” to an “AR-off” phenotype in the majority of CTCs within a month of treatment and complete disappearance of CTCs by 3 months of treatment⁵⁵. However overall, the data on CTC at start of ADT is limited and their analyses in PARADIGM could constitute a novel contribution.

2.7. Whole body diffusion-weighted MRI for hormone sensitive metastatic prostate cancer

Isotope bone scans and computed tomography (CT) remain the SOC imaging modalities for the assessment of metastatic prostate cancer though suffer from widely known limitations in evaluating baseline disease burden as well as monitoring treatment response^{56,57}. Sensitivity for metastatic disease detection on CT is low; sub-centimetre metastatic lymph nodes are incorrectly ascribed as normal and early bone lesions are not visible⁵⁸. Whole-body technetium-labelled bone scan is insensitive for early disease detection as it images the reaction within bone to disease presence rather than tumour itself⁵⁹, as such bone scan are inferior to emerging imaging methods in bone lesion detection⁶⁰. Early response assessment is also problematic, for example, on CT evaluation of response in bony disease is limited in the absence of extra-osseous soft tissue disease but may be seen late when normal trabecular bone is restored⁵⁶.

Adding to the complexity of imaging assessment is that metastatic lesions demonstrate considerable inter-patient as well as intra-patient/inter-metastasis heterogeneity. Whole-genome and deep sequencing analyses on metastatic biopsies from prostate cancer, has demonstrated that after sharing a common clonal origin, individual metastases may differentiate independently of each other and/or the primary tumour⁶¹. There is therefore a clearly recognised requirement for the development of imaging biomarkers to better detect disease, monitor treatment and potentially predict response to guide therapy in concert with ongoing molecular blood-based testing. A potential added benefit of imaging is its ability to assess each tumour site individually (rather than a global assessment afforded by plasma biomarkers for example) to further tailor treatment.

Although nuclear medicine scans such as choline and PSMA PET (Positron emission tomography) -CT have shown promising results for disease detection, their values for response monitoring remains largely unclear and limited to small cohort studies. Furthermore, PET-CT scans impart substantial radiation dose, are largely limited to tertiary centres and are expensive to run⁶².

Whole body diffusion weighted magnetic resonance imaging (WB-DW-MRI) is a non-ionising imaging technique that can be used to assess a variety of cancers, including Prostate cancer at a lower cost compared with PET-CT. It has been shown that WBMRI is superior to conventional imaging techniques (including bone scan, CT, PET-CT) for bony disease detection in variety of cancers^{63,64}, including metastatic prostate cancer⁷⁴. Additionally, using WBMRI derived quantitative imaging biomarkers (QIBs), one can interrogate multiple aspects of tumoral microenvironments including cellularity, vascularity and fat content in one scanning session such that early post-treatment QIB changes have been shown to address current shortcomings of global measurements of response in multiple myeloma⁶⁵. Ongoing work done at UCL Centre for Medical Imaging has looked at WBMRI derived signal fat fraction in lymph nodes as a marker of nodal disease status and treatment response in patients with radio-recurrent prostate cancer capable of providing a QIB to classify pre-treatment nodal disease status whilst change in signal fat fraction may help identify responding lymph nodes (ROC-AUC 0.86). A translational sub-study of WBMRI within the PARADIGM study represents an opportunity to assess the utility of QIB derived from WBMRI in assessing response in concert with other state of the art biomarkers to individualise therapy and their potential to feed into composite biomarker modelling.

2.8. Gut microbiome in metastatic prostate cancer

Through the release of toxins, microbiota can directly affect tumour initiation⁶⁶. The microbiota can contribute to tumour development through the promotion of inflammation⁶⁷ and shaping the tumour immune response⁶⁸. Increasing evidence demonstrates that microbiota is important for the antitumor activity of both chemotherapy⁶⁹ and immune checkpoint inhibitors⁷⁰. Additionally, through the modulation of the microbiota, this may enhance favourable treatment response in cancer patients. There are only a limited number of correlative studies that have investigated the role of the gut microbiota in prostate cancer initiation and progression⁷¹. Shotgun whole-genome metagenome (WGM) sequencing of rectal swabs from mHSPC and mCRPC patients analyses highlighted a specific microbial signature in CRPC patients, with 33 species specifically enriched in CRPC and 10 species enriched in CSPC microbiota. The study identified commensal bacteria species that associated with poor clinical outcome and species that associate with better outcomes⁷².

2.9. Future implications of PARADIGM

PARADIGM will provide Stage 1 clinical qualification⁷³ for the association of ptDNA and worse outcomes after 2-3 cycles of ARSI or docetaxel at start of ADT. If positive, our aim will be to then conduct a second study (PARADIGM-2) that will evaluate improved outcomes by changing treatment early in ptDNA positive patients. This second study would be required to provide the Level 1 evidence to change clinical practice. Use of ptDNA as studied in the PARADIGM studies will have a number of implications for patients:

- Reducing exposure to ineffective treatments: less toxicity and greater cost efficiency
- Maximising treatment efficacy by earlier switch to a potentially more effective therapy
- Implementation of biomarkers for improved treatment selection

3. TRIAL DESIGN

A prospective, observational, biomarker-focused, translational-platform cohort study in newly diagnosed high-risk metastatic prostate cancer patients starting long-term systemic therapy.

3.1. Study Objectives

3.1.1. Primary Objective:

PARADIGM A and D: To determine whether the detection of ptDNA after two or three cycles of ARSI or docetaxel added after the start of ADT is associated with a worse clinical outcome in newly diagnosed polymetastatic prostate cancer.

PARADIGM-E: A separate independent cohort for clinical validation and further evaluation of results from PARADIGM-A (see section 13).

3.1.2. Secondary Objectives:

All cohorts:

1. To compare ptDNA classification at C2D1 and C5D1 with C3D1 or C4D1.
2. To determine whether the detection of ptDNA after five to sixteen weeks of starting ADT and prior to starting ARSI or docetaxel is associated with a worse clinical outcome.
3. To determine the association between clinical outcome and prostate specific antigen (PSA) level (<0.2, 0.2-4, >4ng/dl) after five to sixteen weeks of starting ADT and prior to starting ARSI or docetaxel, prior to treatment at every cycle and at 7 months after start of ADT.
4. To assess whether ptDNA detection is a better predictor of clinical outcome than PSA level (as assessed in objective 3) after five to sixteen weeks of starting ADT and prior to treatment at every cycle.
5. To compare associations with clinical outcome for the change in ptDNA detection and PSA level (as assessed in objective 3) prior to start of ARSI or docetaxel and at C3D1 or C4D1.
6. To evaluate whether ptDNA fraction prior to LHRHa (stratified by no anti-androgen versus 2-3 weeks of anti-androgen) is associated with Progression Free Survival (PFS) and Overall Survival (OS).

3.1.3. Exploratory Objectives:

All cohorts:

1. To develop and test a composite model incorporating different biomarkers for assessing response.
2. To describe ptDNA dynamics and compare to PSA kinetics.

3.1.4. Translational Objectives:

All cohorts:

Translational research will be performed on sub-sets of patients from whom the required evaluations are made. It is expected that not all patients will be able to participate in all the translational studies but as a fundamental aspect of PARADIGM, as many patients as possible should be included in these assessments.

1. Predictors of response to systemic treatment
 - i. To identify molecular signatures in plasma and tumour that associate with PFS or OS with ARSI or docetaxel.
 - ii. To identify a molecular signature in pre-ADT plasma or tumour that associates with plasma AR aberrant status at progression to castration-resistant disease.
2. Tracking of plasma DNA or extracellular vesicles dynamics
 - i. To determine whether detection of ctDNA precedes PFS (or OS)
 - ii. To molecularly characterise resistant clones.
 - iii. To evaluate whether patients who progress with AR gain at the development of mCRPC have a shorter time to PFS (on ADT) and OS.
3. CTC dynamics at initiation of ADT
 - i. To evaluate whether CTC count pre-ADT and after starting ADT associates with shorter PFS and OS.
 - ii. To identify CTC molecular features prior to and after starting ADT that associate with PFS or OS with ARSI or docetaxel.
4. Interrogation of peripheral immune changes secondary to initiation of ADT
 - i. To determine changes in PMN-MDSC (CD11b+CD33+CD15+ cells) and M-MDSCs and immune cells NK cells, T-lymphocytes, CD4+ T-lymphocytes, CD8+ T-lymphocytes and B-lymphocytes) from peripheral blood leukocyte samples after exposure to ADT and subsequently ADT with ARSI or docetaxel.
 - ii. To determine dynamic changes in IL-23 and other cytokines in plasma after exposure to ADT and subsequently ADT with ARSI or docetaxel.
 - iii. To evaluate whether patients with rising PMN-MDSCs, M-MDSCs or cytokines post ADT and during ADT with ARSI or docetaxel have a shorter PFS and rPFS and OS.
 - iv. To define the peripheral blood immune profile and correlate with archival tumour tissue and PFS and OS.
 - v. To correlate peripheral immune changes pre and post ADT with genomic changes in circulation.
 - vi. To determine TCR repertoire changes pre and post ADT and correlate this to PFS and OS.
5. WBMRI derived imaging biomarkers as a surrogate of response
 - i. To determine utility of WBMRI derived quantitative imaging biomarkers in predicting and assessing early response to docetaxel and ARSI as determined by PFS and OS and association with plasma tumour markers both at baseline and changes on treatment.
 - ii. To identify WBMRI imaging biomarkers (at baseline and changes during treatment), which may associate with increased risk of developing mCRPC and may be incorporated into biomarker composite models of response.
6. Evaluating changes in the gut microbiome that occur with institution of testosterone suppression and development of castration-resistance
 - i. To explore the constituent and genomic changes in the gut microbiome at minimal exposure to ADT, after response to ADT with enzalutamide and at progression
 - ii. To test for associations and investigate the role of the gut microbiome in progression to CRPC in patients treated with ADT and enzalutamide

3.2. Study Endpoints

Major Endpoints

PFS and OS for PARADIGM-A and PARADIGM-D and PFS for PARADIGM-E. These will be reported separately for each cohort. OS was defined as an endpoint of major interest in protocols version 1 to 5 but after confirmation of additional funding allowing longer follow-up, protocol 6 was amended to define reporting of OS as a major end-point combined with PFS.

PFS will be defined as the interval from start of ARSI or docetaxel to progression to CRPC, usually necessitating a treatment change, as determined by at least one or more of the following factors:

1. Symptomatic or asymptomatic progression of, or new, distant metastases confirmed by imaging
2. Symptomatic progression of cancer in the prostate confirmed by imaging,
3. Serum PSA progression (in PARADIGM-D only)
4. Death from any cause

The calculation is detailed further in section 13.3.

OS is defined as time from start of ARSI or docetaxel to death from any cause.

3.2.1. Secondary end-point

1. PCSS, defined as time from start of ARSI or docetaxel to death from prostate cancer.

3.3. Study Activation

UCL CTC will ensure that all study documentation has been reviewed and approved by all relevant bodies and that the following have been obtained prior to activating the study:

- Health Research Authority (HRA) approval, including Research Ethics Committee approval
- 'Adoption' into NIHR portfolio
- Adequate funding for central coordination
- Confirmation of sponsorship
- Adequate insurance provision

4. SELECTION OF SITES/SITE INVESTIGATORS

4.1. Site Selection

In this protocol study 'site' refers to a hospital where study-related activities are conducted.

Sites must be able to comply with:

- SOC treatment(s), imaging, clinical care, follow up schedules and all requirements of the study protocol
- Requirements of the UK Policy Framework for Health and Social Care Research, and all amendments
- Data collection requirements, including adherence to electronic case report form (eCRF) submission timelines as per section 14.
- Monitoring requirements, as outlined in protocol section 17 (Trial Monitoring and Oversight)
- Biological sample collection, processing and storage requirements

4.1.1. Selection of Principal Investigator and other investigators at sites

Each site must appoint an appropriate Principal Investigator (PI), i.e. a health care professional authorised by the site to lead and coordinate the work of the study on behalf of the site. Co-investigators must be trained and approved by the PI. The PI is responsible for the conduct of the study at their site and for ensuring that any amendments are implemented in a timely fashion. If a PI plans to take a leave of absence, **UCL CTC must be informed promptly**. For absences greater than three months or where the PI is no longer able to perform their role, a new suitable replacement PI must be identified by the site and UCL CTC notified.

UCL CTC may suspend recruitment at a site where a suitable replacement PI has not been identified within three months.

4.1.2. Training requirements for site staff

All site staff must be appropriately qualified by education, training and experience to perform the study related duties allocated to them, which must be recorded on the site delegation log.

CVs for all staff must be kept up-to-date, signed and dated and copies held in the Investigator Site File (ISF). A current, signed copy of the CV for the PI must be forwarded to UCL CTC upon request.

4.2. Site Initiation and Activation

4.2.1. Site initiation

Before a site is activated, the UCL CTC trial team will arrange a site initiation with the site which the PI and site research team must attend. The site will be trained in the day-to-day management of the study and essential documentation required for the study will be checked.

Site initiation will be performed for each site initially by site visit, telephone or videoconference. This training will include the management of the blood sample collection. Re-initiating sites may be required when there has been a significant delay between initiation and enrolling the first patient.

4.2.2. Required documentation

The following documentation must be submitted by the site to UCL CTC prior to a site being activated by the UCL CTC trial team:

- Trial specific UK Site Registration Form (identifying relevant local staff).
- Relevant institutional approvals.
- A completed site delegation log that is initialled and dated by the PI (with all tasks and responsibilities delegated appropriately).
- Completed site contacts form (with contact information for all members of local staff).
- A signed and dated copy of the PI's current CV (with documented up-to-date GCP training or copy of GCP training certificate).

In addition, the following agreements must be in place:

- A signed Model Agreement for Non-commercial research (mNCA) between the Sponsor and the relevant institution (usually an NHS Trust/Health Board).

4.2.3. Site activation letter/email

Once the UCL CTC trial team has received all required documentation and the site has been initiated, a site activation letter/email will be issued to the PI, at which point the site may start to approach patients.

Following site activation, the PI is responsible for ensuring:

- Adherence to the most recent version of the protocol.
- All relevant site staff are trained in the protocol requirements.
- Appropriate recruitment and medical care of patients in the trial.
- Timely completion and return of electronic case report form (eCRF) forms.
- Timely completion and return of CRFs (pre-ADT sub-study and SARs).
- Prompt notification and assessment of all serious adverse reactions (SARs).

5. INFORMED CONSENT

Sites are responsible for assessing a patient's capacity to give informed consent. There are separate Patient Information Sheet (PIS) and consent forms for this study:

- PIS PARADIGM-D for patients receiving Docetaxel
- PIS PARADIGM-A for patients receiving an ARSI, a separate PIS will be available for each specific ARSI treatment (Abiraterone, Apalutamide and Enzalutamide)
- PIS PARADIGM-E - for patients receiving Enzalutamide.
- Sub-study: pre-ADT (see section 5.2)
- Patient directed collection (see section 5.3)
- Sub study: WB-MRI (see section 5.5)
- Sub-study: Microbiome sub-study (see section 5.2)

There is no minimum time that must pass from first approaching the patient before consent can be taken. If the patient wishes, he can sign the consent form on the same day he is approached. Sites must assess a patient's ability to understand verbal and written information in English and whether an interpreter would be required to ensure fully informed consent. If a patient requires an interpreter and none is available, the patient should not be considered for the study.

