**UNIVERSITY OF OXFORD**

# Phase I/II open label non-randomised safety and efficacy study of the viral vectored ChAd-MVA 5T4 vaccine in combination with PD-1 checkpoint blockade in low- or intermediate-risk localized or locally advanced prostate cancer and advanced metastatic prostate cancer

**Study short title:**
**VAccination in early and ADvanced prostate caNCEr (ADVANCE)**

**Study Code: ADVANCE**

**Protocol Version: 6.0**

**21st August 2019**

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**Sponsor: University of Oxford**

**REC Number: 8/NE/0004**

**EudraCT Number: 2017-001992-22**

**STATEMENT OF COMPLIANCE**

The trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice Guideline E6 (R1) (ICH-GCP) and the applicable regulatory requirements.

**Signatures**

"I have read this protocol and agree to abide by all provisions set forth therein.
I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

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**CONFLICT OF INTEREST**

Adrian Hill is a co-founder of, share holder in and consultant to Vaccitech Ltd who have a commercial interest in the results of this trial and are providing limited co-funding support. Mark Tuthill is a consultant to Vaccitech Ltd.

MODIFICATION HISTORY

| Version | Date | Modifications | Authors |
|---|---|---|---|
| 1.0 | 17.11.2017 | N/A | Irina Redchenko<br>Lucy Carter |
| 2.0 | 17.04.2018 | Trial centres: University Hospital of Wales (Cardiff) was replaced by the Christie NHS Foundation Trust.<br><br>1.Synopsis was modified to reflect changes in the dosing regimens, follow up duration for RP patients, and clarification of primary and secondary endpoint for metastatic prostate cancer patients<br><br>2.2. Rationale for the study design was modified to reflect changes in the vaccine and immune checkpoint inhibitor dose and dosing regimens<br><br>2.5. Previous clinical experience with ChAdOx1-MVA 5T4 vaccine section was modified to reflect an update VANCE01 study progress<br><br>3. OBJECTIVES section was modified to reflect clarification of the primary and secondary objectives for metastatic prostate cancer patients<br><br>4.2. Investigational products section was modified to reflect introduction of the new batch of MVA.5T4 manufactured by IDT<br><br>4.2.2 Anti-Programmed Death Receptor-1 (PD-1) antibody - nivolumab (Opdivo®) section was modified to reflect changes in the Opdivo dose and dosing regimen | Irina Redchenko |

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| | | 4.3. Allocation to study groups and dosing regime section was modified to reflect changes in the dosing regimens of the vaccines and Opdivo<br><br>4.4. Duration of study section was modified to reflect the change in the follow-up of early stage prostate cancer patients from 12 months to 6 months:<br><br>4.6.2 Surgery delay section was modified to reflect a reduction in the delay to 6 weeks instead of 2 months<br><br>4.6.4 Risks associated with checkpoint inhibitor therapy section was modified to reflect reduced number Opdivo doses for early stage prostate cancer patients<br><br>6.1. Study visits section was modified to reflect changes in the dosing regimens<br><br>7. Assessment of scientific objectives section was modified to reflect clarification of the primary, secondary and exploratory endpoint for metastatic prostate cancer patients<br><br>Appendix 1: Schedule of attendance and study procedures was modified to reflect changes in the dosing regimens | |
| 3.0 | 03.09.18 | Trial centres: Leeds, Sheffield and Surrey removed as sites along with local PIs. New trial centre, University College London, and the PI are added.<br>Andrew Protheroe is identified as PI at the Oxford Cancer and Haematology Centre trial centre.<br><br>1.Synopsis was modified to reflect changes in the study design to remove the control no vaccine arm and randomisation, and reduce follow up duration for mCRPC patients.<br><br>2. Background and rationale section was modified to incorparet recent update on the | Irina Redchenko<br>Adrian Hill<br>Mark Tuthill<br>Ian Poulton |

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| | | efficacy of anti-PD1 therapy in mCRPC, an update on VANCE01 progress and a modification to ADVANCE study.<br><br>4.2.2 Anti-Programmed Death Receptor-1 (PD-1) antibody - nivolumab (Opdivo®) section was modified to indicate a supplier pf Opdivo for ADVANCE study.<br><br>4.3. Allocation to study groups and dosing regime section was modified to reflect changes to the study design.<br><br>4.4. Duration of study section was modified to reflect the change in the follow-up of mCRPC patients from 48 months to 12 months.<br><br>4.6.4 Risk associated with exposure to ionising radiation added.<br><br>5.3. Inclusion and Exclusion Criteria section was modified to include additional requirements<br><br>5.7. Interim monitoring and study stopping rules section was added<br><br>6.1. Study visits section was modified to reflect a requirement for on-sudy biopsy and the changes to the study design.<br><br>6.2. Additional study procedures section was modified to reflect changes in participating clinical trial centres, to clarify the sample collection process and to add the details of radiological assessment for mCRPC.<br><br>8.7. Data and Safety Monitoring Committee section was modified to clarify composition of DSMC.<br><br>9.1. Sample size section was modified to reflect changes to the study design. | |

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| | | Appendix 1: Schedule of attendance and study procedures was modified to reflect changes to the study design. | |
| 4.0 | 19.11.18 | Appendix 1: Schedule of attendance and study procedures was modified to reflect changes in blood volumes and timepoints for sample collection for Group 2 | Irina Redchenko |
| 5.0 | 15.02.19 | London and Lausanne trial centres were removed due to their withdrawal from the study. | Irina Redchenko |
| | | 5.3. Inclusion and Exclusion Criteria:<br>Treatment with abiraterone and enzalutamedie will be discontinued after enrolment to the study because of potential risk of liver toxicities.<br>The criterion for on-study tumour biopsy was moved from the criteria for all participants to the criteria for metatstatic patients only. | |
| | | 6.1. Telephone communications:<br>A window for a telephone call after study treatment was changed from 2 days to 2+3 days for logistical reasons. | |
| | | 12.7. Participant Confidentiality<br>The wording has been modified to comply with the General Data Protection Regulation 2018 | |
| | | Appendix 1: Schedule of attendance and study procedures was modified to reflect the following changes. A blood samples for HLA typing will be collected at visit 2 instead of visit 3 for logistaical reasons.<br>Blood samples for ctDNA quantification will be collected at weeks 0, 4, 8, 12 and 24 instead of weeks 0, 24 and 48. Blood samples for CTC quantification will be collected at weeks 0, 8, and 24 instead of weeks 0, 24 and 48. | |
| 6.0 | 21.08.19 | Key roles and general information:<br>PI at the Manchester change – Prof Gillessen replaced by Prof Hoskin | Irina Redchenko |

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| | | Details of the Study Medical Monitor added.<br><br>8.5. Reporting procedures<br>SAEs will be reported to the study medical monitor, not to local safety monitor.<br><br>8.7. Data and Safety Monitoring Committee<br>Responsibilities of the members of the data and safety monitoring committee (DSMC) have been clarified. | |

**CONTENTS**

## KEY ROLES AND GENERAL INFORMATION

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|---|---|
| **Trial Centres** | Centre for Clinical Vaccinology and<br>Tropical Medicine<br>Churchill Hospital<br>Old Road, Headington<br>Oxford, OX3 7LJ, UK<br><br>Department of Oncology<br>Churchill Hospital<br>Oxford University Hospitals NHS Foundation Trust<br>Old Road, Headington, Oxford, OX3 7LE, UK<br><br>Department of Urology<br>Churchill Hospital<br>Oxford University Hospitals NHS Foundation Trust<br>Old Road, Headington, Oxford, OX3 7LE, UK<br><br>Department of Oncology<br>The Christie NHS Foundation Trust<br>Wilmslow Road<br>Manchester<br>M20 4BX |
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**ABBREVIATIONS**

| | |
|---|---|
| **AE** | Adverse event |
| **ALP** | Alkaline Phosphatase |
| **ALT** | Alanine aminotransferase |
| **AST** | Aspartate Aminotransferase |
| **CCVTM** | Clinical Centre for Vaccinology and Tropical Medicine |
| **CBF** | Clinical Biomanufacturing Facility |
| **ChAdOx1** | Chimpanzee Adenovirus Ox1 |
| **CMI** | Cell-mediated immunity |
| **CPI** | Checkpoint inhibitor |
| **CRF** | Case report form |
| **CTC** | Circulating tumour cell |
| **ctDNA** | Circulating tumour DNA |
| **CTL** | Cytotoxic T lymphocytes |
| **DSMC** | Data and Safety monitoring committee |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **ELISPOT** | Enzyme-linked immunospot |
| **ENZ** | Enzalutamide |
| **FBC** | Full blood count |
| **GCP** | Good Clinical Practice |
| **GMP** | Good Manufacturing Practice |
| **HBsAg** | Hepatitis B Surface Antigen |
| **HBV** | Hepatitis B virus |
| **HCV** | Hepatitis C Virus |
| **HIV** | Human Immunodeficiency Virus |
| **HLA** | Human leukocyte antigen |
| **IB** | Investigator's Brochure |
| **ICS** | Intracellular cytokine staining |
| **IFN-γ** | Interferon-gamma |
| **i.m.** | Intramuscularly |
| **IMP** | Investigational Medicinal Product |
| **IRB** | Independent Review Board |
| **i.p.** | Intraperitoneally |
| **i.v.** | Intravenously |
| **LDH** | Lactate dehydrogenase |
| **LFT** | Liver function test |
| **LSM** | Local safety monitor |
| **mAb** | Monoclonal antibody |
| **mCRC** | Metastatic colorectal cancer |
| **mCRPC** | Metastatic castration resistant prostate cancer |
| **MDT** | Multidisciplinary team |
| **MHRA** | Medicines and Healthcare products Regulatory Agency |