The PI, or, where delegated by the PI, other appropriately trained site staff, are required to provide a full explanation of the study and all relevant treatment options to each patient prior to study entry. During these discussions, the current approved patient information sheet for the trial should be discussed with the patient. Written informed consent on the current approved version of the consent form for the study must be obtained before any study-specific procedures are conducted. The discussion and consent process must be documented in the patient notes.

Site staff are responsible for:

- Checking that the current approved version of the PIS and consent form are used
- Giving the patient a copy of the patient information sheet
- Checking that information on the consent form is complete and legible.
- Checking that the patient has initialled all relevant sections and signed and dated the form.
- Checking that an appropriate member of staff has countersigned and dated the consent form to confirm that they provided information to the patient.
- Giving the patient a copy of their signed consent form
- Checking that an appropriate member of staff has made dated entries in the patient's medical notes relating to the informed consent process (i.e. information given, consent signed etc).
- Following registration adding the patients' study number to all copies of the consent form, which should be filed in the patient's medical notes and investigator site file.

The right of the patient to refuse to participate in the study without giving reasons must be respected. All patients are free to withdraw at any time. Also refer to Section 10.

5.1. Remote Consent on PARADIGM

Guidance on conducting remote consent has been provided in Appendix 3 for sites to follow in situations where obtaining physical or face to face consent is not feasible.

5.2. Consent to pre-study (ADT sample)

Collection of blood prior to ADT may be logistically challenging due to the indication to start ADT as soon as possible in men with polymetastatic disease. Explaining the PARADIGM study in detail on the day of diagnosis could over-burden patients with information and may not be feasible. Additionally, the treatment plan for the patient may not have been decided and it would therefore not be feasible to consent them to the main study. However, scientifically the pre-ADT sample is very valuable as it includes tumour DNA from sensitive clones that will rapidly regress after initiation of ADT.

To facilitate the collection of blood prior to starting anti-androgen or LHRHa, patients who have metastatic prostate cancer who are deemed to be potentially eligible for the study will be provided with a pre-study PIS and asked to consent to collect blood for research purposes and data collection. Once they have consented, the pre-ADT sample can be collected. The pre-study PIS will state that approximately 70mls of blood will be collected, processed and stored for future research if patients' consents to this.

At a later date, the patient may be provided with the main study PIS, and they can then decide whether they agree to proceed onto the PARADIGM study. If they do not consent to the main study, the pre-ADT sample will be kept and analysed for future research if patients' have consented to this on the pre-study PIS. Also, if a patient does not consent to the main study and wants the pre-ADT sample destroyed this will be permitted. If patients wish to consent to the Main study at this point or the investigator believes that it is appropriate to do so then they will be provided with the PIS for the main study (see below).

Patients who have previously consented on the pre-study consent form can be approached for the main study at a later date, along with any other potential patients. The PI, or, where delegated by the PI, other appropriately trained site staff, will decide whether the patient will start ARSI (cohort A, or E) or docetaxel (cohort D). There are separate PIS depending on which treatment the patient will receive as part of standard of care and the relevant current approved PIS should be discussed with the patient (see section 5)

5.3. Next-Generation Sequencing (NGS) Results

NGS performed in the translational research may identify molecular information of clinical significance. At consent patients will be specifically asked whether they accept clinically relevant information to be fed back to them. Only results which are of established clinical relevance and for which testing would be available under standard NHS genetic testing guidelines will be fed back e.g. germline pathogenic *BRCA1/2* mutations. Any genetic analysis undertaken does not replace clinically indicated investigations as it cannot be guaranteed that results will be fed back in a timely fashion and tests may not be clinically accredited.

The TMG will decide whether referral to a clinical geneticist is recommended. This is to facilitate access to genetic counselling and the required confirmatory testing, and also necessary in order to offer appropriate advice to biological relatives in the event of detection of a germline (inherited) genetic abnormality.

The TMG will review all detected germline variants detected and make the final decision of which are feedback to patients.

5.4. Consent to Patient Directed sample collection

Patients will be provided the opportunity to consent to having a more active role in the collection of their samples. Patients can be consented at any point during the study. It is the Sites' responsibility to assess a patient's understanding and ensure that they have capability to carry out this task and assess the most appropriate time point to administer the additional patient directed sample collection PIS and consent form, for example when the patient enters the follow up period. The patient may consent to this method, but the site could decide that the patient is not suitable. Further information on what is involved is discussed in section 8.7.1.

5.5. Consent to Whole Body Magnetic Resonance Imaging (WB-MRI)- at selected sites only

Patients who are eligible to receive a WB-MRI scan will be given the opportunity to consent to this at selected sites. The patient can be provided with the WB-MRI PIS and consent form at the same time as the main study PIS or at a later timepoint. For more information, please see section 9.

5.6. Consent to Gut Microbiome sub study

Patients on PARADIGM-E only will be provided the opportunity to consent to collection of stool samples and ask question on antibiotic use and diet by the research team for the Gut Microbiome sub-study as part of the pre-ADT and main-study consent form. For more information see section 8.9.

6. SELECTION OF PATIENTS

6.1. Screening Log

A screening log will not be mandated for the study. Research teams are encouraged to raise screening concerns that arise to the UCL CTC team.

6.2. Patient Eligibility

There will be no exception to the eligibility requirements at the time of registration. Queries in relation to the eligibility criteria must be addressed prior to registration. Patients are eligible for the study if all the inclusion criteria are met and none of the exclusion criteria applies.

Patients' eligibility must be confirmed by an investigator who is suitably qualified and who has been allocated this duty, as documented on the site staff delegation log, prior to registering the patient. Confirmation of eligibility must be documented in the patients' notes and on the registration ECRFs.

Patients must give written informed consent before any study-specific screening investigations are carried out. Refer to section 11.3 for the list of assessments and procedures required to evaluate the suitability of patients prior to entry.

6.2.1 Inclusion criteria

1. Able and willing to provide written informed consent
2. Aged 18 or over
3. Polymetastatic disease defined as one of the following:
 - ≥5 bone metastases,
 - ≥1 unequivocal visceral metastasis
4. Eastern Cooperative Oncology Group (ECOG) Performance status 0 to 2
5. No medical contra-indications to intended treatment (ARSI – abiraterone and prednisolone, apalutamide or enzalutamide (PARADIGM-A), enzalutamide only (PARADIGM-E), or docetaxel and prednisolone (PARADIGM-D).
6. Patients should be either of the following:
 - i. Planned to start long-term Luteinizing hormone Releasing Hormone (LHRH) suppression, or
 - ii. Have started long-term LHRH antagonist within the last 14 weeks, or
 - iii. Have started LHRH agonist within the last 16 weeks. When antiandrogens (e.g. bicalutamide) are used in combination with an LHRH agonist to prevent flare, patients have to have started within 18 weeks of starting antiandrogens.
7. Patients should be planned for addition of ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A, or PARADIGM-E – enzalutamide only cohort), or docetaxel (PARADIGM-D), within 14 weeks after start of LHRH antagonist (or within 16 weeks if LHRH agonist is started without anti-androgen or 18 weeks from start of anti-androgen with a target of 6 cycles or continuation until progression respectively. When patients are started on LHRH antagonist and then converted to a LHRH agonist, the same timelines as for LHRH antagonist apply.
8. No concomitant medical conditions likely to reduce life expectancy.
9. Patient agrees to be followed up in the recruiting site and to having sequential plasma samples collected as per the study protocol.

6.2.2 Exclusion criteria

1. Concurrent or planned for (i.e. prior to development of castration resistance), treatment of the primary tumour with oestrogen radiotherapy or surgery to the primary tumour.
(N.B. Patients may also be eligible if randomised to any of the following experimental drugs; PARPi, PD-1/PD-L1, AKTi, PSMA-lutetium, if given in combination with SOC (i.e. ADT + docetaxel or ARSI) treatment in an open-label clinical trial, or to the placebo arm in an open-label clinical trial following discussion and approval of the CI/delegate. Patients can participate in other observational studies.)
2. Prior systemic therapy for prostate cancer other than for LHRHa +/- anti-androgen (started within the time limits defined in inclusion criterion 7).
3. Metastatic brain disease or leptomeningeal disease.
4. Any surgery planned prior to Cycle 4 Day 1 (C4D1)
5. Other current malignancy or malignancy diagnosed or relapsed within the past 5 years (other than non-melanomatous skin cancer, stage 0 melanoma in situ and non-muscle invasive bladder cancer).
6. Patients who consent to the whole-body magnetic resonance imaging (WB-MRI) translational sub-study should have no contraindications to MRI as per local guidelines.

7. REGISTRATION PROCEDURES

Patients can be registered to the main study prior to starting ADT treatment or within 16 weeks of starting ADT. Patients cannot be registered after they have started treatment on docetaxel or ARSI.

7.1. Registration to pre-study (ADT sample)

Sites will be provided with a log containing pre-study numbers for patients who have consented to the pre-study consent form. Once consented, patients will be added to the log sequentially and a pre-study number provided. UCL CTC should then be emailed to confirm the pre-study patient number, referring site, patient initials, consent date, date sample was taken and whether they have given consent for samples to be kept for research purposes

7.2. Registration to main study

Patient registration to the main study will be performed via a remote EDC system hosted by UCL CTC. Please refer to the PARADIGM Database User Manual for Sites in the site file prior to registering a patient. Patients must be confirmed to be eligible and have given consent prior to registration. Site staff responsible for patient registration must request access to the EDC database by completing their contact details on the site contacts form and delegation log. Access to the database and instructions are provided by UCL CTC. Note that patient initials are required to register a patient. UCL CTC will check all registrations to confirm eligibility and issue a registration email to site where a main study number will be assigned (please note this is a separate study number to the pre-study number). Patients will be registered to either PARADIGM-A or PARADIGM-D depending on which treatment they will receive. Following completion of recruitment to PARADIGM-A cohort, PARADIGM-E will open for patients receiving enzalutamide as SoC. The study number must be recorded in the patient notes. Blood samples cannot be collected unless a study number has been issued by UCL CTC.

Sites should contact UCL CTC if there are any difficulties in accessing the EDC system. If the patient has consented to the patient directed sample collection, UCL CTC will contact the site following registration to collect the patients name and telephone number if patient's have consented to this. This will be stored securely on a permission only folder with an encrypted Excel Spreadsheet at UCL CTC.

CONTACT DETAILS	
PARADIGM Trial Coordinator:	020 7679 9714
UCL CTC Office hours:	09:00 to 17:00 Monday to Friday, excluding Bank Holidays (UK Time)

Once a patient has been registered onto the main study they must be provided with the following:

- Patient samples diary
- Samples kit to include patient instruction, blood tubes, labels, and worksheets (for selected patients see section Patient directed sample collection).

After registration into the trial, the patient's general practitioner (GP) should be informed of the patient's involvement in the trial by the site completing and sending the completed GP letter.

8. STUDY INTERVENTION

8.1. Treatment Summary

All patients will receive SOC treatment and there will be no randomisation. Treatment selection will be based on patient and physician choice, funding availability etc. Toxicity, overdose, allergic reactions and concomitant medicine interactions will be managed according to local guidelines and do not need to be specifically reported to UCL CTC.

8.2. Standard of care - ADT

If LHRH antagonists are used (with no prior anti-androgen), docetaxel or ARSI should be started within 14 weeks. If LHRH agonists are used docetaxel or ARSI should be started within 16 weeks. If antiandrogens (e.g. bicalutamide) are used in combination with an LHRH agonist to prevent flare, docetaxel or ARSI should be started within 18 weeks of starting antiandrogens. When patients are started on LHRH antagonist and then converted to a LHRH agonist, the same timelines as for LHRH antagonist apply. Single-agent anti-androgen is allowed for up to 3 weeks prior to LHRH agonists. Anti-androgen monotherapy is not permitted as a form of long-term ADT.

8.3. Standard of care - Docetaxel (PARADIGM-D)

Docetaxel (with or without prednisolone) will be given according to local protocols as a standard non-trial treatment. One cycle is usually 3 weeks although this can be delayed for medical reasons. ***PARADIGM-D cohort is now closed to recruitment as of February 2023.***

8.4. Standard of care - ARSI (PARADIGM-A)

Abiraterone in combination with prednisolone, enzalutamide* or apalutamide will be given according to local protocols as a standard non-trial treatment. Recruitment to PARADIGM-A will be dependent on availability of local funding. One cycle is always 4 weeks and a delay to treatment does not change the cycle timings.

**Enzalutamide received NICE funding approval on 23/04/2020 as an interim treatment change option for the treatment of prostate cancer in this patient population during the COVID-19 pandemic, as endorsed by NHS England. Enzalutamide is likely to remain an option as a standard of care treatment if local funding arrangements are in place. See below.*

PARADIGM-A cohort is now closed to recruitment as of January 2022.

8.5. Standard of care - Enzalutamide (PARADIGM-E)

In July 2021, extension of the enzalutamide license was approved by the MHRA (and subsequently by NICE) for use to treat metastatic hormone sensitive metastatic prostate cancer. Enzalutamide will be given according to local protocols as a standard non-trial treatment. One cycle is always 4 weeks and a delay to treatment does not change the cycle timings.

8.6. Prednisolone switch to dexamethasone

Some physicians may choose to continue ARSI after failure but change prednisolone to dexamethasone. A progression event on ARSI will be defined when the definition is met, either on first-line or second-line glucocorticoid (see section 13.3 for definition of the major endpoints).

8.7. Research blood sample collection

Blood samples for research will be collected at the following timepoints for the following analyses. Blood samples can be taken up to 120 hours (5 days) prior to pre-specified time points.

Timepoint	Samples to be taken
Pre-ADT ¹	Plasma (ptDNA), Whole blood (CTCs) and Whole blood (immunoprofiling), PAXGene RNA
Prior to Cycle 1 Day 1	Plasma (ptDNA), Whole blood (CTCs) ² and Whole blood (immunoprofiling) ²
Prior to Cycle 2 Day 1	Plasma (ptDNA)
Prior to Cycle 3-day 1	Plasma (ptDNA), Whole blood (CTCs) ² and Whole blood (immunoprofiling) ²
Prior to Cycle 4-day 1	Plasma (ptDNA), Whole blood (CTCs) ^{2,3} and Whole blood (immunoprofiling) ²
Prior to Cycle 5-day 1	Plasma (ptDNA) and Whole blood (immunoprofiling) ²
Prior to Cycle 6-day 1	Plasma (ptDNA)
At completion of 6 cycles	Plasma (ptDNA)
Follow-up visits (frequency as per standard of care) ⁴	Plasma (ptDNA)
At progression	Plasma (ptDNA), and Whole blood (immunoprofiling) ²

¹Selected patients who consent on the pre-study consent form or consent to the main study prior to starting ADT.

²Only patients who had pre-ADT sample taken

³CTCs to be collected at C4 only if not collected at C3D1

⁴Study bloods should not be taken more than once every 3 months

Pre-ADT research blood samples will be collected from approximately 150 patients (the TMG will review this target on a regular basis and may reduce the number if it is thought that it was affecting accrual). A ptDNA sample taken at cycle 3 day 1 or cycle 4 day 1 is required for the major endpoints and any patients who do not have the cycle 3 day 1 or cycle 4 day 1 ptDNA sample taken will not be included in the target 130 patients. A pre-ADT samples is not required for major endpoints analyses.

There are two methods of collecting blood samples for the study, as detailed below. The method chosen will be determined by each site and will take into consideration logistics such as facilities at site as well as the patient's profile (performance status, capability and social support). Eligible patients must be confirmed by a suitable investigator prior deciding the method of collecting samples.

All patients included in the study will be encouraged to bring a 'Patient Sample Diary' to clinic with a record of samples taken. Research teams will ensure the correct record of samples in clinic.