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| **MVA** | Modified Vaccinia virus Ankara |
| **NHS** | National Health Service |
| **OS** | Overall survival |
| **PAP** | Prostatic acid phosphatase |
| **PBMC** | Peripheral blood mononuclear cells |
| **PCa** | Prostate Cancer |
| **PCR** | Polymerase chain reaction |
| **PD-1** | Programmed Death-1 |
| **PFS** | Progression-free survival |
| **Pfu** | Plaque forming units |
| **PD-L1** | Programmed Death-1 ligand |
| **PSA** | Prostate specific antigen |
| **REC** | Research Ethics Committee |
| **RP** | radical prostatectomy |
| **SAE** | Serious adverse event |
| **SFC** | Spot-forming cell |
| **SmPC** | Summary of Product Characteristics |
| **SOP** | Standard Operating Procedures |
| **STEAP1** | Six transmembrane anigen of the prostate |
| **SUSAR** | Suspected unexpected serious adverse reaction |
| **TAA** | Tumour-associated antigen |
| **Vp** | Virus particles |

## 1. SYNOPSIS

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| **Trial Title** | Phase I/II open label non-randomised safety and efficacy study of the viral vectored ChAd-MVA 5T4 vaccine in combination with PD-1 checkpoint blockade in low- or intermediate-risk localised or locally advanced prostate cancer and advanced metastatic prostate cancer |
| **Trial identifier** | ADVANCE |
| **Trial Centres** | Centre for Clinical Vaccinology and Tropical Medicine<br>Churchill Hospital, Old Road, Headington<br>Oxford OX3 7LJ, UK<br><br>Urology Department<br>Churchill Hospital<br>Oxford University Hospitals NHS Foundation Trust<br>Old Road, Headington, Oxford, OX3 7LE, UK<br><br>Oncology Department<br>Churchill Hospital<br>Oxford University Hospitals NHS Foundation Trust<br>Old Road, Headington, Oxford, OX3 7LE, UK<br><br>Department of Oncology<br>The Christie NHS Foundation Trust<br>Wilmslow Road<br>Manchester<br>M20 4BX, UK |
| **Clinical Phase** | Phase I/II |
| **Trial Design** | Open label, non-randomised |
| **Trial Population** | Men aged 18 years and over, diagnosed with low- or intermediate-risk localised or locally advanced prostate cancer scheduled for radical prostatectomy (RP), or diagnosed with metastatic prostate cancer |
| **Planned Sample Size** | 36 subjects<br>Group 1: 12 subjects per group<br>Group 2: 24 subjects per group<br><br>Group 1: Low- or Intermediate-risk localised or locally advanced prostate cancer patients scheduled for RP<br>ChAd-MVA 5T4 vaccine (one cycle) plus 1 dose of anti-PD-1 mAb (nivolumab) |

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| | Group 2: Metastatic prostate cancer patients progressing on the second generation anti-androgens (e.g. abiraterone or enzalutamide) ChAd-MVA 5T4 vaccine (two cycles) plus 3 doses of anti-PD-1 mAb (nivolumab) |
| **Follow up Duration** | 6 months for surgery patients and 12 months for metastatic patients |
| **Planned Study Period** | Approximately 2 years |
| **Co-primary Objectives** | *Low- or intermediate-risk localised or locally advanced prostate cancer patients:*<br>• To assess safety of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb<br>• To determine whether ChAd-MVA 5T4 vaccine in combination with anti-PD-1 will impact on the serum level of PSA<br><br>*Metastatic prostate cancer patients*<br>• To assess safety of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb<br>• To assess efficacy of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb as measured by composite response rate defined as one of the following:<br>- reduction of circulating tumour DNA<br>- serum PSA decrease |
| **Secondary Objectives** | *Low- or intermediate-risk localised or locally advanced prostate cancer patients:*<br>• To assess the magnitude of immune responses in the periphery generated by ChAd-MVA 5T4 vaccine in combination with anti-PD-1 mAb<br>• To assess immune cell subsets in the prostate secondary to treatment<br><br>*Metastatic prostate cancer patients*<br>• To assess the magnitude of immune responses in the periphery generated by ChAd-MVA 5T4 vaccine in combination with anti-PD-1 mAb<br>• To evaluate radiographic progression-free survival at 6 and 12 months post enrolment<br>• To evaluate overall survival at 6 and 12 months post enrolment |
| **Investigational Products** | ChAdOx1.5T4<br>MVA.5T4<br>PD-1 mAb (nivolumab) |

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| **Forms** | ChAdOx1.5T4<br>MVA.5T4<br>PD-1 mAb (nivolumab) | liquid<br>liquid<br>liquid |
| **Doses** | ChAdOx1.5T4<br>MVA.5T4<br>PD-1 mAb (nivolumab) | 2.5 x $10^{10}$ vp<br>2.0 x $10^{8}$ pfu<br>480mg |
| **Route** | ChAdOx1.5T4 and MVA.5T4: Intramuscular (i.m.) injection in a thigh<br>PD-1 mAb (nivolumab): Intravenous (i.v.) infusion | |

## 2. BACKGROUND AND RATIONALE

### 2.1. The need for a new vaccine against prostate cancer

Prostate cancer is the most common non-skin cancer in men and the second leading cause of cancer-related death in the Western world, including the UK. At present, prostate cancer is most often diagnosed at a clinically localised disease stage following blood testing for prostate-specific antigen (PSA). However, approximately one third of men treated with surgery or radiotherapy for clinically localised prostate cancer will experience recurrence of the disease. If the disease progresses to metastatic castration-resistant prostate cancer (mCRPC), the treatment options are limited and mainly palliative. Second generation anti-androgen agents (i.e. abiraterone and enzalutamide) have recently been approved by the FDA for treatment of mCRPC. However, resistance to these drugs often ensues, with the disease becoming lethal.

There is a substantial body of evidence that immune responses can control and eradicate cancer. Numerous studies have demonstrated an increase in spontaneous and carcinogen-induced cancers in immunocompromised mice[1]. Epidemiological studies show that there is an increasing risk of cancer among people treated with immuno-suppressants following transplantion[2]. It is now accepted that immune responses can be directed to antigens expressed in tumours (tumour-associated antigens, or TAAs) which can lead to long-term tumour control or destroy tumours.

Over the last decade, cancer immunotherapy has been evolving as a viable option for treatment of advanced stage prostate cancer patients. The first and to date only one therapeutic cancer vaccine, Provenge, has been approved by the FDA, and is indicated for an asymptomatic or minimally symptomatic metastatic castration resistant prostate cancer[3]. This immunotherapy targets one of the prostate-associated TAAs – prostatic acid phosphatase (PAP). The treatment entails three rounds of leukapheresis per patient, *in vitro* stimulation of the cells shipped to a centralized manufacturing facility and re-infusion of the cells. The vaccine has shown statistically significant though modest efficacy in clinical trials, increasing overall survival in treated patients by four months. However, no effect on time to tumour progression compared to placebo group has been observed, and the induced T cell responses appeared to be rather weak[4]. Additionally,

there are substantial cost implications for this individualised immunotherapy in that the vaccine is custom-made and currently the treatment costs over $90,000 per patient. Given the high price for a modest effectiveness, Provenge is unlikely to be cost-effective, in particular because individually tailored approaches are logistically impractical on a population basis.

Another clinically advanced vaccine, PROSTVAC, was until recently in a Phase III clinical trial (NCT01322490). This vaccine comprises two recombinant viral vectors, replication competent vaccinia- and fowlpox viruses, each encoding a transgene for PSA. The treatment consists of a priming injection with vaccinia vector followed by 6 boosting injections with the fowlpox vector. In a prospective randomized, double-blind, placebo-controlled phase II study in patients with metastatic prostate cancer, men in the PROSTVAC group had a significant increase in median overall survival (8.5 months) compared to the control group. However, there was no antibody response to PSA, and T cell immune responses were very modest[5]. Recently, it was announced that no significant efficacy was observed in the phase III trial. Taken together, the available data on immunogenicity of prostate cancer vaccines indicate the need for more effective vaccine capable of eliciting sustained immune responses of high magnitude which can promote tumour control and lead to anti-tumour responses.

The efficacy of immunotherapy may be best at the early stages of prostate cancer, when cancer cell deposits are comparatively few and small, and are more accessible to the cytotoxic T cells (CTL), which are the major players in anti-tumour protection. If an effective prostate cancer vaccine is administered before or soon after radical treatment with curative intent to patients without clinical evidence of metastatic disease, but who could later develop recurrence because of undetected micrometastases, there would be a minimal tumour burden for the CTLs to destroy and, therefore, the treatment may be more likely to be curative.

A new class of anti-cancer drugs termed immune checkpoint inhibitors (CPIs) has recently emerged. These belong to a category of antigen non-specific immunotherapies, in contrast to Provenge and PROSTVAC. The immune system is capable of recognising and eliminating cancer but it is held in check by inhibitory receptors and ligands. These immune checkpoint pathways, which normally maintain self-tolerance and down-regulate immune responses to pathogens to limit collateral tissue damage, can be co-opted by cancer to evade immune destruction. The novel drugs are the molecules that interrupt these immune checkpoint pathways and thus unleash anti-tumour immunity. The monoclonal antibodies (mAbs) against CTLA-4 (Cytotoxic T-lymphocyte–associated antigen 4), PD-1 (Programmed Death-1) and PD-L1 (Programmed Death Ligand-1) are the first drugs having entered the clinic as immunotherapies, and they have been licenced for advanced melanoma, lung, bladder, kidney cancers and other cancer types[6,7].

It is noteworthy that cancer types with the highest response rate to PD-1 blockade are the ones with the highest mutational load. Mutated proteins can give rise to neoantigens that are likely to be recognized by the host immune system and generate a spontaneous anti-tumour immune

response. Prostate cancer is known to have a relatively low mutational burden and this may, at least in part, explain the observation that checkpoint inhibitors have shown limited clinical benefit in prostate cancer to date[8,9]. However, the data from the ongoing KEYNOTE-199 study (NCT02787005) reported (by de Bono et al.) at the ASCO annual meeting in June 2018 (abstract number 5007) demostrate that anti PD-1 therapy had clinical activity in 11% of 258 metatstatic castration-resistant prostate cancer patients. These drugs are even more likely to be clinically effective in prostate cancer if they are delivered in combination with an immunogenic vaccine, thus circumventing the need for tumour-specific neoepitopes in order to induce spontaneous anti-tumour immune responses.

### 2.2. Rationale for the study design

*Heterologous ChAdOx1-MVA prime boost regime*

ADVANCE will investigate a vaccination regime comprising a simian adenovirus ChAdOx1 as a priming agent and an MVA vector as a boosting vaccine. Both viral vectors will encode the same antigen, the TAA 5T4. Previous studies have shown that heterologous prime-boost regimens (rather than homologous prime-boost regimes) employing different genetic vaccine vectors are better means to induce potent T cell responses in nonhuman primates and humans[10-12]. Adenovirus vectors have attracted considerable attention as potential viral vectors for genetic vaccination given their favourable safety profile and potent transduction efficiency following intramuscular injection. However, the neutralising antibody response against adenoviral capsid proteins following adenovirus vectors injection limits the success of vaccination protocols based on multiple administrations of the same adenoviral serotype. This has lead to the consideration of simian adenoviruses, which are not known to cause pathology or illness in humans and to which the prevalence of antibodies is low. There is already considerable clinical experience with this vector expressing influenza [13] and tuberculosis antigens.

A therpauetic vaccine in the form of MVA.5T4 viral vector was originally developed by Oxford BioMedica Ltd under a trade name of TroVax® as a homologous prime boost vaccination regime and to date has been administered to over 600 cancer patients[14-16] (details in section 2.4). More recently Oxford has developed a new MVA.5T4 vector with the same transgene insert, but expressed from a different likely more potent poxvirus promoter.

The ChAdOx1-MVA 5T4 vaccination regime has undergone the first clinical testing as a prostate cancer vaccine in the VANCE01 study, which was completed in May 2018 (NCT02390063). Preliminary data analysis from low- and intermediate-risk localised prostate cancer patients enrolled in this study to date have demonstrated that the vaccine has a good safety profile and elicits substantial T cell immunogenicity (section 2.5).

*Selection of the 5T4 antigen as a prostate cancer vaccine transgene*

The vaccine transgene, 5T4 antigen, is a transmembrane glycoprotein of 72 kDa normally expressed on the embryonic tissue but also on various malignant tumour cells, whereas tissue from all essential organs are negative for 5T4 expression. Weak to moderate staining was observed in some specialized epithelia; however, this staining was weak compared to the human placenta positive control.

The 5T4 antigen plays a vital role in the multiple biological and pathological processes including massive cellular migration during embryogenesis, cell invasion associated with implantation, and neoplastic metastasis in the progression of tumour genesis. Expression of 5T4 decreases cell-substratum attachment and increases cellular motility[17]. The 5T4 antigen is expressed in a wide spectrum of human solid malignancies including prostate cancer, and high expression of 5T4 is associated with poor prognosis[18]. Significant expression of 5T4 has been detected in the majority of primary prostate cancers (16/19, 84%) studied. In addition, although access to biopsy material from metastatic prostate cancer is limited, three samples confirmed as PSA positive were also positive for 5T4 expression, whereas expression of this protein is not evident in normal prostate tissues (screen of three donors) but is present at low levels in benign prostatic hyperplasia (BPH). 5T4 antigen expression in BPH contrasts with that identified in prostatic carcinomas; expression is generally diffuse and at low level with localisation to basal epithelial cells, whereas in primary prostate cancer samples expression is generally high and does not show any apparent polarization. These data indicate that 5T4 antigen is not expressed in normal prostatic epithelia, but is expressed at low levels in non-malignant hyperplasia and at high levels in prostate cancer. These observations support the hypothesis that 5T4 expression increases in association of malignant epithelial progression and suggest that the 5T4 antigen is a promising target for prostate cancer immunotherapy.

*PD-1 checkpoint blockade in prostate cancer*

The clinical activity of PD-1 checkpoint monotherapy has been confirmed in a number of malignancies, including melanoma, kidney, lung, urothelial, head and neck cancer and Hodgkin's lymphoma. This approach may also be advantageous for targeting prostate cancer, a disease for which anti-tumour vaccines have demonstrated clinical benefit, and yet PD-1 pathway inhibitors alone or in combination with vaccines have not been studied extensively.

The main two reasons for prostate cancer being left out from the list of suitable targets for PD-1 axis inhibition are reported prostate tumour negativity for PD-L1 expression and low tumour mutational load resulting in a limited number of potentially immunogenic neoantigens. The latter issue could be circumvented if checkpoint blockers are co-administered with a vaccine delivering a specific antigen for an immune attack rather than relying on spontaneous immune responses to neoantigens.

Regarding prostate cancer and PD-L1 expression, there are several arguments in support of anti-PD1 treatment in prostate cancer settings. The full activity spectrum of PD-1 pathway-blocking drugs is not yet known. Although PD-L1 presence appears to enrich for response to anti-PD1/L1 therapy, it has been documented that patients with PD-L1 negative tumours can also respond to treatment, and in urogenital cancers (such as bladder carcinoma and renal cell carcinoma), patients with both PD-L1 positive and negative tumours responded at the same rate[19]. Efficacy of PD1 therapy in PD-L1 negative tumours may be attributed to several factors. Firstly, the immunohistochemistry assays for detecting PD-L1 expression in tumours are still under development, and some methods and antibodies used in these assays may be more sensitive and specific than others. Also, there may be differences in definition of positivity (tumour cells only or expression on other cells in the various studies)[20].

Technical aspects aside, given the focal nature of PD-L1 expression within many tumours and information about intra-tumoural genetic heterogeneity[21], if small needle biopsies are screened, a false-negative evaluation could potentially ensue. Also, PD-L1 expression is likely to fluctuate over time in response to changes in tumour microenvironment, e.g. PD-L1 is up-regulated on tumour cells following IFNγ secretion by vaccine-induced T cells. All of these factors may lead to a poor correlation between PD-L1 expression levels at any one time point and response to therapy.

Consideration also has to be given to the PD-L1 expression on various immune cell types in addition to tumour cells. IFNγ has been demonstrated to induce the up-regulation of PD-L1 by many cell types including myeloid-derived cells, regulatory T cells and endothelial cells[22-24]. Other cytokines including IL-2, IL-7, IL-15 and IL-21 have also been shown to up-regulate PD-L1 expression by various cell types[25]. Moreover, a significantly increased number of PD-L1/2-positive dendritic cells and a high frequency of PD-1 positive T cells have recently been detected in blood samples of prostate cancer patients progressing on enzalutamide compared to treatment-naïve patients or those responding to treatment[26].