8.7.1. Patient directed sample collection

The patient-directed sample collection gives patients a more active role in the study. The site will supply empty blood test tubes to the patient to take home and bring to the next blood test appointment. It can be advantageous for the patient to take part in the patient directed collection when the patient is seen less frequently, for example in the follow up period. Patients can consent at any time point to take part in the patient directed sample collection (please see section 5.3). Once the patient has consented, they will receive a patient collection diary at the time of registration.

Once a patient has consented to the patient directed sample collection, the site must record consent information on the EDC detailing date of consent, patient directed sample collection version of PIS and informed consent form, name of person taking consent and whether the patient has agreed for their name and telephone number to be sent to UCL CTC. They will also be given patient instructions sheet and sample kit(s) with blood tubes, labels and worksheets.

If the patient agrees for their name and telephone number to be sent to UCL CTC, the site must notify the UCL CTC that the patient has agreed to this via email. The PARADIGM team will contact the site to obtain the patient's name and number, and this will be recorded securely. The PARADIGM team will telephone the patient for a welcome call. Prior to each clinic visit, patients will receive a call from the PARADIGM team to remind them to bring blood tubes to phlebotomy. Following collection, tubes will be stored at phlebotomy. The research team will coordinate the samples for collection and will be sent on the same day via post or courier. To maximise efficiency, PARADIGM team will be in regular contact with each site to ensure the patients are contacted at the appropriate time.

If the patient does not consent for their personal details to be passed onto UCL CTC, then the site must maintain oversight of the patient directed collection ensuring patients are given suitable lab supplies to take home and to remind these patients to bring the supplies to their next blood test appointment. To maximise efficiency, the PARADIGM team will be in regular contact with each site to ensure the proper collection of samples and to help outline a suitable process for each site to manage oversight of the patient directed sample collection.

8.7.2. Site directed sample collection

If the patient does not consent to the patient directed sample collection the research team will coordinate the collection of the research blood samples. Sample kits will be kept at site, and study samples will be taken directly by the research team or via phlebotomy as agreed at site initiation. All samples will be sent on the same day via post or courier. All patients will receive a copy of the 'sample collection diary'.

8.8. Research blood sample and stool sample processing

For detailed information on the preparation, storage and shipping of blood samples please refer to the PARADIGM Lab Manual.

8.8.1. Collection of ptDNA sample

Whole blood will be collected into 4 x 10ml plasma collection tubes per time point and handled according to the PARADIGM lab manual/patient instructions sheet. It is important that plasma collection tubes are repeatedly inverted 8-10 times after collection to ensure adequate mixing of additives. Samples are to be shipped to:

Shipping address:

ECMC GCLP Facility
Ground Floor
UCL Cancer Institute
Paul O'Gorman Building
72 Huntley Street
London WC1E 6DD

ptDNA samples will be analysed in batches by team members blinded to clinical outcome. Analytically validated and "fixed" custom targeted next-generation assays performed in a GCLP environment will be used. Patients will be classified into ptDNA positive or negative based on a pre-defined threshold. For pre-ADT samples, the plasma tumour fraction DNA will also be reported.

8.8.2. Collection of CTC sample

Whole blood will be collected in 1 X 10ml CTC tubes and gently inverted 8 to 10 times. Samples should be kept at room temperature and shipped on the same day of collection to Epic Sciences, Inc by courier. ***CTC sample collection is no longer required as of April 2022.***

Shipping address:

Labcorp Central Laboratory Services Sàrl
KIT receipt- CENTERLINX Rue Moises- Marcinhes 7
Meyrin1217
Switzerland

8.8.3. Collection of Immunoprofiling sample

Whole blood will be collected in 2 x 9ml immunoprofiling tubes and 2.5ml PAXGene RNA tube and shipped to:

Shipping address:

ECMC GCLP Facility
Ground Floor
UCL Cancer Institute
Paul O'Gorman Building
72 Huntley Street
London WC1E 6DD

8.8.4. Collection of stool sample

Stool samples will be collected and shipped to the address below, further instructions can be found in the laboratory manual.

Shipping address:

ECMC GCLP Facility
Ground Floor
UCL Cancer Institute
Paul O'Gorman Building
72 Huntley Street
London WC1E 6DD

8.9. Gut Microbiome

A stool sample will be collected where possible from all patients recruited to the pre-ADT sub-study and PARADIGM-E main-study.

A sample will be collected at the following timepoints:

Prior to ADT

- pre-ADT if possible (ideally prior to prostate biopsy)

At main-study registration

- prior to start of enzalutamide at cycle 1 day 1 (C1D1)

Whilst on enzalutamide treatment

- one sample at either cycle 3 day 1 (C3D1)
or, at C4D1 or C5D1 if not taken at C3D1
- at progression

Questions on antibiotic use and diet will be asked by the research team at the time that the first sample is collected (i.e. at the pre-ADT timepoint), or otherwise at the time of main-study registration (C1D1) if not previously completed. Subsequent questions on antibiotic use at each cycle as highlighted in appendix 2 will be documented on the eCRF.

8.10. Archival diagnostic block collection

Patients will be asked to consent for the use of remaining tissue samples if available e.g. those obtained at prostate biopsy or following surgery, for use in translational research. These samples are usually stored as Formaldehyde Fixed-Paraffin Embedded (FFPE) tissue blocks at the hospital where the procedure was performed. Recruiting sites will be asked to retrieve tissue samples stored in pathology stores or referring hospitals and send to the UCL Cancer Institute.

Up to 15 sections will be cut and the tumour blocks will be returned to sites if required or when analyses completed.

Shipping address:

ECMC GCLP Facility
Ground Floor
UCL Cancer Institute
Paul O'Gorman Building
72 Huntley Street
London WC1E 6DD

8.11. Central review of imaging

At the end of recruitment, central review of SOC imaging may be requested from UCL CTC to evaluate eligibility and to confirm disease response and progression. The statistician and TMG will review the data collected from the EDC to assess if central review of imaging is needed. Imaging data should be transferred electronically via IEP. At each site, SOC scans used to evaluate eligibility and to confirm disease response and progression will be transferred securely within the NHS and stored on a secure NHS network. Once the scans are read and the reports are created, then the scan will be fully anonymised before sending results to UCL CTC.

9. WB-MRI

WB-MRI scans will be performed at selected sites in a subset of 50 patients. Patients will have two WB MRIs, the first baseline scan ideally within 4 weeks of starting ADT and prior to commencing systemic ARSI or docetaxel and the second scan will be scheduled at any point between 2 weeks prior to cycle 3 day 1- and 2-weeks post cycle 4 day 1 (8-week window). Scans will take place at selected MRI scanning sites.

The WB-MRI sub-study is now closed to recruitment as of September 2023.

9.1. Referral to WB-MRI scanning site

The WB-MRI PIS can be given at the same time as the main study PIS at the referring site or a later date (see section 5.5). Consent to the WB MRI will be undertaken by a trained member of the research or imaging team, a radiologist at the MRI scanning site or MRI referring site. If the patient agrees to take part in the MRI sub study, the MRI referring site must send an email to the MRI scanning site in order to book the MRI scan. Please refer to WB-MRI referral and booking process document on how to refer the patient for the WB-MRI sub study. The MRI referring site will then send out the appointment letter to the patient.

9.2. Site qualification process and quality checks

The MRI scanning sites are required to undergo a site qualification process and approval by the PARADIGM imaging team. At each site, a named person (and their deputies) responsible for scanning, quality assurance (QC) and data transfer should be identified and included onto the study delegation log, which will be signed off by the Principal Investigator.

A documented MRI scanner quality assurance program must be in place. The full process of the site qualification process, pre- scan patient preparation, patient positioning and quality checks are listed in the PARADIGM imaging manual.

9.3. Data format transfer and archiving

Data from the scans should be uploaded by Digital Imaging and Communications in Medicine (DICOM) at the MRI scanning site. Instructions for transfer of research WB MRI including accessing the online platform, registration and image upload/submission will be made available to the site during site qualification process and this will be performed securely and anonymisation will be undertaken during the secure upload process- this will typically be the study number. All acquired WB MRI data will also be saved/archived locally on an approved data storage device (e.g. picture archiving and communication system; PACS). All data must be archived according to local protocol and stored at least until confirmation by the PARADIGM imaging team that all data has been received for the study. As detailed in the UK site registration form, the referring site should transfer images when requested to lead WB MRI Site (UCLH) for subsequent central review.

9.4. Results

At MRI scanning sites, MRI scans will be reviewed for significant additional or incidental findings only, by an appropriately experienced and qualified radiologist who will generate a report, and the patients local care team at the MRI referring site will be informed of any incidental findings.

10. WITHDRAWAL OF PATIENTS

In consenting to the study, patients are consenting to assessments, collection of biological samples, follow-up, and data collection.

10.1. Further data collection

If a patient explicitly states they do not wish to contribute further data to the study their decision must be respected, with the exception of essential safety data (see section 15) and recorded on the relevant EDC form. In this event data due up to the date of withdrawal must be submitted but no further data, other than essential safety data, sent to UCL CTC.

10.2. Losses to follow up

If a patient moves from the area, every effort should be made for the patient to be followed up at another participating trial site and for this new site to take over the responsibility for the patient, or for follow-up via GP. Details of participating trial sites can be obtained from the UCL CTC trial team, who must be informed of the transfer of care and follow up arrangements. If it is not possible to transfer to a participating site, the registering site remains responsible for submission of eCRF.

If a patient is lost to follow-up at a site every effort should be made to contact the patient's GP to obtain information on the patient's status.

10.3. Loss of Capacity

Patients who lose capacity during the study would continue in the study for the purposes of data collection only. The data would be sourced from the medical notes and no further contact would be made with the patient. If the patient regained capacity, an Investigator would discuss with the patient their continued participation in the study and together, the patient and Investigator would decide what action, if any, to take.

If a patient moves from the area, every effort should be made for the patient to be followed up at another participating trial site and for this new site to take over the responsibility for the patient, or for follow-up via GP. Details of participating trial sites can be obtained from the UCL CTC trial team, who must be informed of the transfer of care and follow up arrangements. If it is not possible to transfer to a participating site, the registering site remains responsible for submission of forms.

If a patient is lost to follow-up at a site every effort should be made to contact the patient's GP to obtain information on the patient's status.

10.4. Pre-study patients

The site will email UCL CTC if a pre-study patient does not consent or is not eligible for the main study. Any samples collected as part of pre-study will be kept for research purposes if the patient consented to this or destroyed. UCL will notify the relevant central laboratory(ies) of these patients and instruct them regarding the use or destruction of the samples

11. ASSESSMENTS AND DATA COLLECTION

All assessments should be carried out as part of standard of care (SoC) (except for WB-MRI) and local guidelines should be followed. Data from the assessments listed below will be collected if they are part of the patient's routine assessments and no additional assessments are required for the study.

However, there are a minimum number of assessments required for patients to be confirmed as eligible for the study and in order to appropriately monitor for response and disease progression.

Please also see schedule of events table in Appendix 2.

11.1. Assessments prior to starting ADT

Patients must consent on either the pre-study consent form or the main study consent form before any samples can be taken:

- 4x 10ml blood for ptDNA analysis
- 2 x 9ml blood for immunoprofiling
- 1 x 2.5ml PAXGene RNA
- 1 x 10ml blood for CTC analysis
- Stool Sample
- Questions relating to antibiotic use and diet (if not done at the ADT timepoint please complete at main study registration)

Patients must consent on MRI sub study PIS and consent form before the WB-MRI can be performed.

- WB-MRI pre-ADT (only patients who have consented to the imaging translational sub-study and to be performed ideally within 4 weeks of starting ADT and before ARSI or docetaxel). See section 9 for more details.

11.2. Pre-registration of pre-study patients

The following data will be collected for patients who do not enter the main study and patients will be asked to consent to this on the pre-study PIS/consent form:

- Number of bone metastases
- Visceral metastases

This information will be requested on a pre-study CRF.

11.3. Pre-registration for main study

The following is required to evaluate the suitability of patients for the study and should be carried out as part of the patient's SOC:

- Histological confirmation of prostate adenocarcinoma (*optional - to be recorded in the EDC if biopsy was done as per site NHS local policy*) and Gleason score
- Age
- ECOG Performance status (within 1 month of registration)
- Relevant medical history
- Patients self-reported race
- Review of prior treatment for prostate cancer
- Review and documentation of ongoing medication taken within 30 days of registration

- Start date of LHRH agonist or antagonist and of anti-androgens if used as cover
- Planned start date of docetaxel or ARSI
- For ARSI (PARADIGM-A), type of ARSI to be used
- A whole-body technetium labelled bone scan.
Use of other imaging to assess number of bone metastases may be permitted after discussion with the UCL CTC.
- CT scans of the chest, abdomen and pelvis are required to assess visceral disease performed prior to registration.
Use of other imaging to assess visceral disease volume status is permitted after discussion with the UCL CTC
- Serum PSA prior to ADT
- Questions relating to antibiotic use and diet (*if not already done at pre-ADT visit*)

11.4. Assessments prior to starting treatment

The following assessments may be carried out before the patient starts treatment (~within 4 weeks) on ARSI or docetaxel and results will be recorded on the study EDC forms if performed. Blood samples collected for research must be taken.

Note: If there is a delay in treatment sample collection on the scheduled date, the sample can be taken when patient is next seen in clinic and an appropriate timepoint assigned by the site. Further samples must be taken as per schedule.

Cycle 1 day 1

- 4 x 10ml blood for ptDNA analysis
- 2 x 9ml blood for immunoprofiling (only patients who had pre-ADT sample taken)
- 1 x 10ml blood for CTC analysis (only patients who had pre-ADT sample taken)
- Stool sample
- Serum PSA
- Serum testosterone confirming castration and as close as possible to collection of the research blood samples
- Serum Lactate Dehydrogenase (LDH) if physicians considered relevant
- Serum Alkaline phosphatase (ALP) if physicians considered relevant
- Full Blood Count including differential of components
- Serum creatinine
- Height and weight
- Questions relating to antibiotic use

11.5. Assessments during first six cycles of treatment

During treatment with ARSI or docetaxel the patient may be seen at every cycle as per standard practice and the following assessments may be performed as per local practice, however all blood samples collected for research must be taken

Cycle 2 day 1

- Serum PSA
- 4 x 10ml blood for ptDNA analysis

Cycle 3 day 1

- Serum PSA
- Serum LDH if physicians considered relevant
- Serum ALP if physicians considered relevant
- 4 x 10ml blood for ptDNA analysis
- 2 x 9ml blood for immunoprofiling (only patients who had pre-ADT sample taken)
- 1 x 10ml blood for CTC analysis ((only patients who had pre-ADT sample taken)
- Stool Sample
- Questions relating to antibiotic use
- WBMRI (+/- 2 weeks) from cycle 3 day 1 and only patients participating in imaging translational sub-study only)

Cycle 4 day 1

- Serum PSA
- 4 x 10ml blood for ptDNA analysis
- 1 x 10ml blood for CTC analysis (only patients who had pre-ADT sample taken and C3D1 CTC sample was missed)
- 2 x 9ml blood for immunoprofiling (only patients who had pre-ADT sample taken)
- Stool Sample (only required if not collected at cycle 3)
- Questions relating to antibiotic use
- Whole body technetium labelled bone scan or equivalent where indicated in line with standard or care (see section Symptomatic or asymptomatic new or unequivocal progression of prior distant metastases confirmed by imaging)
- CT, chest, abdomen and pelvis or equivalent where indicated in line with standard or care
- WBMRI +/- 2 weeks if not taken at cycle 4 day 1, and only patients participating in imaging translational sub-study only)

Cycle 5 day 1

- Serum PSA
- Serum LDH if physicians considered relevant
- Serum ALP if physicians considered relevant
- 4 x 10ml blood for ptDNA analysis
- 2 x 9ml blood for immunoprofiling (only patients who had pre-ADT sample taken)
- Stool Sample (only required if not collected at cycle 3 or cycle 4)
- Questions relating to antibiotic use

Cycle 6 day 1

- Serum PSA
- Serum LDH if physicians considered relevant
- Serum ALP if physicians considered relevant
- 4 x 10ml blood for ptDNA analysis

11.6. Assessments after first six cycles of treatment and follow up

After completion of six cycles of ARSI (C7D1) or docetaxel (end of treatment), it is not proposed to routinely assess patients for response. However, in order that objective progression can be assessed, it is necessary to have imaging taken at time of best response as judged by the treating clinician and in accordance with SOC.