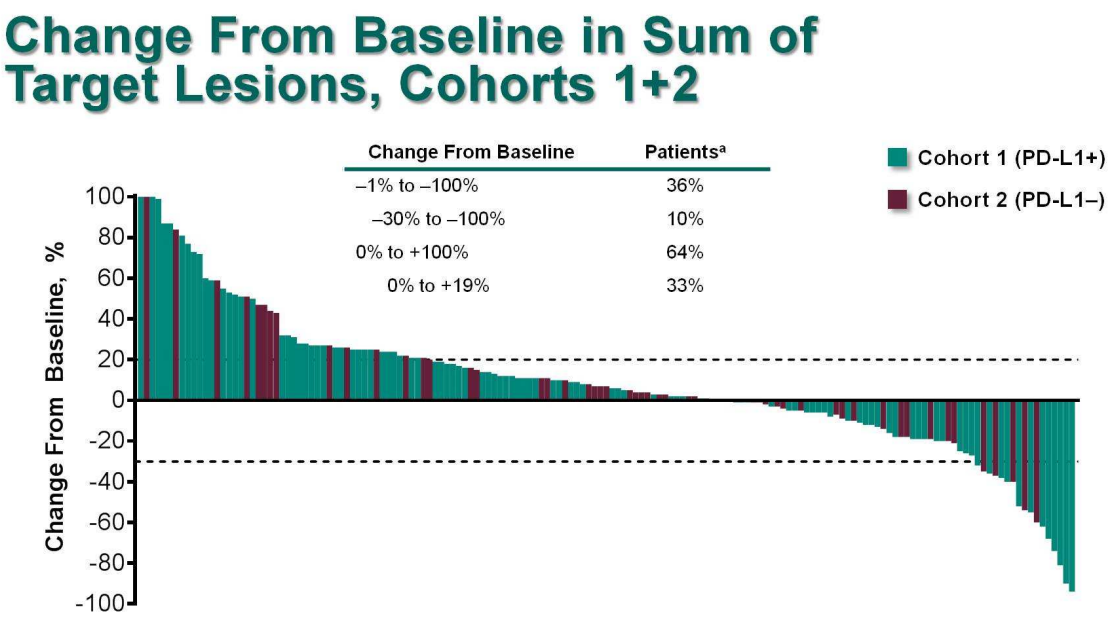
With further development and validation of PD-L1 antibodies for immunohistochemistry, the new data have recently emerged regarding PD-L1 expression on primary prostate tumours. The PD-L1 expression was reported in prostate tumour epithelial cells in 92% of cases[27]. More importantly, expression of the PD-L1 was not only highly prevalent in primary prostate cancer cells but was also an independent indicator of biochemical recurrence, suggesting a biologic relevance in primary tumors and potential clinical benefit for hormone-naïve prostate cancer[28].

Based on all of this information, prostate cancer patients may derive clinical benefit from PD-1 blockade in combination with the ChAdOx-MVA vaccine. Despite the apparent negative PD-L1 expression status of prostate tumours, there are some clinical trials evaluating the effect of anti-PD-1 mAbs in the advanced prostate cancer stage setting (NCT02312557, NCT00730639, NCT01968109) and some of these studies are testing the efficacy of anti-PD-1 mAbs in combination with vaccines (NCT02325557, NCT0142096).

Encouragingly, the first meaningful evidence of PD-1 clinical activity in advanced metastatic prostate cancer has recently emerged. Graff and colleagues enrolled 27 men with mCRPC progressing on the androgen receptor antagonist enzalutamide (ENZ) and treated them with anti-PD-1 mAb pembrolizumab. Five of the 27 treated patients demonstrated a strong response to the treatment, with a 99% PSA reduction and observed shrinkage of measurable tumour lesions (ESMO 2017).

A large multi-centre international trial, KEYNOTE-199, to evaluate pembrolizumab as a monotherapy in 370 patients with metastatic castration resistant prostate cancer previously treated with docetaxel-based chemotherapy is currently ongoing (NCT02787005). Groundbreaking preliminary results from 258 patients already recruited to the trial were reported by De Bono and colleagues at the ASCO annual meeting in June 2018 (J Clin Oncol 36, 2018 (suppl; abstr 5007). It was demonstrated that pembrolizumab has antitumour activity in both PD-L1 positive and PD-L1 negative patients with mCRPC, as shown in the figure below. Across all cohorts, disease control rate lasting ≥6 months was 11% in both RECIST-measurable and non-measurable disease.

Metastatic castration-resistant prostate cancer is genetically heterogeneous and includes DNA repair defective subtypes. DNA repair defects were suggested to be associated with pembrolizumab anti-tumour activity in this study. These data support further evaluation of anti PD-1 therapy in mCRPC and, in particular, in combination with vaccines that would provide a tumour-associated antigen as an alternative to naturally occurring mutated antigens.




[a]Percentages are calculated out of the **163 patients** who had ≥1 post-baseline scan evaluable per RECIST v1.1 by independent, central review.
Data cutoff date: Oct 13, 2017.

Presented By Johann De Bono at 2018 ASCO Annual Meeting

In ADVANCE, we are targeting a 25% or greater response rate to the vaccine in combination with PD-1 blockade in mCRPC patients. The sample size of 24 patients in Group 2 will require that 6 out of 24 treated patients will have complete response, partial response or stable disease. The patient cohorts recruited to the KEYNOTE-199 study will serve as a control group for ADVANCE and 11% overall response rate to PD-1 blockade as a monohterapy will be compared with the response rate to the vaccine in combination with anti-PD-1 treatment in the ADVANCE patients. If six of 24 treated patients do respond as described the would be a significantly (P,0.05, two-tailed) higher rate than in the KEYNOTE-199 series and suggest added value to use of the viral vectors, supporting their assessment in a future larger scale trial.

*Route of injection*

The choice of the intramuscular route for ChAdOx1 vector is based on the assumption that no co-infection of natural human adenovirus could occur at this site. Furthermore, there is a large body of data from human clinical trials using replication defective Ad5- and Ad6-based HIV vaccines injected intramuscularly showing an excellent safety profile, no viral shedding, and high levels of immunogenicity. MVA.5T4 will also be given by the intramuscular route following data from A. Hill showing enhanced immunogenicity and less local reactivity (study VAC033) when MVA vectors are administered via the intramuscular compared to subcutaneous route.

Anti-PD-1 monoclonal antibody will be administered by intravenous infusion as per the SmPC.

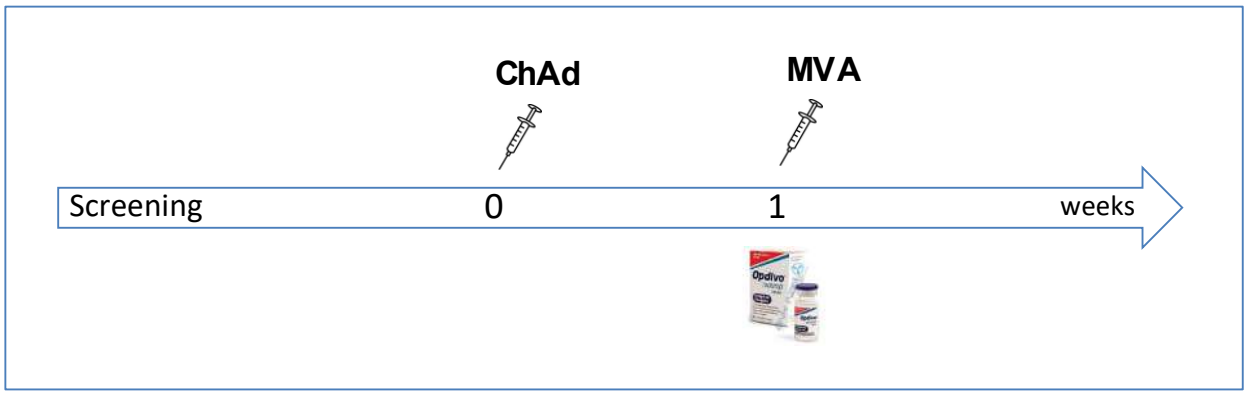
*Vaccine and immune checkpoint inhibitor dose*

In this study ChAdOx1.5T4 and MVA.5T4 vaccines are administered in a single injection at the dose of $2.5x10^{10}$ vp and $2x10^{8}$ pfu respectively. Low- or intermediate-risk prostate cancer patients will receive one cycle of ChAd and MVA immunisation with the vaccines given one week apart. Metastatic prostate cancer patients will receive two cycles of ChAd-MVA immunisations with the vaccines given four weeks apart. This is intended to boost the elicited immune response and to maintain the pool of antigen-specific memory T cells. The interval between the cycles will be approximately 2 months. In the recently compelted phase I trial there was similar immunogenicity with intervals between the ChAd and MVA vector administration of 1 and 4 weeks. The 1 week interval is likely to be preferred by low and intermediate risk patients as this reduces time to surgery.

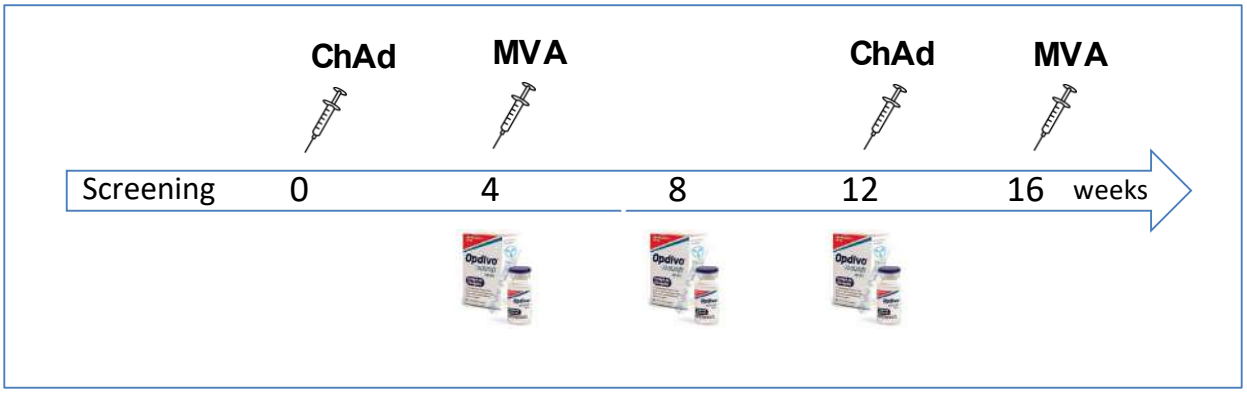
PD-1 mAb (nivolumab, Opdivo®) is administered intravenously at a uniform dose of 480mg per infusion. Low- or intermediate-risk prostate cancer patients will receive one intravenous infusion of nivolumab concomitantly with MVA.5T4 vaccine (i.e. on the same day as the vaccine). Metastatic prostate cancer patients will receive three intravenous infusion of nivolumab four week apart, with the first dose given concomitantly with the first MVA.5T4 vaccine.

The dosing regimens for each arm are shown in the diagram below.

Group 1 dosing regimen




Group 2 dosing regimen




*Selection of the study patient cohort*

Low- or intermediate-risk localised or locally advanced prostate cancer patients undergoing RP.

One trial cohort in this study will comprise patients who have been diagnosed with low- or intermediate-risk non-metastatic prostate adenocarcinoma and who are scheduled for radical prostatectomy. Surgery is one of the standard treatment options for localised or locally advanced prostate cancer. This patient cohort has been chosen for the study for a number of reasons. Firstly, these patients are likely to benefit from immunotherapy that may prevent relapse after primary treatment for localised or locally advanced prostate cancer. Their immune system has not been compromised by either immunosuppressive drugs or the immunosuppressive effects arising from a significant tumour burden, and therefore, these men are more likely to mount a tumour-specific immune response following vaccination, and therein benefit from a vaccine that induces long-lasting prevention of tumour recurrence via induction of immune memory. Importantly, this patient population has been shown to mount 5T4-specific immune responses following ChAdOx1-MVA 5T4 vaccination as a monotherapy in the VANCE01 study. In the current study, we will investigate the possibility that a combination of the vaccine with anti-PD-1 mAb will result in stronger immunity and / or enhanced clinical benefit compared to the vaccine as a single agent tested in VANCE01.

This trial design will allow a rapid assessment of the vaccine's immunological effect, and not only in circulation but, more importantly, in samples from a surgical specimen. Tissue from the whole prostate following surgical removal will be studied using immunohistochemistry to investigate the impact of vaccination on the tumour characteristics, including the number of CD8+ and CD4+ T cells and Gleason grading, by comparing these post-treatment surgical samples against baseline pre-treatment diagnostic biopsy samples. In addition, the specificity of tumour-infiltrating lymphocytes in the prostate will be compared against that of those in the blood, to investigate if T cells are enriched for those targeting the 5T4 antigen (if a fresh surgical specimen is available).

Metastatic prostate cancer patients

A second trial cohort in this study will comprise patients with metastatic castration resistant prostate cancer (mCRPC) with evidence of progression on anti-androgens. In the CRPC setting, the primary successful therapeutic target remains the androgen receptor. Intensive research into androgen receptor resistance mechanisms has enabled effective new treatments - androgen synthesis pathway targeting agents, such as abiraterone, and direct inhibitors of the androgen receptor, such as enzalutamide (ENZ). All patients with CRPC will ultimately succumb to the disease in the absence of a competing cause of mortality. The majority of prostate cancer vaccine monotherapies have to date been tested in this patient cohort and have demonstrated significant but only modest survival benefit.

Whilst CRCP patients have shown resistance to immune checkpoint blockade[29-31] until recently, there are several lines of evidence to indicate that metastatic patients who are resistant to the second generation anti-androgens (particularly enzalutamide), can be particularly susceptible to immunotherapies. In one case study, a durable complete PSA response was reported in a patient progressing on ENZ following treatment with Provenge[32]. More recently, in a small trial of ten mCRPC patients progressing on ENZ and treated with anti-PD-1 mAb, three patients experienced rapid PSA reduction to ≤ 0.2 ng/ml[33]. Although the mechanisms behind efficacy of the vaccine and checkpoint blockade in ENZ-resistant patients remain to be elucidated, there are considerable data showing that androgen-ablation may augment an anti-tumor immune response.

Second generation anti-androgen therapy represents a more potent form of androgen suppression and may therefore be associated with previously under-appreciated immune modulatory effects. Interestingly, patients progressing on ENZ had significantly increased PD-L1/2 positive dendritic cells in blood compared to treatment-naïve or those responding to treatment[26]. These data would be consistent with the notion that responses to immunotherapy might be more common in men progressing on enzalutamide and potentially abiraterone and thus provide a rationale for selecting this patient cohort for this trial.

Early evidence of vaccine efficacy in combination with PD-1 blockade compared to PD-1 inhibition on its own will be assessed by novel genomic analysis methods, in addition to

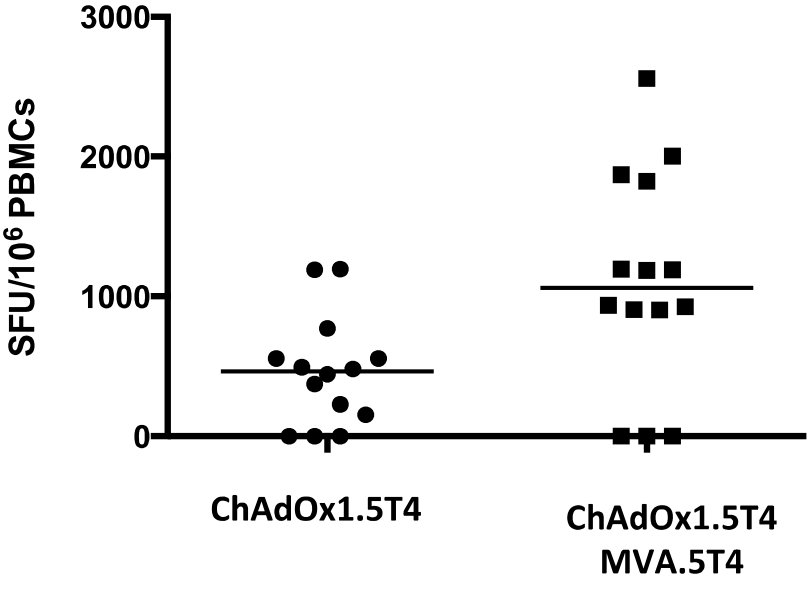
established efficacy endpoints such PSA reduction, progression-free survival (PFS) and overall survival (OS). Circulating tumour DNA (ctDNA) and circulating tumour cells (CTCs) quantification will be performed at baseline and following the immunotherapy course to monitor tumour burden secondary to intervention.

### 2.3. Pre-clinical studies involving ChAdOx1-MVA 5T4 vaccine as a monotherapy and in combination with PD-1

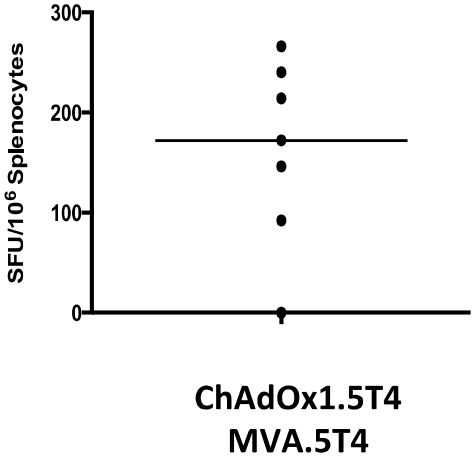
**ChAdOx1.5T4 -MVA.5T4 prime boost vaccination regime as a monotherapy**

The ChAdOx1.5T4 vaccine has shown robust immunogenicity in mouse studies. A summary of representative data is described below. The ChAdOx1.5T4 vaccine has been used most commonly in a heterologous prime-boost regimen with MVA.5T4. In a typical experiment, 6-8 week old C57BL/6 mice were primed with ChAdOx1.5T4 on day 0 and boosted with MVA.5T4 four weeks later on day 28. All vaccines were delivered intramuscularly. The dose of adenovirus was 5 x $10^9$ vp and the dose of MVA was 1 x $10^7$ pfu. Anti-5T4 cellular immune responses were assessed two weeks after the prime on day 14 and one week after the boost on day 35 by an IFNγ ELISPOT assay performed on PBMCs (Figure 1). An IFN-γ ELISPOT assay was also performed on the splenocytes 2 weeks after the MVA boost (Figure 2). The dissection of the responses to the individual 5T4 peptide pools demonstrated a broad specificity of the induced immune responses (Figure 3). In conclusion, the ChAdOx1-MVA 5T4 prime-boost vaccination regime is immunogenic in a mouse model.