As part of their routine care, patients will have baseline radiological examinations. To assess response, the same imaging should be used throughout for staging assessments. In addition, whenever clinically appropriate, patients could have scans repeated at 24 weeks if they were abnormal at baseline, particularly if they have a low PSA value on entry into the study making biochemical assessment of treatment failure difficult.

Patients will be followed up for at least 36 months and up to 120 months for PFS and OS and documentation of subsequent treatments.

After the completion of 6 cycles (Docetaxel) or at C7D1 for (ARSI)

- Serum PSA
- Serum testosterone
- Serum LDH if physicians considered relevant
- Serum ALP if physicians considered relevant
- CT chest, abdomen and pelvis or equivalent where indicated in line with standard or care
- Whole body technetium labelled bone scan or equivalent where indicated in line with standard or care
- 4 x 10ml blood for ptDNA analysis

Follow up (frequency as per standard of care)

- Serum PSA
- CT chest, abdomen and pelvis or equivalent where indicated in line with standard or care
- Whole body technetium labelled bone scan or equivalent where indicated in line with standard or care
- 4 x 10ml blood for ptDNA analysis (study bloods should not be taken more than once every 3 months)

11.7. Recording disease progression

Progression is defined as at least an increase of 20% in the sum of the longest diameter of target lesions, including a maximum of five target lesions, or the appearance of new lesions in keeping with Response Criteria for Solid Tumour assessment.

The following assessments should be carried out as part of SOC, where indicated to confirm progression:

- Serum PSA
- A whole-body technetium labelled bone scan or equivalent
- CT scans of the chest, abdomen and pelvis or equivalent

In addition, the following research samples should be taken, and questions completed:

- 4 x 10ml blood for ptDNA analysis
- 2 x 9ml blood for immunoprofiling (only patients who had pre-ADT sample taken)
- Stool sample
- Questions relating to antibiotic use

The following outcomes should be reported on the progression EDC form:

- PSA nadir and PSA progression values
- Local (primary tumour or pelvic lymph nodes) progression (symptomatic and confirmed radiologically)
- Unequivocal progression or development of new distant metastases

11.8. Assessments after Disease Progression

Every 6 months (+/- 2 weeks) the following data will be collected:

- Review of start and end date of subsequent treatment for mCRPC
- Survival data

12. TRANSLATIONAL RESEARCH

12.1. Predictors of response to systemic treatment

Objective 1:

Molecular analyses of archival diagnostic tissue (mostly formalin fixed paraffin embedded) will be performed, including but not exclusive to low-pass whole genome NGS, targeted high-coverage NGS, whole genome expression array analysis and targeted methylation studies. Areas of tumour will be micro-dissected from 10-micron thick sections to enrich for cellularity. It is anticipated that tumour blocks will be retrieved and successfully sequenced from 70-80% of patients.

Genomic analyses on ptDNA will be performed and the gene-specific or molecular sub-type specific correlation with patient-matched tumour tissue will be evaluated. Based on this finding, a decision will be made (for every molecular question separately) whether to define patients by tissue alone or plasma alone or a combination of tissue and plasma. Pre-ADT plasma is targeted for collection from 30-40% of patients.

Correlations with outcome will be performed for molecular sub-groups defined in ongoing studies in the STRATOSPHERE collaboration.

Objective 2:

Plasma AR status at progression will be defined as in Section 12.2, objective 3. Molecular sub-classes will be described as in Objective 1 and whether patients harbouring a specific molecular signature are more likely to progress with AR gained versus AR normal disease will be assessed. These analyses will be performed only on patients from whom a progression samples are obtained. AR gain is expected to occur at a higher prevalence (~50%) in patients who progress within the follow-up timelines of the study.

12.2. Tracking of plasma DNA or extracellular vesicles dynamics

Blood for plasma analysis will be collected at follow-up every 3 months or as clinically indicated. Sequential plasma samples will be subjected to targeted custom next-generation sequencing.

Objective 1:

To define progression by ptDNA criteria and ascertain whether this precedes progression as defined by the criteria utilised in the defining PFS. The target is to collect ~60% of 3-monthly plasma samples from ~80% of patients. PtDNA progression is defined as:

1. For patients who remain positive on treatment, the date of progression is recorded as the C3D1.
2. For patients who become negative, a second ptDNA positive reading is required. The date of first detection of ptDNA is considered the day of progression.

Objective 2:

Plasma DNA and extra-cellular vesicles will be subjected to custom or whole-genome/transcriptome NGS that will define both tumour content and clonal and sub-clonal aberrations. Technical validation of NGS calls could be performed using an orthogonal approach such as droplet digital Polymerase chain reaction (PCR).

Objective 3:

AR status will be ascertained from NGS data and defined as the ratio of AR copies to control regions on chromosome X (including ZXDB). AR copy number normal is defined as a ratio <1.93 and gain is defined as 1.93 and greater. The patient will be categorised by the highest AR copy number value observed in progression samples.

12.3. CTC dynamics at initiation of ADT

Objective:

1. To evaluate whether CTC count pre-ADT and after starting ADT associates with shorter PFS and OS.
2. To identify CTC molecular features prior to and after starting ADT that associate with PFS or OS with ARSI or docetaxel.

CTC from 10mls fresh blood (within 72 hours of blood draw) will be collected prior to ADT, prior to start of ARSI or docetaxel and at C3D1 or C4D1, (CTCs collected at C4D1 only if C3D1 sample was not collected). Blood will be shipped to a central laboratory and captured onto slides by industry collaborators Epic Sciences using a proprietary technology and slides will be frozen. Slides will be analysed in batches prior to correlations with clinical outcome. CTC count will be reported as the number of cells per ml of blood and CTC will be characterised by feature classes, including but not exclusive to the Epic CTC Heterogeneity or Genome Instability signatures, identified in ongoing prostate cancer studies.

The primary analysis of correlations of CTC features with outcome will be performed by the UCL CTC and Epic Sciences will be blinded to clinical data. Clinical outcome data could be shared with Epic Science for secondary analyses.

12.4. Interrogation of peripheral immune changes secondary to initiation of ADT

Objective:

1. To determine changes in myeloid-derived suppressor cells (PMN-MDSC) (CD11b+CD33+CD15+ cells) and M-MDSCs and immune cells (NK cells, T-lymphocytes, CD4+ T-lymphocytes, CD8+ T-lymphocytes and B-lymphocytes) from peripheral blood leukocyte samples after exposure to ADT and subsequently ADT with ARSI or docetaxel
2. To determine dynamic changes in IL-23 and other cytokines in plasma after exposure to ADT and subsequently ADT with ARSI or docetaxel
3. To evaluate whether patients with rising PMN-MDSCs, M-MDSCs or cytokines post ADT and during ADT with ARSI or docetaxel have a shorter PFS and rPFS and OS.
4. To correlate archival tumour tissue immune profile with peripheral blood.
5. To correlate peripheral immune changes pre and post ADT with genomic changes in circulation.
6. To determine TCR repertoire changes pre and post ADT and correlate this to PFS and OS.

Whole blood from will be collected in ptDNA tubes prior to ADT, prior to start of ARSI or docetaxel, and at progression. A sample of whole blood prior to ADT will also be collected in PAXGene RNAtubes. Samples will be shipped centrally and processed for mRNA extraction, peripheral immune cell profiles and cytokine levels. Approximately 30-40% of patients are expected to donate pre-ADT and subsequent samples for these studies.

Peripheral blood will be immunophenotyped to define B-cells, T-cell populations NK, PMN-MDSC and M-MDSC. Phenotypic analyses of immune cell populations will be performed with fluorescently labelled antibodies to HLA-DR, CD45, CD15, CD33, CD14 and CD11b, CD3, CD4, CD8, CD19, CD20 and CD56. PMN-MDSCs will be defined as CD15+ HLA-DR low CD14-/+ and M-MDSCs as HLA-DR low CD14+ CD15- CD11b+ CD33+. Plasma cytokines, including IL-23, will be tested using validated enzyme-linked immunosorbent assay (ELISA) assays. Next generation TCR beta sequencing will be performed on peripheral blood mononuclear cells. Whole-blood samples collected in PAXGene RNA tubes will be subjected to a 6 gene prostate cancer specific gene expression model that will consist of *ABL2*, *SEMA4D*, *ITGAL*, *C1QA*, *TIMP1*, *CDKN1A*.

Archival tumour tissue will be immunophenotyped and may include RNA sequencing for IL-23 and other cytokines. Additionally, multiplex immunofluorescence will be performed on FFPE sections. Correlations with peripheral blood analyses will be performed.

Plasma genomic changes will be identified using custom next-generation sequencing as planned in Section 12.3 on plasma collected at the same time as blood for immunoprofiling.

12.5. Evaluating the changes in the gut microbiome in hormone sensitive prostate cancer

Objectives:

1. To explore the constituent and genomic changes in the gut microbiome at minimal exposure to ADT, after response to ADT with enzalutamide and at progression
2. To test for associations and investigate the role of the gut microbiome in progression to CRPC in patients treated with ADT and enzalutamide

Stool samples will be collected in accordance with the International Human Microbiome Standards (IHMS)⁷⁴ at the pre-ADT timepoint (if possible prior to biopsy collection), C1D1, at cycle 3 day 1 (C3D1), or, at C4D1 or C5D1 if not taken at C3D1, and at progression. Samples will be shipped to the central lab and processed for DNA extraction. Samples will be subjected to deep shotgun metagenomic sequencing in order to detect the changes of the intestinal ecosystems on treatment. These changes will be correlated with clinical outcome. Approximately 60-70% of patients from PARADIGM-E only are expected to provide stool samples for this sub-study.

12.6. Whole-body MRI derived imaging biomarkers as a surrogate of response at selected sites only

Due to funding and logistical limitations, this translational sub-study will be conducted solely in UCLH and other selected sites. Patients who are eligible for WB-MRI and who have consented to this will undergo two WB-MRI examinations – baseline scan will be scheduled prior to initiation of ARSI or docetaxel and ideally no later than 4 weeks after commencing ADT, and the second scan will be scheduled at any point between 2 weeks prior to cycle 3 day 1- and 2-weeks post cycle 4 day 1 (8 week window)). All WB-MRIs will be performed on either a 1.5T or a 3T MR scanner and will be limited to maximum of around 60 minutes duration and acquisition will include for example (but not limited to) the following sequences:

- T2-weighted axial free-breathing turbo spin echo images from vertex to mid-thigh using multiple stacks
- Multi-echo Dixon axial breath-hold proton density fat fraction (PDFF) with multiple stacks covering vertex to mid-thigh
- Axial fat-suppressed free breathing echo planar imaging diffusion weighted MRI (with at least 2 b-values)

This will afford the evaluation of at least three QIB (apparent diffusion co-efficient, proton density fat fraction and T2* mapping) and allow the measurement of these at baseline, on treatment and the change between these at each identified disease site (measured by regions or volumes of interest on their respective parametric maps).

Response will be defined based on conventional morphological imaging assessment (e.g. Response Evaluation Criteria in Solid Tumours (RECIST) definitions and/or working group modifications thereof as appropriate) and informed by clinical, histopathological and SOC radiological follow-up as per usual

pathways at the end of the study follow-up by consensus including, but not limited to, appropriate radiologist, oncologist and/or pathologist.

13. STATISTICS

The primary aim of this study is to determine whether the detection of ptDNA after two or three cycles of ARSI or docetaxel added after start of ADT is associated with a worse clinical outcome in newly diagnosed polymetastatic prostate cancer. The clinical outcome measures used will be PFS and PCSS.

The PARADIGM-E (Enzalutamide added after the start of ADT) cohort will commence once recruitment to PARADIGM-A has finished, and PARADIGM-E will be used as a validation cohort for PARADIGM-A.

13.1. Calculation of sample size

We require about 130 patients for our primary analyses (65 in PARADIGM-D, 65 in PARADIGM-A cohorts) but we allow (conservatively) for about 25% of patients to not be evaluable for the primary endpoints due to, for example, dropping out of the study (see section 1.1). The target total number of patients to be recruited (cohorts A and D) is therefore 170. After recruitment to PARADIGM-A has finished, PARADIGM-E would start recruiting, with a target of 65 evaluable patients. The TMG will regularly review the proportion of patients who are ptDNA positive, and the number eligible for the primary analyses, and may revise the accrual targets accordingly. The TMG will also review the allocation of patients to the separate cohorts and can change the target numbers based on, for example, drug funding access, local guidelines, and changing landscape.

For PARADIGM-D, we aim to recruit around 65 evaluable patients. About 20% of patients are expected to be ptDNA positive, among whom the 12-month PFS rate is expected to be 50%. The table below shows power for different 12-month PFS rates among ptDNA negatives (and therefore different hazard ratios), using a two-sided log-rank test with 10% statistical significance, 18-month accrual period and follow-up period of approximately 48 months (using PASS sample size software, v2020.0.5).

PARADIGM-D and PARADIGM-A could be reported separately as they have been powered as stand-alone cohorts. The target number of events may be achieved earlier in PARADIGM-D due to i) the shorter time to PFS given the inclusion of PSA progression and previous studies reporting this is shorter than for AAP REF ii) delay to accrual to PARADIGM-A due to funding restrictions. This could lead to PARADIGM-D being reported first.

PARADIGM-D and PARADIGM-A will be reported for PFS when an expected 50% of events have occurred in the ptDNA positive.

For PARADIGM-D, we assume a 50% PFS rate at 12 months in ptDNA positive patients and 80% PFS rate at 12 months in ptDNA negative patient.

	PFS rate in ptDNA negatives (vs 50% rate in ptDNA positives*)		
	70%	75%	80%
Hazard rate (in ptDNA negatives)	0.0297	0.0240	0.0186
Hazard ratio (ptDNA negative versus positive)	0.514	0.415	0.322
Total number of evaluable patients	63	60	61
Approx. number of PFS events	49	43	39
Power	60%	80%	95%

*Hazard rate 0.0578

Power will be higher with more evaluable patients

For PARADIGM-A, we also aim to recruit around 65 evaluable patients, using the same design assumptions as for PARADIGM-D except for the 12-month PFS rates. Among ptDNA positive patients, the expected PFS rate is 60%, and the table below shows power for different PFS rates among ptDNA negatives (and therefore different hazard ratios):

	12-month PFS rate in ptDNA negatives (vs 60% rate in ptDNA positives*)	
	80%	85%
Hazard rate (in ptDNA negatives)	0.0186	0.0135
Hazard ratio (ptDNA negative versus positive)	0.437	0.317
Total number of evaluable patients	66	64
Approx. number of PFS events	41	34
Power	73%	93%

*Hazard rate 0.0426

Power will be higher with more evaluable patients

The samples size is also large enough to detect similar hazard ratios for OS with longer follow-up and sufficient events. OS will be reported at the first report together with PFS. A second later report after sufficient follow-up could be considered to increase confidence for the association with OS.