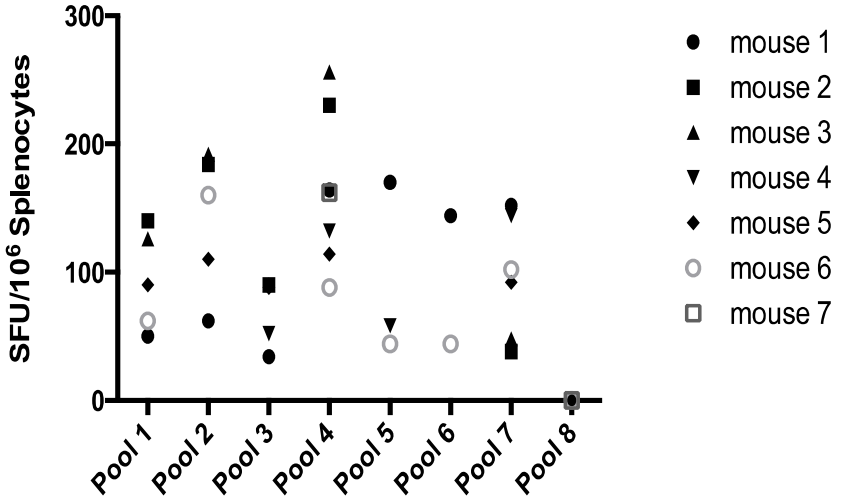
**Figure 1. Murine blood IFNγ ELISPOT assay after ChAdOx1.5T4 prime and MVA.5T4 boost**



## Figure 2. Murine spleen IFNγ ELISPOT assay after ChAdOx1.5T4 prime and MVA.5T4 boost




## Figure 3. Murine spleen IFNγ ELISPOT assay: Response to individual 5T4 peptide pools

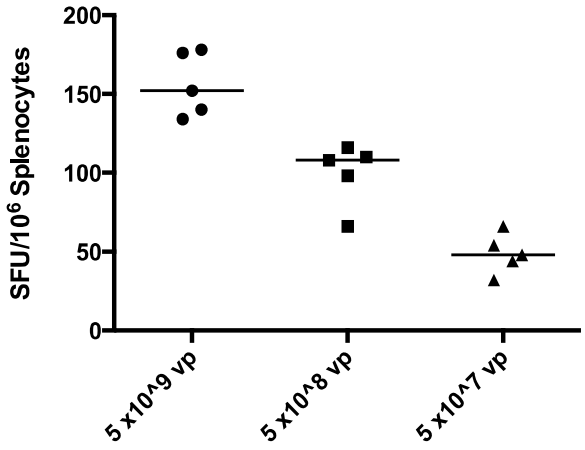


**Immunopotency of the clinical grade ChAdOx1.5T4 batch**

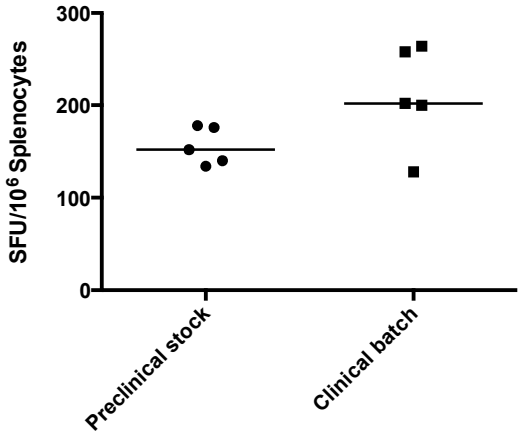
The immunopotency assay was performed to compare a pre-clinical stock of the vaccine with the clinical grade ChAdOx1.5T4 vaccine manufactured by the Oxford Clinical Biomanufacturing Facility (CBF).

Two groups of 6-8 week old C57BL/6 male mice were vaccinated intramuscularly with 5 x $10^9$ vp of the pre-clinical stock of ChAdOx1.5T4 vaccine and 5 x $10^9$ vp of the clinical grade vaccine. For additional information, two sets of 5 mice were vaccinated with 5 x $10^8$ vp and 5 x $10^7$ vp per mouse. Thirteen days later the mice were sacrificed and the spleens harvested for an ex-vivo IFNγ ELISPOT assay. This assay demonstrated that ChAdOx1.5T4 elicited a strong cellular immune response, with all mice responding, at all three doses tested (Figure 4). The assay showed equivalent immunopotency of the pre-clinical stock and clinical grade vaccines (Figure 5).

# Figure 4. Murine spleen IFNγ ELISPOT assay after ChAdOx1.5T4 vaccination: dose response



## Figure 5. Immunopotency of ChAdOx1.5T4 pre-clinical Stock and clinical batch at 5 x 10^9 vp dose




**Safety of the clinical grade ChAdOx1.5T4 batch**

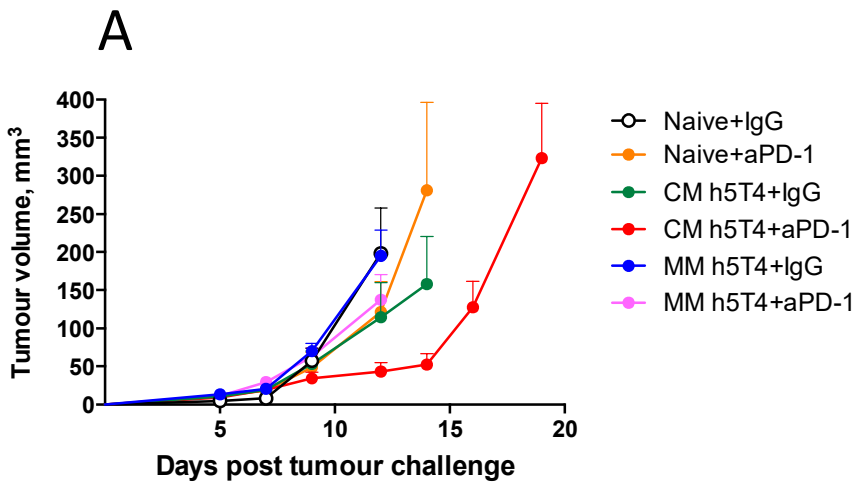
A GLP repeat dose toxicity study of the ChAdOx1.5T4 vaccine has been performed at the Huntingdon Life Sciences test facilities. The objective of this study was to investigate the potential toxicity of ChAdOx1.5T4 to BALB/c mice when administered by intra-muscular injection on 2 occasions with a 14 day interval followed by a 13 day observational period. One group comprising ten male and ten female mice have received 55 µL of ChAdOx1.5T4 (1 x 10^10 vp) into the right hind limb on Day 1 and Day 15. The control group consisting of five male and five female mice have received phosphate buffered saline (PBS), at the same volume-dose as the treated group. During the study, clinical conditions, body weight, food consumption, haematology (peripheral blood), blood chemistry, organ weight, macropathology and histpathology investigations were undertaken.

There was no effect of treatment on bodyweight gain or food consumption. There was no effect of treatment on blood chemistry parameters. Haematological examination on Day 28 revealed slightly higher group mean neutrophil and lymphocyte counts for treated males and females when compared with control males. This resulted in a slightly higher group mean total white blood cell count for treated animals. Values for other parameters, some of which attained a level of statistical significance were considered to be within the expected ranges for mice of this age and strain. There was no effect of treatment on blood chemistry parameters.

There were no unscheduled deaths during the study. There were no clinical findings and no reaction to treatment at the dose site during the course of the study. It was concluded that treatment with ChAdOx1.5T4 was well tolerated and was not associated with any adverse effects.

**ChAdOx1.5T4 -MVA.5T4 prime boost vaccination regime in combination with anti-PD-1 monoclonal antibody**

Previously, we have shown that the ChAdOx1-MVA vaccine encoding the prostate-associated antigen STEAP1 in combination with PD-1 blockade enhances efficacy and significantly improves survival of mice in the TRAMP-C1 subcutaneous tumour model[34]. Further research has been conducted to assess the immunogenicity and tumour protective efficacy of ChAdOx1-MVA 5T4 vaccine as a single agent and in combination with checkpoint blocking antibodies. First, we have demonstrated that the vaccine alone mediated complete protection against challenge with the B16 tumour cell line expressing 5T4 antigen in a prophylactic setting. However, in a therapeutic setting the tumour progression has been modestly delayed, so we have evaluated whether the vaccine efficacy can be improved by a combination with anti PD-1 mAb[35]. In a typical experiment, C57BL/6 mice were challenged with B16.h5T4 cells and primed with ChAdOx1.h5T4 or MVA.h5T4 vectors, or were left untreated (naïve mice). One week after, mice received MVA.h5T4 boost and PD-1 or isotype control antibodies intraperitoneally (i.p.). A comparative efficacy of the vaccine and PD-1 blockade as monotherapies and their combinations is shown in Figure 6 A,B. Firstly, blockade of PD-1 on its own offered just marginally better survival over isotype control mAb. As expected, ChAdOx1-MVA vaccine on its own delayed tumour progression to some extent. Notably, the administration of this vaccine in combination with anti-PD-1 was significantly more effective at suppressing tumour growth and prolonging survival of challenged mice than ChAdOx1-MVA vaccination used as monotherapy. In comparison, B16.h5T4 challenged mice prime-boosted with the MVA.5T4 vector, succumbed to tumours at the same time as naïve controls. Importantly, the analysis of IFNγ responses in PBMCs isolated from the different groups demonstrated no effect of anti PD-1 therapy on the magnitude of h5T4-specific T cell responses in circulation, suggesting that anti PD-1 antibodies are acting at the tumour site (Figure 6C).