If accrual rates are higher than expected during the recruitment period, we may continue to enrol beyond 65 evaluable patients to increase power for potentially less extreme hazard ratios (e.g. 0.4 to 0.5).

PARADIGM-E will serve as a clinical validation of PARADIGM-A. We aim to enrol about 85 patients (to yield 65) after allowing for 25% drop out. Of these, 34-41 PFS events are expected (as in the table above). The main statistical analyses for validation involve estimating sensitivity (percentage of patients who have a PFS event during the follow up period, who were classified as ptDNA positive) and false-positive rate (percentage of patients who do not have a PFS event during the follow up period but were classified as ptDNA positive); with the goal of achieving relatively high sensitivity and low false-positive rate. For sensitivity of, for example, 50% the margin of error (i.e. standard error) is $\pm 8.6\%$ (with 34 events) to $\pm 7.8\%$ (with 41 events); which are fairly reasonable. For sensitivity of 70% the margin of error (i.e. standard error) is $\pm 7.8\%$ (with 34 events) to $\pm 7.2\%$ (with 41 events). If the dropout rate is lower than expected this will increase the number of patients available for the analyses, leading to smaller standard errors. Also, if the accrual rate is quicker than expected or more funding becomes available to allow us to extend the recruitment period, we may be able to recruit more than 85 patients with known outcomes with the intention of minimising the standard error of the sensitivities.

13.2. Definition of criteria for a patient to be included in the major endpoints analysis

As the main analyses are based on the association of ptDNA at C3D1, or C4D1 with outcome, only patients who meet all of the following criteria (as applicable) will be considered evaluable:

- Received ADT followed by ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A, or PARADIGM-E enzalutamide only cohort) or docetaxel (PARADIGM-D) as defined in Sections 8.2 - 8.5.
- PARADIGM- D only: Received a minimum of two cycles of docetaxel within 8 weeks or three cycles within 11 weeks from start of treatment
- PARADIGM- A or E only: Received at least 7 days continuous and a minimum of 42 days of ARSI prior to C3D1 sample or 63 days prior to C4D1.

- Received ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A, or PARADIGM-E - enzalutamide only cohort) or docetaxel (PARADIGM-D), with no switch of treatment, until progression
- Plasma sample collected at Cycle 3 Day 1 or Cycle 4 Day 1.
- No radiotherapy, surgery or prostate intervention after consent until C3D1 or C4D1 (if a C3D1 sample is not available). Patients who have an intervention after C3D1 and prior to C5D1 will be included in the primary analysis but dropped from comparisons between C5D1 and C3D1/C4D1 values.
- Plasma testosterone in the castrate range measured in the central lab on the sample used for ptDNA assessment.

Any patients who do not fulfil all of the above criteria (as applicable) will continue to be followed up for PFS and OS, but no further sequential bloods will be mandated. Their existing samples previously obtained will be retained and can be used for translational research.

13.3. Outline of the analyses

The major endpoints are PFS and OS defined in Section 0. Patients will be defined as ptDNA positive in either a C3D1 or C4D1 sample. If a sample is available for, or positive at only one of these time-points, the patient will be classified as ptDNA positive. Final reports may include the split of C3D1 and C4D1 positive patients, but no distinction will be made for the primary analysis.

The primary report of a cohort will be driven by progression events, and we aim to report the primary analysis when approximately 30% of patients have been reported to have progressed in a cohort. Both PFS and OS will be reported and a second report for OS could be considered when 30% of patients have reported a death.

An estimate of the HR for PFS or OS will be computed using Cox Regression, providing the assumption of proportional hazards is satisfied. As a sensitivity analysis, we will also perform an adjusted Cox regression where site and patient/tumour factors can be included as covariates in the model to assess if including study site materially affects the HR estimate. Comparison of PFS or OS between ptDNA positive and ptDNA negative patients will be performed using a log-rank test (separate analyses per treatment arm). Kaplan-Meier (KM) curves will be plotted for each treatment arm to show PFS or OS for ptDNA positive and ptDNA negative patients.

We will also explore whether the difference in hazards (for PFS or OS) between the ptDNA positive and negative patients is larger earlier on, because positive patients may progress rapidly while those who are negative or become negative may develop secondary resistance and so progress later on. It is possible that the ptDNA test may be associated with a smaller difference in PFS or OS at, for example, 18-24 months, than the difference in the first 12-18 months. The Wilcoxon test for time-to-event analyses gives a higher weight to events that occur earlier, so we will use this method as well as the logrank test and Cox regression.

In keeping with clinical practice, serum PSA progression does not constitute a progression event in PARADIGM-A or PARADIGM-E but does in PARADIGM-D. If second-line treatment is started without progression confirmed by the following definitions, the date of start of 2nd-line treatment is used as the date of progression.

The primary analyses for PARADIGM-E involves estimating the prognostic performance of ptDNA using a model established in PARADIGM-A:

- sensitivity (percentage of patients who have a PFS event during the follow up period, who were classified as ptDNA positive).
- false-positive rate (percentage of patients who do not have a PFS event but are classified as ptDNA positive)-this is the same as 1 minus specificity.
- These analyses would be repeated using OS (i.e. the performance of ptDNA to predict deaths).

The observed survival times (distinguished from survival rates) from the validation set of PARADIGM-E patients will also then be compared to those predicted from PARADIGM-A. Criteria such as ROC analysis and simpler descriptive analysis, such as the predicted value being within +5%, +10% etc. of the observed will also be used. In all analyses, models with and without adjustment of clinical prognostic factors could be used.

13.3.1. Symptomatic or asymptomatic new or unequivocal progression of prior distant metastases confirmed by imaging

To assess objective progression, imaging is required in addition to clinical progression. Metastases are of two types:

Measurable lesions. These can be accurately measured in at least one dimension and the longest diameter is used to calculate progression compared to the smallest lesion detected on scans at best response. The imaging used is at the discretion of the investigator, but the same technique should be used throughout. The investigator should be certain that the lesions are prostate cancer metastases. Progression is defined as at least an increase of 20% in the sum of the longest diameter of target lesions, including a maximum of five target lesions, or the appearance of new lesions in keeping with Response Criteria for Solid Tumour assessment.

Non-measurable lesions. All other lesions are included as non-measurable. Progression is defined as the appearance of one or more new soft tissue lesions or two or more lesions on bone scan and/or the unequivocal progression of existing non-target lesions. In PARADIGM-A or PARADIGM-E, when two or more new bone lesions are detected on scans performed at or before CSD1, a second scan is required after at least 6 weeks to exclude this is secondary to a flare phenomenon.

If a confirmatory scan is required after the initial scan is conducted, and progression is confirmed, then the date of progression will be that of the first scan.

13.3.2. Symptomatic progression of cancer in the prostate confirmed by imaging

Local disease progression in the pelvis accompanied by symptoms and confirmed radiologically will constitute a progression event. The date of scans should be used to denote the date of progression. Pelvic progression in the absence of symptoms does not constitute progression.

13.3.3. Serum PSA progression (PARADIGM-D only)

Serum PSA progression is defined as an increase in PSA to more than 50% of nadir and >4ng/mL taking as reference the lowest recorded PSA level since starting ADT. A second, confirmatory PSA value should be obtained between one week and 3 months later. The timing of assessments needs to be considered because spurious rises in PSA can occur e.g. following procedures involving the urinary tract. PSA failure is confirmed if the second value is around the same level or higher i.e. the trend is confirmed. The date of PSA progression should be provided as the date of the first raised PSA that fulfilled the study definition of progression. A confirmatory PSA is not required if there is associated radiological progression as defined in section 12.7.1. When progression is defined by radiological progression rather than PSA, the date of CT scans should be used.

Second-line treatment commenced specifically for a PSA rise should not start until the study definition for serum PSA progression has been met. However, if second line treatment does start before the study definition is met then report the closest PSA value prior to the treatment start date as the progression value. This is not required if second line treatment is being started for radiological signs of progression when the date of scans confirming progression should be used.

If the lowest recorded PSA value on docetaxel is more than 4ng/ml and more than 50% of the pre-treatment PSA level, then C3D1 (or C4D1 if a C3D1 plasma sample is not available) are defined as the progression time-point.

13.3.4. Prostate cancer specific death

Death attributed by investigators as probably related to prostate cancer progression and not related to toxic death or death from any other cause.

13.4. Secondary endpoints detailed definition and analyses

The secondary endpoints of interest are:

- PCSS defined as time from start of ARSI or docetaxel to death from prostate cancer.

Patients recruited to both treatment arms could be combined and an estimate of the HR for PCSS and OS by ptDNA status will be calculated. With reference to the secondary objectives, secondary analyses will be conducted on each survival outcome (PFS, PCSS and OS) as follows:

1. ptDNA classification at C2D1 and C5D1 with C3D1 or C4D1: the number of patients in each treatment arm who are deemed ptDNA positive at C2D1, C3D1, C4D1 and C5D1 will be presented in tables. Comparisons to the number at C3D1 or C4D1 will be performed using Fisher's exact tests.
2. To determine whether the detection of ptDNA after five to sixteen weeks of ADT and prior to starting ARSI or docetaxel (timepoint "pre-ADT") associates with a worse survival outcome: Number of patients who are ptDNA positive and negative at this timepoint will be presented. KM estimates for each ptDNA status at this timepoint will be computed. Comparison between groups will be performed using a log-rank test.
3. To assess the association between each survival outcome and PSA level (<0.2, 0.2-4, >4ng/dl) at every cycle and at 7 months after start of ADT, KM estimates of PFS/PCSS/OS rates at the specified timepoints will be calculated. PFS/PCSS/OS rates per PSA level will be compared using Cox regression up to and including the timepoint of interest (assessing HR of PSA levels).
4. To assess whether ptDNA detection is better for predicting each survival outcome than PSA level after five to sixteen weeks of starting ADT and prior to starting ARSI or docetaxel at every cycle. Differences in time between PSA rise and ptDNA detection in progressors and non-progressors will be assessed (distribution and summary statistics).
5. To compare associations with each survival outcome for the change in ptDNA detection prior to start of ARSI or docetaxel (timepoint "pre-") with ptDNA detection at C3D1 or C4D1, the number of patients who belong to each of the following four groups will be presented; (+ve pre-, +ve on treatment), (+ve pre-, -ve on treatment), (-ve pre-, +ve on treatment), (-ve pre-, -ve on treatment). PFS/PCSS/OS status will be evaluated at C3D1 (or C4D1) compared across the four groups (use Cox regression to obtain hazard ratios against a reference group of interest).
6. To evaluate whether ptDNA fraction prior to ADT associates with worse survival outcomes, continuous measurement of ptDNA taken prior to ADT will be assessed (histogram and summary statistics). The association with each survival outcome will be done using time-to-event analyses (accounts for censoring of observations).

13.5. Exploratory endpoint

To develop and test a composite model incorporating different biomarkers for assessing response: this exploratory analysis will use regression methods, with response category/survival status as outcome variables and biomarkers as covariates.

13.6. Statistical plan for Translational Research

Translational research will be performed on sub-sets of patients from whom the required evaluations are made. It is expected that not all patients will be able to participate in all the translational studies but as a fundamental aspect of PARADIGM, as many patients as possible should be included in these assessments.

13.6.1 Predictors of response to systemic treatment

Objective 1

To identify molecular signatures in plasma and tumour that associate with PFS or OS with ARSI or docetaxel: KM estimates of survival outcomes across different molecular signature subgroups will be calculated and plotted for comparison. Pairwise comparisons to a reference subgroup category via log-rank tests may be performed to estimate the effect of different subgroups on survival outcomes.

Objective 2

To identify a molecular signature in pre-ADT plasma or tumour that associates with plasma AR aberrant status at progression to castration-resistant disease: distribution of AR gain and AR normal status across molecular subgroups will be presented to assess which subgroups account for at least 90% of the AR gain study population.

13.6.2 Tracking of plasma DNA or extracellular vesicles dynamics

Objective 1

To determine whether detection of ptDNA precedes clinical, biochemical or radiological progression: amongst men who have confirmed clinical, biochemical or radiological progression, time between ptDNA detection and confirmation of progression will be calculated. Data may be left-censored (i.e. date of recorded ptDNA detection is an upper-bound for when it reached a detectable level, due to timing of visits/scans). Similarly, we may not detect ptDNA at the time of progression. Using appropriate survival analysis methods to account for this potential missingness, we will estimate the expected time between ptDNA detection and progression confirmation and test to see if this is significantly different from 0.

Objective 2

Genomic changes in sequential plasma samples will be reported for each patient separately although changes recurrent across patients could be grouped. A progressive rise in the abundance of an aberration supports its association with progression. The probability of falsely detecting a change that associates with resistance is reduced when detected in >1 sequential samples, improving the power for testing of multiple genes to identify ones that temporally associate with resistance.

Objective 3

To evaluate whether patients who progress with AR gain at the development of mCRPC have a shorter time to PFS (on ADT) and OS: Log-rank test effect of AR status on survival outcomes will be performed.

13.6.3 CTC dynamics at initiation of ADT

Objective 1

To evaluate whether CTC count pre-ADT and after starting ADT associates with shorter PFS and OS: Cox regression with survival outcome and CTC count as a time-varying covariate will be performed to assess possible associations.

Objective 2

To identify CTC molecular features prior to and after starting ADT that associate with PFS or OS with ARSI or docetaxel. KM estimates of survival outcomes across subgroups defined by CTC molecular features will be calculated and plotted for descriptive comparison. Interrogation of peripheral immune changes secondary to initiation of ADT.

13.6.4 Interrogation of peripheral immune changes secondary to initiation of ADT

To determine changes in myeloid-derived suppressor cells, immune cells and cytokines from peripheral blood leukocyte samples after exposure to ADT and subsequently ADT with ARSI or docetaxel. Mean differences in cell counts between ADT exposure and ADT with ARSI or docetaxel will be calculated and summarised, complete with 95% confidence intervals. T-test/sign tests of differences from pre-study therapy (after ADT but before ARSI or docetaxel) will be conducted.

13.6.5 Novel imaging as a surrogate of response

Differences in baseline and on treatment QIBs and proportional changes between the two may be compared between response groups e.g. with Mann Whitney U test and pairwise QIB changes between these could be compared with Wilcoxon signed rank test. As part of an 'all lesion' QIB analysis- accounting for multiple samples per patient will be required e.g. using linear mixed method modelling using response as the fixed factor and patient as the random factor.

13.6.6 Evaluating the changes in the gut microbiome in hormone sensitive prostate cancer

To explore the bacterial and genomic changes in the gut microbiome prior to the exposure to ADT, subsequently in response to ADT with enzalutamide and at progression, and to investigate the role of the gut microbiome in progression to CRPC in patients treated with ADT and enzalutamide. A heat map representing differentially abundant bacterial species at pre-ADT, at the start of enzalutamide and at progression will be plotted. A supervised partial least square discriminant analysis (PLS-DA) and the subsequent variable importance plot (VIP) will be used to determine the most discriminant bacterial species among at the pre-defined time points. Two-tailed Mann-Whitney and Kruskal-Wallis tests will be employed to assess the significance of pairwise and multiple comparisons.

14. DATA MANAGEMENT AND DATA HANDLING GUIDELINES

14.1. Entering data into Electronic Data Capture (EDC) system

The eCRFs must be completed by staff who are listed on the site staff delegation log and authorized by the PI to perform this duty. Data must be accurately transcribed into the eCRF and must be verifiable from source data at site. Examples of source documents are hospital records, which include patient's medical notes, laboratory and other clinical reports etc.

Each authorized staff member will have their own unique login details for the EDC system. They must never be shared among staff as the EDC audit trail will record all entries/changes made by each user. The PI is responsible for the accuracy of all data reported in the eCRF.

The use of abbreviations and acronyms must be avoided.

Where copies of supporting source documentation (e.g. autopsy reports, pathology reports, CT scan images etc.) are being submitted to UCL CTC, the patient's study number must be clearly indicated on all material and any patient identifiers removed/blacked out prior to sending, to maintain confidentiality.

14.2. Corrections to electronic data capture forms (eDCFs)

Corrections can be made to data on the eCRFs where necessary, as long as the eCRF has not been locked/frozen by UCL CTC. The eCRF audit trail will record the original data, the change made, the user making the change and the date and time. Site staff should contact UCL CTC if changes need to be made to a locked/frozen eCRF.

14.3. Missing Data

To avoid the need for unnecessary data queries, fields should not be left blank on the eCRF. If data is unavailable, please refer to the EDC manual for information on how to indicate that data is "Not Done," "Not Applicable," "Not Available" or "Not Known" (only use if every effort has been made to obtain the data).

14.4. Timelines for Data Entry

The relevant eCRFs must be completed within 1 month after a patient's visit, and baseline form within 2 weeks of patient registration being confirmed

Sites who persistently do not enter data within the required timelines may be suspended from recruiting further patients into the study by UCL CTC and subjected to a 'triggered' monitoring visit. See section 17 for details.

14.5. Data Queries

Data entered onto the EDC system will be subject to some basic checks at the time of entry, and any discrepancies will be flagged to the user in the form of a warning. The data can be corrected immediately, or where this is not possible, the warning can be saved, and the data amended at a later stage.

Further data review will be carried out at UCL CTC, and queries raised where necessary. Further guidance on the process for handling data queries can be found in the EDC manual.

15. SAFETY REPORTING

15.1. Definitions

The following definitions have been adapted from Directive 2001/20/EC, ICH E2A “Clinical Safety Data Management: Definitions and Standards for Expedited Reporting” and International Conference of Harmonisation-Good Clinical Practice (ICH GCP E6).

This is a low-risk prospective observational cohort study.

15.1.1. Adverse Reactions (AR)

All untoward and unintended events related to a ‘Study Procedure;’ where a causal relationship between a ‘Study Procedure’ and an event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

15.1.2. Related & Unexpected SARs

A serious adverse reaction, the nature or severity of which is not consistent with the applicable reference safety information for the Study Procedure.

Main study/pre-ADT sub-study

Study Procedure means the blood sampling procedure for the purposes of obtaining research samples within the study.

WB-MRI sub-study

Study Procedure means the scanning procedure patient undergoes for imaging data to be acquired.

15.1.3. Serious Adverse Reactions (SARs)

SARs are not anticipated in this study however should they occur, they will be reported immediately to the UCL CTC and documented on the study database.

SARs are adverse reactions that meet any of the following criteria:

- Results in death.
- Is life-threatening (the term “life-threatening” refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalisation or prolongs existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Is otherwise medically significant (e.g. important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above).

15.2. Reporting of Serious Adverse Reactions (SARs)

As this is an observational cohort study where patients follow their normal clinical pathways, and the study introduces a procedural intervention of blood sampling and scanning procedure the PI, other delegated site investigator or imaging practitioner should monitor each participant at each visit and only report to UCL CTC events that are serious and related (i.e. a SAR) to the study procedures highlighted in this protocol.

All SARs that occur between the between the start of the first study procedure and 7 days post the last study procedure must be submitted via email, within **24 hours** of observing or notification/occurrence of the event, using the study specific SAR Report.

All sections on the SAR Report must be completed. If the SAR report is **not sent within 24 hours** to UCL CTC, the circumstances that led to this must be detailed in the SAR Report to avoid unnecessary queries.

Causality

The PI, or other delegated site investigator, must perform an evaluation of causality for each event.

- Related (reasonable possibility) to a study procedure.
- Not related (no reasonable possibility) to a study procedure.

Severity

Severity of each event must be determined by using the Common Terminology Criteria for Adverse Events (CTCAE) v5 as a guideline, wherever possible. The criteria are available online at:

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Completed SAR Reports must be or emailed within 24 hours of becoming aware of the event to UCL CTC:

Email : ctc.paradigm@ucl.ac.uk

SAR Follow-Up Reports

All SARs must be followed-up until resolution and until there are no further queries.

Sites must ensure any new and relevant information is provided promptly. If the reaction term changes or a new reaction is added, the causality must be re-assessed by an Investigator.

SAR Processing at UCL CTC

On receipt of the SAR Report, UCL CTC will check for legibility, completeness, accuracy and consistency. There are no expected serious adverse reactions for the study, therefore all SARs will be considered Related and Unexpected Serious Adverse Reactions.

The CI, or their delegate (e.g. a clinical member of the SMG), may be contacted to review the SAR and to perform an evaluation of causality on behalf of UCL CTC.

Related and Unexpected Serious Adverse Reaction

If the event is evaluated as a related and unexpected Serious Adverse Reaction, UCL CTC will submit a report to the REC within the required timeline.

Wherever possible, evaluations of causal relationship by both the site and the Sponsor's clinical reviewer will be reported.

16. INCIDENT REPORTING AND SERIOUS BREACHES

16.1. Incident Reporting

Organisations must notify UCL CTC of all deviations from the protocol or GCP immediately. An incident report may be requested and will be provided, but an equivalent document (e.g. Trust Incident form) is acceptable where already completed.

If site staff are unsure whether a certain occurrence constitutes a deviation from the protocol or GCP, the UCL CTC trial team can be contacted immediately to discuss.

UCL CTC will use an organisation's history of non-compliance to make decisions on future collaborations.

UCL CTC will assess all incidents to see if they meet the definition of a serious breach.

16.2. Serious Breaches

A "serious breach" is defined as a breach of the protocol or of the conditions or principles of Good Clinical Practice (or equivalent standards for conduct of non-CTIMPs) which is likely to affect to a significant degree the safety or physical or mental integrity of the trial subjects, or the scientific value of the research.

Systematic or persistent non-compliance by a site with the principles of GCP and/or the protocol, occurring on study within the specified timeframe, may be deemed a serious breach.

In cases where a serious breach has been identified, UCL CTC will inform the REC within 7 calendar days of becoming aware of the breach.

17. STUDY MONITORING AND OVERSIGHT

Participating sites and PIs must agree to allow study-related on-site monitoring, Sponsor audits and regulatory inspections by providing direct access to source data/documents as required. Where permitted by site policy, remote access to source data/documents may also be provided by participating sites for remote monitoring by UCL CTC or its representatives.

Patients are informed of this in the PIS and are asked to consent to their medical notes being reviewed by appropriate individuals on the consent form. UCL CTC staff or its representatives will conduct all monitoring in compliance with the participant consent, site policy and data protection requirements.

UCL CTC or its representatives will determine the appropriate level and nature of monitoring required, based on the objective, purpose, phase, design, size, complexity, endpoints, and risks associated with the study. Risk will be assessed on an ongoing basis and adjustments made accordingly.

Details of monitoring activities will be included in the study monitoring plan and conveyed to sites during initiation. The study monitoring plan will be kept under review during the study and updated information provided to sites as necessary.

17.1. Centralised Monitoring

UCL CTC performs centralised monitoring, which requires the submission of documents by sites to UCL CTC for review, including but not limited to: PI CV and GCP, biological sample logs and staff delegation logs. Expectations for document submission will be explained during site initiation and UCL CTC or its representatives will send emails to sites requesting the documents when required.

The documents will be checked for consistency and completeness. Also refer to section 4.2. Data received at UCL CTC will be subject to review in accordance with the data queries section 14.5.

Sites will be requested to conduct quality control checks of documentation held within the Investigator Site File annually. Checklists detailing the current version/date of version-controlled documents will be provided by UCL CTC for this purpose.

17.2. Triggered On-Site /Remote Monitoring

On-site/remote monitoring visits may be scheduled following UCL CTC review and/or where there is evidence or suspicion of non-compliance at a site with important aspect(s) of the study protocol/GCP requirements.

On-site Monitoring

Sites will be sent an email in advance outlining the reason(s) for the visit and confirming when it will take place. The email will include a list of the documents that are to be reviewed, interviews that will be conducted, planned inspections of the facilities and who will be performing the visit.

Remote Monitoring

UCL CTC defines remote monitoring as monitoring activities conducted at a location remote from the research site which replicate some on-site activities e.g. source data review. Remote monitoring may be conducted in response to exceptional circumstances preventing access to participating sites (e.g. global pandemic) or conducted routinely. Details of remote monitoring will be agreed with participating sites, conducted in accordance with site policy and documented in the monitoring plan.

Sites will be sent an email in advance, confirming when remote monitoring is scheduled to take place and how the source documents will be remotely accessed. The email will include a list of the documents to be reviewed, interviews that will be conducted via telephone/videoconference and who will be performing remote monitoring.

Remote monitoring will be conducted by UCL CTC or its representatives via a device with adequate security. Patient confidentiality will always be maintained, and monitoring activities will be conducted in an appropriate environment where no unauthorised viewing or overhearing of conversations is possible by third parties. Also refer to section 14 Data Management and Data Handling Guidelines for details of how source documentation may be submitted to UCL CTC.

Monitoring Follow up

Following on-site/remote monitoring, the Trial Monitor/Trial Coordinator will provide a follow up email to the site, which will summarise the documents reviewed and a statement of findings, incidents, deficiencies, conclusions, actions taken and/or actions required. The PI at each site will be responsible for ensuring that monitoring findings are addressed in a timely manner, and by the deadline specified.

17.3. Escalation of monitoring issues

Where monitoring indicates that a patient may have been placed at risk, the matter will be raised urgently with site staff and escalated as appropriate.

UCL CTC will assess whether it is appropriate for the site to continue participation in the study and whether the incident(s) constitute a serious breach. Refer to section 16 (Incident Reporting and Serious Breaches

17.4. Oversight Committees

17.4.1. Trial Management Group (TMG)

The TMG will include the Chief Investigator, clinicians and experts from relevant specialties and PARADIGM trial staff from UCL CTC (see page 2). The TMG will be responsible for overseeing the study. The group will meet regularly (approximately 2-3 times a year) and will send updates to PIs (via newsletters or at Investigator meetings) and to the National Cancer Research Institute (NCRI) Prostate Cancer name Clinical Studies Group.

The TMG will review substantial amendments to the protocol prior to submission to the REC. All PIs will be kept informed of substantial amendments through their nominated responsible individual and are responsible for their prompt implementation.

All TMG members will be required to sign the PARADIGM TMG charter and to declare all potential conflicts of interest.

Full responsibilities of the TMG will be detailed in the TMG charter and will include:

- Oversee and take responsibility for the conduct of the study according to the study protocol.
- Review number of patients with pre-ADT samples.
- Review proportion of recruited patients who are eligible for the main analyses.
- Review of number of patients allocated to PARADIGM-D versus PARADIGM-A, and PARADIGM-E as a separate cohort.
- Feedback of clinically relevant germline and other molecular variants.

17.4.2. Translational Research Committee

The role of the TRC will be to oversee the analysis of plasma DNA and the translational sub-studies. The committee will include members with specific expertise related to this work and will report to the TMG with results that are of interest.

All members will be required to sign a charter and declare all potential conflicts of interest.

17.4.3. Trial Steering Committee (TSC)

The role of the TSC is to provide overall supervision of the study. The TSC will review the recommendations of the Independent Data Monitoring Committee and, on consideration of this information, recommend any appropriate amendments/actions for the study as necessary. The TSC acts on behalf of the funders and the Sponsor.

All TSC will be required to sign the TSC charter and to declare all potential conflicts of interest.

17.4.4. Role of UCL CTC

UCL CTC on behalf on the sponsor will be responsible for the day-to-day coordination and management of the study and the UCL CTC Director will act as custodian of the data generated in the study (on behalf of UCL). UCL CTC is responsible for all duties relating to safety reporting (which are conducted in accordance with section 15 (Safety Reporting)).

18. STUDY CLOSURE

18.1. End of Study

For regulatory purposes the end of the study will be 120 months after the first patient has been registered, or once all patients have died, whichever is sooner. This timeline also incorporates completion of analyses of samples for the study. If the event rate to report study endpoints is reached prior to this timepoint, and analyses of samples is also completed, then the end of study will be declared earlier.

At this point the 'declaration of end of trial' form will be submitted to and Ethics Committee, as required.

Following this, UCL CTC will advise sites on the procedure for closing the study at the site.

Once the end of trial has been declared, no more prospective patient data will be collected but sites must co-operate with any data queries regarding existing data to allow for analysis and publication of results.

18.2. Archiving of Study Documentation

At the end of the study, UCL CTC will archive securely all centrally held study related documentation for a minimum of 5 years. Arrangements for confidential destruction will then be made. It is the responsibility of PIs to ensure data and all essential documents relating to the study held at site are retained securely for a minimum of 5 years after the end of the study, and in accordance with national legislation.

Essential documents are those which enable both the conduct of the study and the quality of the data produced to be evaluated and show whether the site complied with the principles of GCP and all applicable regulatory requirements.

UCL CTC will notify sites when study documentation held at sites may be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

18.3. Early Discontinuation of Study

The study may be stopped before completion on the recommendation of the TSC (see section Trial Steering Committee (TSC)). Sites will be informed in writing by UCL CTC of reasons for early closure and the actions to be taken with regards the treatment and follow up of patients.

18.4. Withdrawal from trial participation by a site

Should a site choose to close to recruitment the PI must inform UCL CTC in writing. Follow up as per protocol must continue for any patients recruited into the trial at that site and other responsibilities continue as per the site agreement.

19. ETHICAL CONSIDERATIONS

In conducting the study, the Sponsor, UCL CTC and sites shall comply with all relevant guidance, laws and statutes, as amended from time to time, applicable to the performance of clinical trials including, but not limited to:

- The principles of Good Clinical Practice
- Human Rights Act 1998
- Data Protection Act 2018, and General Data Protection Regulation (EU)2016/679 (GDPR)
- Freedom of Information Act 2000
- Mental Capacity Act 2005
- UK Policy Framework for Health and Social Care Research, issued by the Health Research Authority

19.1. Ethical Approval

The trial will be conducted in accordance with the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (1996 version) and in accordance with the terms and conditions of the ethical approval given to the trial.

The trial has received a favourable opinion from the London-Brighton and Sussex Research Ethics Committee (REC) and Health Research Authority (HRA) approval for conduct in the UK.

UCL CTC will submit Annual Progress Reports to the REC, commencing one year from the date of ethical approval for the trial.

19.2. Site Approvals

Evidence of assessment of capability and capacity by the Trust/Health Board R&D for a trial site must be provided to UCL CTC. Sites will only be activated when all necessary local approvals for the trial have been obtained.

19.3. Protocol Amendments

UCL CTC will be responsible for gaining ethical for amendments made to the protocol and other study-related documents. Once approved, UCL CTC will ensure that all amended documents are distributed to sites as appropriate.

Site staff will be responsible for acknowledging receipt of documents and for implementing all amendments promptly.

19.4. Patient Confidentiality & Data Protection

Patient identifiable data, including full name/initials, and telephone number may be collected by UCL CTC if patients consent to this. UCL CTC will preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified.

Patient identifiable data, including full name, hospital number, date of birth, NHS number, home address, and GP details may be recorded on imaging scans sent within the NHS to different hospitals. These will be transferred via the secure trust NHS network and patient information will be transmitted and stored securely.