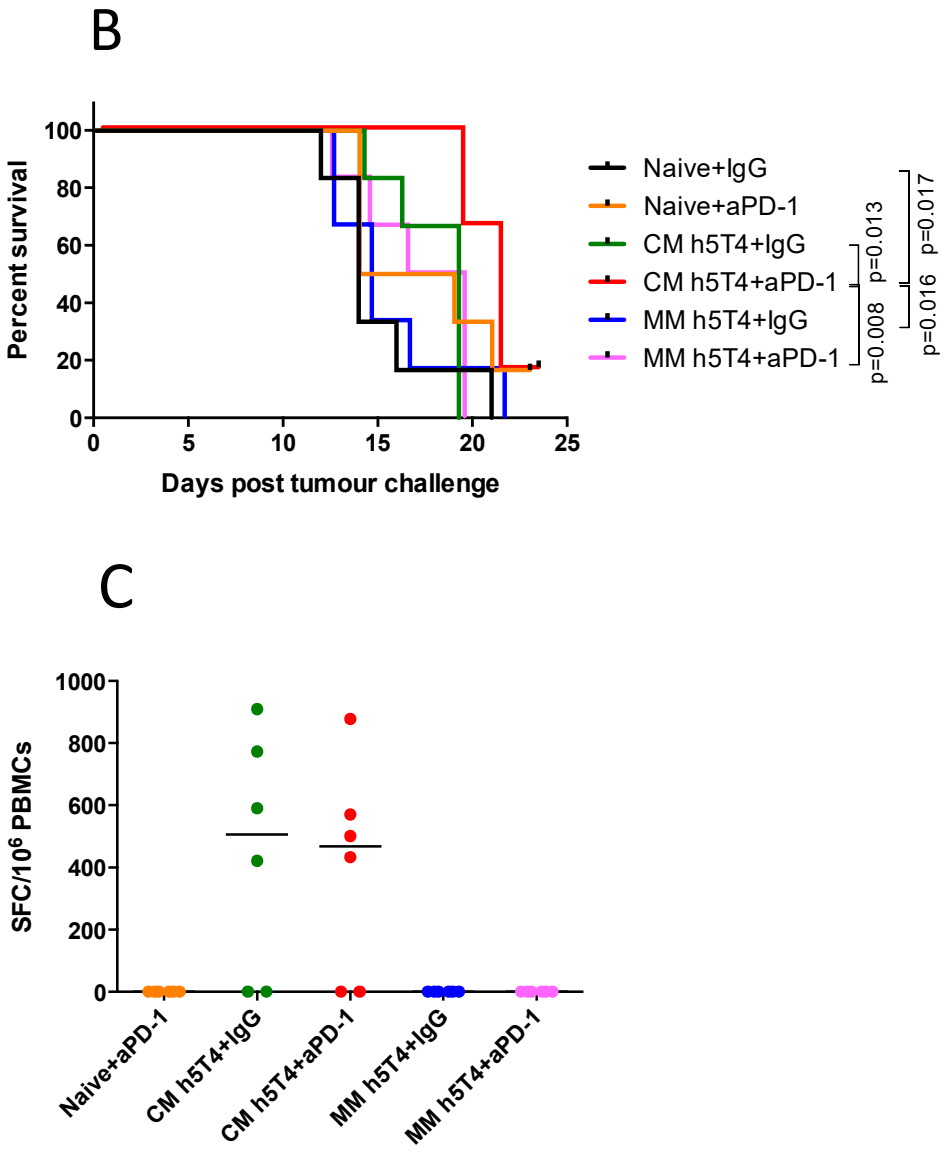



**Figure 6. Heterologous ChAdOx1-MVA h5T4 vaccination regime in combination with anti PD-1 therapy significantly improves survival in B16.h5T4 melanoma tumour model compared with homologous MVA h5T4 vaccination combined to anti PD-1 therapy**

C57BL/6 mice were challenged subcutaneously with $0.5x10^6$ B16.h5T4 cells and immunised intramuscularly with $10^7$ IU of ChAdOx1.h5T4 or $10^6$ pfu of MVA.h5T4. One week later, mice were boosted with $10^6$ pfu of MVA.h5T4 with PD-1 antibody or isotype control antibody. Naïve control mice were challenged and given only antibodies (IgG or PD-1) at the timepoint for boosting immunisation. Tumour size was measured three times per week and volumes were calculated as described above. Representative data of two biological replicate experiments are shown. (A) Tumour growth kinetics for each group expressed by mean tumour volume ± SEM. (B) Kaplan-Meyer survival curves of the different groups of mice. (C) Graph shows representative data of ex vivo blood ELIspot performed after prime-boost immunisations in the different groups of mice. Bars represent median spot forming cells (SFC) per $10^6$ PBMCs.

## 2.4. Previous clinical experience with MVA.5T4 vaccine

An MVA vector expressing the 5T4 antigen has been developed by Oxford BioMedica Ltd under the trade name of TroVax®. TroVax® has been administered to over 600 cancer patients. 12 studies of TroVax® in colorectal, renal and prostate cancer have been completed to date, and few serious adverse events have been attributed to TroVax® by investigators or the sponsor. Mild transient injection site reactions were reported in the majority of subjects together with mild transient pyrexia. No other notable, common or serious adverse events have been reported in studies using TroVax® as a single agent in heavily pre-treated subjects or in studies combining TroVax® with chemotherapy, interferon-α and IL-2[15,36-41].

**Single agent studies**

A single agent study (TV1) was conducted in 22 mCRC patients in objective response or stable disease (SD) 2.5 months after the end of first line chemotherapy. Four dose levels and two routes of administration (i.m. or i.d.) were tested in this study. TroVax® was well tolerated at all dose levels with a similar safety profile at each dose level. The highest dose level tested was $6.83 \times 10^8$ pfu. The safety profile was comparable to the usual safety profile of a vaccine. The main AEs considered to be related to TroVax®, all Grade 1, were injection site erythema and injection site reaction. Sixteen out of 17 patients evaluable for immune response had a cellular or humoral immune response. No radiological objective response was reported.

A second single agent study (TV-CR-UK) was conducted in 20 patients with resectable liver metastases of colorectal cancer. Patients received two injections before and after surgery. Two additional injections were administered in case of immune response. Sixteen out of 19 evaluable patients for immune response had a 5T4 cellular or humoral immune response prior to surgery. All 16 patients who completed four TroVax® injections and underwent surgery had a 5T4 cellular or humoral immune response after the surgery. Overall, taking into account immune response before and after surgery, 18 out of 19 evaluable patients had a 5T4 cellular or humoral immune response. The most common AE considered related to TroVax® was Grade 1-2 injection site reaction.

**Combination studies**

*In colorectal cancer*

Two Phase II combination studies were conducted in colorectal cancer (TV2-cr-ch-Oxaliplatin and TV2-cr-ch-Irinotecan studies). Chemotherapy did not abrogate the immune response to TroVax®. In both studies, the safety profile of TroVax® was similar to the safety profile reported in the single agent study. The safety profile of chemotherapy in combination with TroVax® was similar to the safety profile of chemotherapy alone.

*In advanced renal cell cancer*

Four Phase II and 1 Phase III studies were initiated in advanced renal clear cell cancer. One Phase II study assessed TroVax® in combination with interleukin-2 high dose (IL-2 HD) (TV2 Renal), 1 Phase II study of TroVax® in combination with IL-2 low dose (IL-2 LD) (TV-B2-001-05); 1 Phase I/II feasibility study of TroVax® with IFN-α (TV2-003-06); and 1 Phase II study of TroVax® with or without IFN-α (TV2-002-06).

A randomized Phase III double blind study was initiated in 2006 (TV3-001-06). This study compared TroVax® to placebo combined to standard of care (IL-2, IFN-α, sunitinib) in first line advanced (locally advanced or metastatic) renal clear cell cancer. The primary endpoint was overall survival; however, following the fourth interim review by the data safety monitoring board, the study was judged to be futile and was terminated early.

*In hormone refractory prostate cancer*

Three randomized Phase II studies were initiated in hormone refractory prostate cancer; one study evaluated TroVax® either alone or in combination with granulocyte macrophage-colony stimulating factor (GM-CSF) (TV-PHPRC-001-06), one study evaluated TroVax® plus docetaxel and docetaxel alone (PCa-07-101) but was stopped prematurely due to the termination of the TRIST study, and the third study (TV2-001-09) compared TroVax® plus docetaxel and docetaxel alone, but was also stopped prematurely owing to slow recruitment.