Data will be stored in a secure manner and UCL CTC trials are registered in accordance with the Data Protection Act 2018 and GDPR, with the Data Protection Officer at UCL.

Patient identifiable data, including initials will be provided to the UCL Cancer Institute including the GCLP laboratory in order to process the samples. Both Laboratories will preserve patient confidentiality and will not disclose or reproduce any information by which patients could be identified.

20. SPONSORSHIP AND INDEMNITY

20.1. Sponsor Details

Sponsor Name: University College London
Address: Joint Research Office
4th Floor West
250 Euston Road
London,
NW1 2PG
Contact: Managing Director, UCLH/UCL Research
Tel: 020 3447 9995/2178 (unit admin)
Fax: 020 3447 9937

20.2. Indemnity

UCL holds insurance against claims from participants for injury caused by their participation in the clinical trial/study. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial/study is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial/study. UCL does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial/study without the need to prove negligence on the part of UCL or another party. Participants who sustain injury and wish to make claim for compensation should be advised to do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this study shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

21. PUBLICATION POLICY

All publications and presentations relating to the study should be authorised by the TMG. The TMG will form the basis of the writing committee and advise on the nature of the publications. All collaborators who have actively contributed to the study will be named authors on all main study papers and anyone else who has had a significant input into the conduct, analysis and interpretation of the study.

Specialist papers focusing on a particular aspect of translational research may not require all collaborators to be authors. Data from all sites will be analysed together. Participating sites may not publish study results prior to the first publication by the TMG or without prior written consent from the TMG.

The Chief Investigator will make the final decision on authorship. The study data is owned by the Sponsor. The ClinicalTrials.gov number should be quoted in any publications resulting from this study.

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83. UK CR: Prognostic/predictive Biomarker Roadmap, Cancer Research UK
84. Standard IHM: International Human Microbiome Standards, 2015

Appendix 1: Abbreviations

AAP	Abiraterone acetate with prednisolone/prednisone
ADT	Androgen Deprivation Therapy
ALP	Alkaline Phosphatase
AR	Androgen receptor
ARSI	Androgen receptor signalling inhibitors
BP	Base pairs
CEA	Carcinoembryonic antigen
CRPC	Castrate resistant prostate cancer
ctDNA	Circulating Tumour DNA
CTC	Circulating Tumour Cells
CT	Computerised Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CN	Copy Number
CXCR2	CXC chemokine receptor 2
Ctla-4	Cytotoxic T lymphocyte antigen-4
DICOM	Digital Imaging and Communications in Medicine
Doc	Docetaxel with or without prednisolone/prednisone
DFS	Disease Free Survival
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic data capture
eDCF	Electronic data capture form
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EPCAM	Epithelial Cell Adhesion Molecule
ESMO	European Society of Medical oncology
FFPE	Formaldehyde Fixed-Paraffin Embedded
GCP	Good clinical practice
GDPR	General Data Protection Regulation

GNRH	Gonadotropin-releasing hormone
HR	Hazard ratio
HRA	Health Research Authority
IQR	Interquartile range
ISF	Investigator site file
LDH	Lactate Dehydrogenase
LH	Luteinizing hormone
LHRHa	Luteinizing hormone-releasing hormone agonist/antagonist (full text used when specifically referring to either agonist or antagonist)
mHSPC	Metastatic hormone sensitive prostate cancer
mCRPC	Metastatic castration-resistant prostate cancer
MDSCs	Myeloid-Derived Suppressor Cells
M-MDSC	Monocytic myeloid-derived suppressor cells
mNCA	Model clinical trial agreement
MRD	Minimal residual disease
NK	Natural Killer
NGS	Next generation Sequencing
OS	Overall Survival
PET	Positron emission tomography
PFS	Progression Free Survival
PI	Principal Investigator
PIS	Patient information sheet
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumours
rPFS	Radiographic Progression free survival
SAR	Serious Adverse Reaction
SOC	Standard of care
SNVS	Single nucleotide variants
TAMs	Tumour associated macrophages
TCR	T cell receptor
TMF	Trial Master File

UCL CTC	CR UK and UCL Cancer Trials Centre
WB-MRI	Whole body Magnetic Resonance Image

APPENDIX 2: SCHEDULE OF ASSESSMENTS

Assessment	Pre- ADT	Registration to main study	C1D1	C2 D1	C3D1	C4D1	C5D1	C6D1	At completion of 6 cycles	Follow-up visit (frequency as per standard of care)	At disease progression	After Disease Progression	Death
Standard of Care Assessments													
Informed consent	X	X											
CT chest, abdomen and pelvis ¹		X				X ¹¹			X	X ¹²	X		
Whole body technetium labelled bone scan ¹		X				X ¹¹			X	X ¹²	X		
Relevant medical history		X											
Review ongoing medication ²		X											
ECOG Performance Score ²		X											
Serum PSA ¹⁴		X ³	X	X	X	X	X	X	X	X	X		
Serum testosterone ¹⁴			X ¹⁰						X				
Serum LDH (if physicians consider relevant)			X		X		X	X	X				
Serum ALP (if physicians considers relevant)			X		X		X	X	X				
Full blood count, including differential			X										
Serum creatinine			X										
Height and weight			X										
Survival follow-up												X	X
Patient self-reported race		X											
Gleason Score		X											
PARADIGM-Specific Assessments¹⁶													
Plasma (ptDNA) 4 x 10 ml	X ^{4,6}		X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ^{15,18}	X ^{13,15}		
Whole blood (Immunoprofiling) 2 x 9 ml	X ^{4,6}		X ^{4,9}		X ^{4,9}	X ^{4,9}	X ^{4,9}				X ^{13,9,15}		
Whole Blood (CTCs) 1 x 10 ml	X ^{4,6}		X ⁹		X ⁹	X ^{9,17}							
PAXGene RNA 1 x 2.5 ml	X ^{4,6}												
WB-MRI	X ^{7,8}				X ^{7, 11}	X ^{7, 11}							
Retrieve archival tumour blocks ⁵		X											
Stool sample collection	X ¹⁹		X		X	X ²⁰	X ²¹				X		
Antibiotic use and diet questions	X ²²	X ²²											
Antibiotic use			X		X ²³	X ²³	X ²³				X		
Notes													
- Alternative imaging is permitted after discussion with UCL CTC - Within 30 days of registration - Prior to ADT - All samples can be collected up to 120 hours prior to pre-specified time points - Archival tumour blocks to be shipped as soon as possible after entering patient onto trial - For a subset of patients (~150) and can be collected after consent to the pre-study consent form - Subset of patients who have consented on MRI consent form at UCLH and selected centres - Ideally within 4 weeks ADT and before starting ARSI/Doc - Only patients who had the pre-ADT blood sample - Confirming castration and as close as possible to collection of pre ARSI/Doc research blood sample - To be taken at any point between 2 weeks prior to C3D1 or 2 weeks after C4D1 (8-week window) - Recommended to be repeated at 24 weeks and whenever clinically appropriate - Progression samples missed at the time of progression can be collected when new treatment is commenced - Data from samples can be collected where samples are taken per standard of care - Samples can be collected 7 days before pre-specified time points. - If a decision is made to delay treatment after sample collection, the site can collect PARADIGM samples at the delayed treatment date - Only patients who had pre-ADT sample taken and C3D1 CTC sample was missed													

18.

Study bloods should not be taken more than once every 3 months
19.

Where possible to be taken prior to biopsy (pre-ADT)
20.

Stool sample to be taken if not taken at cycle 3
21.

Stool sample to be taken at cycle 5 if not taken at cycle 3 or 4
22.

Antibiotic and diet questions to be done once at either main-study or pre-ADT registration only
23.

Complete antibiotic use questions at cycle 3, 4, or 5 in line with the relevant patient visit when patient is expected to collect a stool sample

APPENDIX 3: REMOTE CONSENT GUIDANCE

To be followed when physical consent cannot be given by the patient for main study and all sub-studies. All steps performed need to be clearly documented and filed in patient notes.

Important notes:

- Sites must keep a **patient consent log** where the consent procedure is recorded as “remote” or “in person.”
- All patient recruited remotely for main study and/or sub-studies must be **re-consented in person** at the first visit

Remote Consent Process

1. **The PIS and ICF should be provided to the patient via email or post.**
2. **There must be an interview between the patient and the delegated site staff via phone or videocall** which involves a 2-way communication, allowing the patient the opportunity to ask questions and for the site staff to assess comprehension.
3. **The patient must have ample time to decide** so the PIS/ICF should be provided to the patient before the tele/video interview to allow time to read (this can be on the same day).
4. **The delegated site staff should document in the medical notes:**
 - Date PIS and ICF were emailed/posted to the patient
 - Version of the PIS and ICF used
 - When and how the interview was conducted (e.g. phone or videocall), the discussions held and who was present, questions raised by the patient if any, date and time of interview etc
 - Confirmation that the patient had the PIS and ICF documents with them for the tele/video interview
 - Patient has confirmed verbally that they are happy to proceed to consent
 - Date of the patient’s signature - this is the date of consent
5. **Confirming the patient’s consent:**
 - The patient should have the PIS and ICF documents with them for the tele/video interview
 - The delegated site staff should guide the patient through how to agree (or disagree, if optional) to each item of the ICF

Following the tele/video call then patient can sign consent by either:

Via email:

- The patient should initial the applicable boxes, type their name and date on the consent form and save the document
- The patient should email the completed ICF back to the site
- The delegated site staff will print the email and ICF completed by the patient, counter-sign and date the ICF

Via post:

- The patient should initial and sign the paper copy of the ICF and send back to the site via post
- The delegated site staff should counter-sign and date the ICF when received

6. **Filing the consent documentation:** The site should provide a fully completed copy of the ICF back to the patient, file a copy in the medical notes and in the ISF (with patient email as applicable).

APPENDIX 4: PROTOCOL VERSION HISTORY

Protocol		Amendments:		
Version no.	Date	Amendment no.	Protocol Section (no./title)	Summary of main changes from previous version.
1	14.01.2019	N/A	N/A	N/A
2	20.12.2019	4.0	General	Administrative changes, including correction of typographical and grammatical changes.
2	20.12.2019	4.0	General	Update to TMG membership.
2	20.12.2019	4.0	General	Change of wording from eCRF to EDC.
2	20.12.2019	4.0	General	Translational research wording changed to tracking of plasma DNA dynamics and extracellular vesicles.
2	20.12.2019	4.0	General	Secondary objectives changed in line with inclusion criteria.
2	20.12.2019	4.0	1.1	Addition of Clinical trials.gov number.
2	20.12.2019	4.0	1.1 & 6.2	Modifications to inclusion and exclusion criteria- Changes made to the definition of polymetastatic disease, extending the time limits of starting LHRA agonist or antagonist, extending the time limits to start docetaxel and abiraterone after stating LHRA antagonist or agonist, patients must be 18 and over, removal of medically unsuitable for either abiraterone, prednisolone or docetaxel as this is already mentioned in the inclusion criteria.
2	20.12.2019	4.0	2.3	Addition of background to nomenclature and categorisation related to disease metastatic status.
2	20.12.2019	4.0	2.7	Clarification of wording to WB DW MRI for hormone sensitive metastatic prostate cancer.
2	20.12.2019	4.0	4.1	Addition of definition of serious breach.
2	20.12.2019	4.0	4.1	Update to wording- addition of 'Framework for Health and Social Care'.
2	20.12.2019	4.0	4.2.3	Site activation can be via letter or email
2	20.12.2019	4.0	5.0	Updated number of patient information sheets.
2	20.12.2019	4.0	7.1	Addition of data collected about pre-study consent.
2	20.12.2019	4.0	7.2	Clarification regarding registration to main study procedure.
2	20.12.2019	4.0	8.6	Timepoint and Samples to be taken updated
2	20.12.2019	4.0	8.6.1	Clarification of patient directed sample collection.
2	20.12.2019	4.0	8.7.1	Removal of lab processing instructions.
2	20.12.2019	4.0	8.7.1	Name of central laboratory changed.
2	20.12.2019	4.0	8.9	Addition of central review of imaging.
2	20.12.2019	4.0	9.0	Addition of information about WB-MRI timelines, site qualification process, transferring of WB-MRI data and WB-MRI results.

2	20.12.2019	4.0	10.4	Addition of pre-study patient withdrawal of consent.
2	20.12.2019	4.0	11.0	Clarification that data and assessments collected must be standard of care unless these are research blood samples or research tissue samples.
2	20.12.2019	4.0	11.0	Addition of immunoprofiling tube
2	20.12.2019	4.0	11.1	Collection of data for patients who have registered onto pre-study.
2	20.12.2019	4.0	11.2	Collection of self-reported race.
2	20.12.2019	4.0	12.5	Addition of 1.5T scanner used for WB-MRI imaging
2	20.12.2019	4.0	13.2	Modification of secondary endpoints in line with changes to inclusion criteria.
2	20.12.2019	4.0	15.1	Changes in definition of SAR.
2	20.12.2019	4.0	15.2	Addition of SARS submitted via email and fax. Removal of SARS submitted electronically.
2	20.12.2019	4.0	17.1	Details of study specific logs added- Biological sample logs and PI GCP and CV.
2	20.12.2019	4.0	17.2.2	Explanation of role or TRC.
2	20.12.2019	4.0	19.4	Collection of patient identifiable data for central review of imaging and how data for central imaging will be transferred and stored.
2	20.12.2019	4.0	Appendix 2.0	Gleason score and self-reported race data collected.
2	20.12.2019	4.0	Appendix 2.0	Testosterone and PSA data can be collected if performed as per SOC.
2	20.12.2019	4.0	Appendix 2.0	Follow up research samples can be collected before 72 hours.
3	26.05.2020	7.0	General	Addition of PCUK movember logo.
3	26.05.2020	7.0	General	Update to TMG membership.
3	26.05.2020	7.0	General	Throughout-Abiraterone has been replaced by Androgen receptor signalling inhibitors (ARSI) as applicable.
3	26.05.2020	7.0	General	Throughout - C3D1 collection for primary analysis modified to C3D1 or C4D1 as applicable.
3	26.05.2020	7.0	General	Sample collection at C2D1, C3D1 and C5D1 have been revised to every cycle.
3	26.05.2020	7.0	1.1 ,6.2.1 & 6.2.2	Clarification of wording in inclusion criterion 7, to include patients planning to start LHRH or having LHRH treatment. Pre-ADT and LHRHa treatment timelines defined. Previous inclusion criterion 10 is now criterion 2. Rewording of exclusion criterion 1 'Concurrent or planned for (within the first 5 cycles of docetaxel or abiraterone...' to 'Concurrent or

				planned for (i.e. prior to development of castration resistance)'. Exclusion criterion 4 revised to 'Any surgery planned prior to Cycle 4 Day 1'
3	26.05.2020	7.0	1.2	Study summary diagram amended & updated to include reference to ARSI and addition of C4D1.
3	26.05.2020	7.0	2.1	Additional background information provided for Enzalutamide and Apalutamide in PARADIGM-A.
3	26.05.2020	7.0	2.2	Typographical errors corrected and references updated.
3	26.05.2020	7.0	2.3	Removal of "Approval of abiraterone and funding access in the UK will be restricted to this population".
3	26.05.2020	7.0	2.4	PARADIGM justification - reference to ARSI instead of Abiraterone - overall justification unchanged.
3	26.05.2020	7.0	3.1.1 & 3.1.2	Primary objective amended to include detection of ptDNA after two, or three cycles. Secondary objective amended to include C4D1 sample.
3	26.05.2020	7.0	4.1	Removal of serious breach details (cross reference formatting error).
3	26.05.2020	7.0	5.1	Clarification added that each specific ARSI treatment will have a separate PIS.
3	26.05.2020	7.0	8.2	Addition of anti-androgen use and LHRAa timelines defined.
3	26.05.2020	7.0	8.3	Addition of 'with or without prednisolone' and docetaxel cycles defined.
3	26.05.2020	7.0	8.4	Standard of care abiraterone has been amended to Standard of care ARSI - <i>footnote added to include NICE funding approval for Enzalutamide treatment option.</i>
3	26.05.2020	7.0	8.6	Table updated to include collection of samples at C4D1.
3	26.05.2020	7.0	11.1	Removal of Gleason Score for pre-registration of pre-study patients
3	26.05.2020	7.0	11.2	Addition of Gleason Score for pre-registration for Main Study. Addition of type of ARSI to be used for patient receiving ARSI treatment.
3	26.05.2020	7.0	11.5	Sample collection at C2D1, C3D1, C5D1 and C6D1 revised to also include sample collection at C4D1. Addition of CTC bloods at C4D1 are taken if missed at C3D1.
3	26.05.2020	7.0	12.3	Clarification and justification of CTC sample collection at C4D1 if not taken at C3D1.

3	26.05.2020	7.0	13.1	Statement to clarifying statistics analysis in PARADIGM-A cohort will include patients receiving all ARSI treatments.
3	26.05.2020	7.0	13.2	Definition of patients to be included in primary end-point analysis revised: Point 1 -Delays in treatment extended from 1 to 2 weeks. Point 2 wording revised from Received a minimum of two cycles of docetaxel or abiraterone within 9weeks from start of treatment' to 'Received a minimum of two cycles within 8 weeks or three cycles within 11 weeks of docetaxel from start of treatment. Addition of point 3 Received at least 7 days continuous and a minimum of 42 days of ARSI prior to C3D1 sample or 63 days prior to C4D1. Addition of point 4- Received a minimum of 4 cycles of docetaxel or ARSI until progression. Addition of point 7 - Plasma testosterone in the castrate range measured in the central lab on the sample used for ptDNA assessment. Amended follow-up procedures for patients who do not meet the criteria of primary end-point analysis.
3	26.05.2020	7.0	13.3	Clarification of how positive ptDNA primary endpoint samples at C3D1 & C4D1 will be analysed. Serum PSA progression for PARADIGM-D patients defined.
3	26.05.2020	7.0	13.3.1	Paradigm A and D included in title
3	26.05.2020	7.0	13.3.3	Addition of PARADIGM -D to title. Statement has been removed in section 13.3.3 and added to 13.3 'In keeping with clinical practice, serum PSA progression does not constitute a progression event in PARADIGM-A but does in PARADIGM-D.' Removal of Methods for PSA calculation. Additional wording in text to clarify radiological progression and when C3D1 or C4D1 can be used to define progression.
3	26.05.2020	7.0	15.1	Removal of definition ' <i>Definitions and Standards for Expedited Reporting</i> ' and ICH GCP E6' – text here in error.

3	26.05.2020	7.0	17.1	Removal of text relating to site initiation visits (cross reference formatting error).
3	26.05.2020	7.0	22	References updated.
3	26.05.2020	7.0	Appendix 1.0	Table updated with addition of ARSI and LHRAa
3	26.05.2020	7.0	Appendix 2.0	Corrections made in wording of carcinoma to adenocarcinoma.
3	26.05.2020	7.0	Appendix 2.0	Samples collected at C4D1 added in assessment table.
3	26.05.2020	7.0	Appendix 2.0	Footnote 16 - added to confirm when samples should be collected if treatment is delayed.
3	26.05.2020	7.0	Appendix 2.0	Footnote 17 - conditions of when C4D1 CTC sample collection should be taken is defined.
4	17.07.2020	8.0	1.1 & 6.2	Inclusion criteria point 7 & 8 amended to patients have to have started within 18 weeks of starting antiandrogens with a minimum of 5 weeks of LHRH agonist, instead of 12 weeks of LHRH agonist.
5	22.09.2020	9.0	1.1 & 6.2	Removal of inclusion criterion point 3. Exclusion criteria point 1 has been revised to clarify that patients may be eligible for PARADIGM if randomised to receive an experimental drug or to the placebo arm of an open-label clinical trial after discussion and approval of the CI/delegate.
5	22.09.2020	9.0	1.1 & 6.2.1	Inclusion criterion 3 revised from '≥1 measurable visceral metastasis' to '≥1 unequivocal visceral metastasis.
5	22.09.2020	9.0	1.1,6.2 and 8.2	Removal of minimum time required for LHRH agonist and antagonists.
5	22.09.2020	9.0	4.2.1	Site initiation statement revised to include SIV via teleconference.
5	22.09.2020	9.0	7 & 15.2	Trial team contact details updated.
5	22.09.2020	9.0	8.6	Sample collection window has been increased from 72 hours prior to each cycle to 120 hours (5 days).
5	22.09.2020	9.0	8.8	Statement revised to confirm that consent can be requested if biopsy tissue is available.
5	22.09.2020	9.0	11.2	Recording histological information on the EDC made optional for patient registration.
5	22.09.2020	9.0	11.6	Clarification added that after completion of 6 cycles of treatment is the end of treatment for docetaxel and is C7D1 for ARSI.
5	22.09.2020	9.0	17.1	Removal of duplicated information refer to section 3.3. Refer corrected to 4.2.
5	22.09.2020	9.0	Appendix 2	Removal of histological confirmation of prostate adenocarcinoma from the assessment table.
5	22.09.2020	9.0	Appendix 3	Correction of protocol version 3 dates amended from 15.05.2020 to 26.05.2020.
6	11/02/2022	14.0	General	Throughout - CTC protocol template changes included in sections applicable.
6	11/02/2022	14.0	General Appendix 2	Throughout – ARSI – abiraterone, apalutamide or enzalutamide (PARADIGM-A), or

				enzalutamide (PARADIGM-E) or docetaxel (PARADIGM-D).
6	11/02/2022	14.0	General	Throughout -primary end-point (PFS) changed to major endpoint(s) which now included OS.
6	11/02/2022	14.0	Cover Page	Astellas added as a funder of separate PARADIGM-E cohort.
6	11/02/2022	14.0	Cover Page	Removal of sponsor authorisation signature from the Director.
6	11/02/2022	14.0	Cover Page	Trial statistician details removed.
6	11/02/2022	14.0	Cover Page	Update to TMG membership.
6	11/02/2022	14.0	1.1,1.3	Astellas details added to the funders and reference section. Funders for CTC sample analysis and WB-MRI sub-study included.
6	11/02/2022	14.0	1.1	Target accrual for cohort stipulated as 85 men to have 65 men evaluable for the primary endpoint.
6	11/02/2022	14.0	1.1	Inclusion of target of 6 cycles for docetaxel and until progression for ARSI
6	11/02/2022	14.0	1.1,2.4. & 3.1	Primary objective for cohort E outlined which will be used for validation of cohort A.
6	11/02/2022	14.0	1.1 and 18.1	Cohort E recruitment set for 18 months All cohort patients will be followed up for up to 120 months (from the first patient registered) for PFS and OS and subsequent treatments will be recorded.
6	11/02/2022	14.0	1.1& 3.2	Study major endpoints defined as PFS and OS. PFS amended to include death from any cause instead of prostate cancer specific death. OS included as a major end-point for docetaxel and ARSI cohorts.
6	11/02/2022	14.0	1.1,3.2.2 and 13.4	Replacement of OS with PCSS as the secondary end-point.
6	11/02/2022	14.0	1.1	Treatment summary for the enzalutamide cohort included in study summary.
6	11/02/2022	14.0	1.1	Number of sites participating increased to 20 and confirmation of pre-ADT participating sites included.
6	11/02/2022	14.0	1.2	Study summary updated to include sample collection for cohort E patients.
6	11/02/2022	14.0	1.3	Astellas funding information added in section.
6	11/02/2022	14.0	3.1	Translational objective point 2 primary end-point has been substituted by OS.
6	11/02/2022	14.0	4.1	Removal of monitoring requirements statement
6	11/02/2022	14.0	4.2	Astellas funding information included as well other funders for sub-studies.
6	11/02/2022	14.0	5.0	Number of PIS removed. Main-study and sub-study PIS listed.
6	11/02/2022	14.0	5.1	Cohort E PIS included
6	11/02/2022	14.0	5.1 Appendix 3	Addition of guidance outlining remote consent processes for patients recruited onto the main-study and sub-studies remotely.
6	11/02/2022	14.0	7.2	Contact details updated fax number removed.

6	11/02/2022	14.0	8.4	Removal of statement that enzalutamide interim treatment change will be reviewed after 3 months.
6	11/02/2022	14.0	8.5	Standard of care procedure for PARADIGM -E outlined.
6	11/02/2022	14.0	8.2.2	Epic science central lab address updated to reflect their new name 'Labcorp Central Laboratory Services Sàrl'.
6	11/02/2022	14.0	8.7	Pre-ADT recruitment target increased from 50 to 100 patients. Follow-up visits frequency set to be as per SoC. Follow- up samples should not be taken more than once every 3months.
6	11/02/2022	14.0	9,12.5,11.5,12.5 & Appendix 2	WB-MRI scanning window extended from 2 weeks of cycle 3 day 1 to + 2 weeks of cycle 4-day 1.
6	11/02/2022	14.0	11.2	Type of ARSI specified for PARADIGM-A
6	11/02/2022	14.0	11	Throughout section 11 statement added that procedures and assessments will be conducted in in line with standard of care.
6	11/02/2022	14.0	11.4	Statement added to confirm that delayed treatment samples at any timepoint can be collected at later time and a suitable timepoint/visit assigned.
6	11/02/2022	14.0	13	Cohort E recruitment starting point added and clarification on use as validation cohort for A. Calculation sample size section revised to tailor analyses for PARADIGM-A, PARADIGM-D and include PARADIGM-E
6	11/02/2022	14.0	13.1	Outlining of PARADIGM, A, D & E statistical analyses for PFS and OS using hazard ratios across different samples sizes.
6	11/02/2022	14.0	13.2	Major end-points for each cohort specified.
6	11/02/2022	14.0	13.3	Inclusion of OS as major endpoint in analysis. Analyses reporting section for primary ptDNA positive cohorts revised. Statement revised to state that confirmation for a progression date of the first scan should be used is second scan is positive.
6	11/02/2022	14.0	13.4	Statement removed on analyses of PFS.
6	11/02/2022	14.0	14	Added request for baseline forms 2 weeks after registration.
6	11/02/2022	14.0	15.2	Removal of fax number.
6	11/02/2022	14.0	21	Removal of statement on data being published after primary end-point is reached.
7	09/03/2022	14.0	Throughout	Clarification added in sections applicable that PARADIGM E will be activated after recruitment has been completed for PARADIGM A.
8	28/10/2022	18.0	Throughout	Protocol updated to include gut microbiome analysis and stool sample collection in metastatic prostate cancer patient.

8	28/10/2022	18.0	Throughout	Typographical errors corrected throughout the protocol.
8	28/10/2022	18.0	Throughout	Reference to the PARADIGM CTP has been replaced by PARADIGM team.
8	28/10/2022	18.0	Throughout	Clarification added throughout the protocol that mHSPC stands for metastatic hormone sensitive prostate cancer.
8	28/10/2022	18.0	Throughout	CTC Template changes from version 13 dated 08/07/2022 have been incorporated into protocol v8. In Section 4.12 to Clarify that GCP training is not required for all staff responsible for study activities. Section 5: Patient should be given a copy of the PIS & signed ICF. Section 7.2: Instruction for sites to send GP letter added. Section 17:4: updated to clarify that the CTC Director is the custodian of the data. Section 20: Updated with new address of UCL JRO.
8	28/10/2022	18.0	Cover Page	Trial Management Group members updated to removal PARADIGM Clinical Trial Practitioner, Salahah Ahmed Laskar and include Memuna Rashid as the Trial Statistician.
8	28/10/2022	18.0	Cover page	Fax number removed and Memuna Rashid added as the study statistician.
8	28/10/2022	18.0	Section 1.1 & 1.3:	Protocol Summary and Summary of Trial Design updated to include gut microbiome sub-study funder, John Black Foundation.
8	28/10/2022	18.0	Section 1.1, 3.2.4 12:5 & 13.6.6	Translation research updated to include objectives for gut microbiome sub-study.
8	28/10/2022	18.0	Section 2.8	Justification added for conduct of the microbiome analysis in metastatic prostate cancer patients.
8	28/10/2022	18.0	Section 5:3	Title for Next-Generation Sequencing (NGS) Results added.
8	28/10/2022	18.0	Section 5:6	Consent for the gut microbiome sub-study include explaining timepoints where this will occur.
8	28/10/2022	18.0	Section 8.8	Stool sample collection and processing updates included.
8	28/10/2022	18.0	Section 8:9	Stool sample timepoints outlined and explanation provided for when questionnaire should be completed.
8	28/10/2022	18.0	Section 11:1	Assessment prior to ADT have been added into section. Clarification included to explain that WB-MRI consent on the MRI PIS before MRI can be performed.
8	28/10/2022	18.0	Section 11:3 & 11:4:	Stool sample and questionnaire completion added to applicable timepoints.

8	28/10/2022	18.0	Section:13	Corrections made to Hazard ratio tables for PARADIGM A & D in statistical section 13.
8	28/10/2022	18.0	Section:13.6.6	Analysis tests which will be used to evaluate changes in gut microbiome defined.
8	28/10/2022	18.0	Section:15.2	Reporting SAR procedures updated to highlight inclusion of WB-MRI.
8.1	15/11/2022	18.0	Section 5.6 Throughout section 11	Removal of terminology referring to patient completing questionnaire. Clarification added to state that questions will be asked by the research team.
8.1	15/11/2022	18.0	Section 8.9 Appendix 2	Header added to differentiate the timeline of stool sample collection from ADT to Progression. C1D1 added to main-study registration statement. Appendix 2 updated to clarify that there are only 4 collection timepoints for stool sample collection from ADT to Progression. Removal of main-study registration timepoint as sample is being taken at C1D1.
8.2	25/04/2024	28.0	Throughout	Typographical errors corrected throughout the protocol.
8.2	25/04/2024	28.0	Section 1	In protocol version 8.1, SPM was updated from Marian Duggan to Ka Man Mak. SPM has been changed from Ka Man Mak to Rubina Begum in protocol v8.2. Laura White has been replaced by Temi Adedoyin the Clinical Programme Lead Manager. TMG members list updated to reflect members current on the committee. Translational research sub-committee removed in protocol v8.2. Pre-ADT recruitment target increased from 50 to 100 patients. Recruitment duration extended to 24months from 18months.
8.2	25/04/2024	28.0	Section 5	Microbiome sub-study added
8.2	25/04/2024	28.0	Section 8	Statements added under cohort A and D to confirm recruitment closure dates. Pre-ADT recruitment target increased from 50 to 100 patients.

				Note added to confirm that CTC sample collection is no longer required for patients recruited in the pre-ADT sub-study as of April 2023.
8.2	25/04/2024	28.0	Section 9	WB-MRI sub-study closure date confirmed
8.2	25/04/2024	28.0	Section 11	Removal of statement requesting completion of antibiotic use questions at cycle 2-day 1
8.2	25/04/2024	28.0	Appendix 2	Added death column to table in Appendix Transfer of antibiotic use and diet questions from the Standard of Care assessment section to PARADIGM-specific assessments. Appendix has been updated to incorporate minor changes reflecting the accurate sample collection timelines.

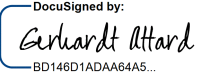
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