**Overall conclusions from TroVax clinical studies**

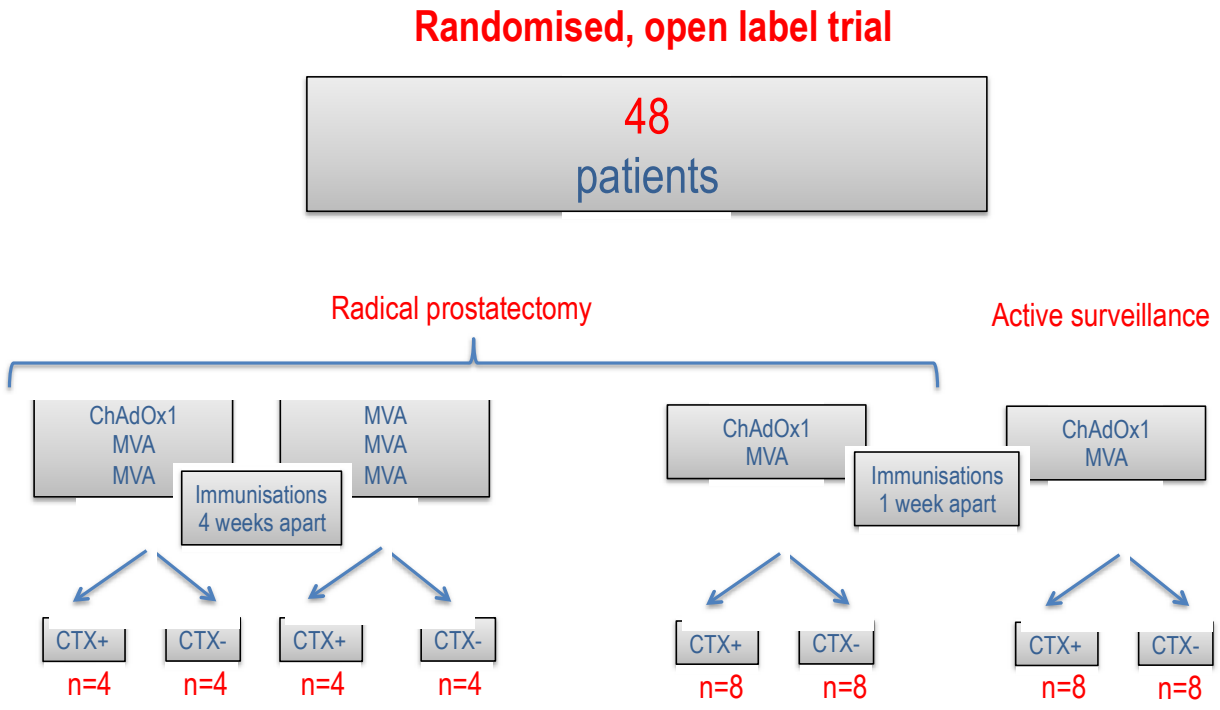
The administration of TroVax® is safe with mild to moderate vaccine reactions, chills, pyrexia, arthralgia and myalgia being the only adverse events of note. The safety profile of TroVax® is similar to that of a vaccine containing an attenuated vaccinia virus. The vector (MVA) has been widely used and no complications were reported when it was administered to over 120,000 subjects, including subjects at risk from vaccine complication. The safety profile of TroVax® allows combination with chemotherapy without increase in treatment toxicity and does not abrogate the immune response.

### 2.5. Previous clinical experience with ChAdOx1-MVA 5T4 vaccine

VANCE01 was the first phase I clinical trial to assess the safety and immunogenicity of ChAdOx1-MVA 5T4 vaccine. VANCE01 began in Oxford in June 2015 (NCT02390063) and completed in May 2018. It compares ChAd-MVA vaccination to MVA alone, with and without low dose cyclophosphamide in low- and intermediate-risk localised prostate cancer.

An initial clinical assessment of the vaccine has shown it to be both safe and immunogenic for cellular and antibody responses to the 5T4 protein. The study design and further details of this

trial are given below.




To date, 40 patients have been enrolled and 39 of these have completed the vaccination course comprising either a standard immunisation protocol of 3 immunisations given at 4 week intervals or an accelerated protocol of 2 immunisations given at a 1 week interval.

The majority of adverse events were mild in nature and the interim clinical safety review was satisfactory. Both ChAdOx1.5T4 and MVA.5T4 vaccines have been well tolerated in both the standard prime-boost and the accelerated prime-boost vaccination regimes. Systemic reactogenicity appears most pronounced following the accelerated vaccination regime after the MVA.5T4 boost, but all symptomatic manifestations have been self-limiting and quickly resolving. Derangements in clinical haematology and biochemistry have all been mild and transient in nature. No additional safety findings are apparent following vaccination in combination with low dose cyclophosphamide. There has been one serious adverse event (SAE) which was deemed unlikely to be related to the investigational medicinal products employed in this study.
Overall, ChAdOx1-MVA 5T4 vaccine was safe and well tolerated. An initial assessment of immunogenicity is very encouraging given that 5T4 is a self-antigen against which an immunological tolerance is likely to exist. More than 60% of the patients in VANCE01 have mounted cellular 5T4 specific responses that are readily detectable *ex vivo*. A summary of the cellular immune responses observed to date are shown in Figure 7. Of note, the elicited immune responses were comparable between the standard 4 week vaccination regime and the accelerated 1 week immunisation schedule, but CTX pre-condtioning has not improved vaccine immunogenicity. Therefore, in ADVANCE study CTX will not be administered and a 2 week interval between ChAdOx1.5T4 prime and MVA.5T4 boost will be deployed in order to minimise surgery

delay for intermediate-risk prostate cancer patients and at the same time to allow sufficient time for the immune cells to mediate their effect on the target organ.

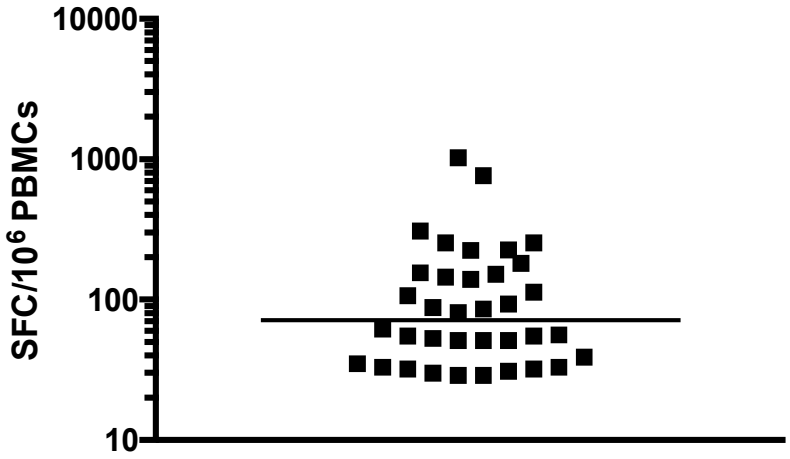



**Figure 7. Summary of 5T4-specific cellular immune responses.** Each dot represents the magnitude of the largest response at any post vaccination time point for each responding subject.

### 2.6. Previous clinical experience with PD-1 blockade in prostate cancer

**PD-1 blockade as a monotherapy**

Results to date examining PD-1 blockade alone have suggested little role for these treatments as monotherapy for patients with prostate cancer. Clinical efficacy of PD-1 therapy (nivolumab) in mCRPC has been assessed in two independent phase 1 trials. Despite evidence showing that CD8 T cells infiltrating prostate tumours express PD-1, no objective responses to single-agent PD-1 blockade were reported in the 25 patients with prostate cancer who were treated in both of these trials[8,9].

More recently, however, an unexpected clinical activity of PD-1 blockade was seen in mCRPC patients progressing on ENZ[33]. Patients with evidence of progression on enzalutamide were treated with anti-PD-1 mAb (pembrolizumab) 200 mg IV every 3 weeks for 4 doses. Anti-PD-1 mAb was added to standard dose of enzalutamide. Three of the first ten patients enrolled in this ongoing phase II trial experienced rapid PSA reductions to ≤ 0.2 ng/ml. Two of these three patients had measurable disease upon study entry and both of these patients achieved a partial response. A phase II trial is currently underway that more thoroughly examines the antitumor efficacy of PD-1 blockade in patients with mCRPC (NCT02312557).

As mentioned earlier, a large multi-centre international trial, KEYNOTE-199, evaluating pembrolizumab as a monotherapy in 370 patients with metastatic castration resistant prostate cancer is currently ongoing (NCT02787005). Preliminary results from 258 patients already

recruited to the trail were reported by De Bono and colleagues at the ASCO annual meeting in June 2018 and showed that pembrolizumab had antitumour activity in 11% of both PD-L1 positive and PD-L1 negative patients with mCRPC.

**PD-1 blockade in combination with vaccines**

It remains to be determined whether PD-1 blockade will be more effective in patients with prostate cancer when used in combination with other treatments. To address this question, several trials are currently underway to the assess clinical activity of PD-1 in combination with vaccines.

In one trial, mCRPC patients are treated with anti-PD-1 (pembrolizumab) in combination with plasmid DNA vaccine (pTVG-HP) encoding prostatic acid phosphatase (PAP). This pilot phase I study will recruit 32 patients who will be randomized to two arms to receive 6 doses of the vaccine and four infusions of anti-PD-1 either concurrently or sequentially (NCT02499835).

In the second trial, anti-PD-1 mAb (nivolumab) is administered in combination with PROSTVAC vaccine. Three doses of nivolumab (240mg per dose) are administered at the same time as the booster vaccinations given 3 weeks apart. This study is recruiting cancer patients who are surgical candidates for radical prostatectomy and the experimental treatment is given as a neoadjuvant prior to surgery (NCT02933255).

Data collection for both studies is ongoing and the final results are not expected within the next couple of years.

## 3. OBJECTIVES

**Primary objectives**

*Low- or intermediate-risk* PCa *patients:*

- To assess safety of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb
- To determine whether ChAd-MVA 5T4 vaccine in combination with anti-PD-1 will impact on the level of PSA in the blood

*Metastatic PCa patients:*

- To assess safety of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb
- To assess efficacy of the viral vectored ChAd-MVA 5T4 vaccine when administered in combination with anti-PD-1 mAb as measured by composite response rate defined as one of the following:

- reduction of circulating tumour DNA of ≥50%
- serum PSA decrease of ≥50%

**Secondary objectives**

*Low- or intermediate-risk* PCa *patients:*

- To assess the magnitude of immune responses in the periphery generated by ChAd-MVA 5T4 vaccine in combination with anti-PD-1 mAb
- To assess immune cell subsets in the prostate secondary to treatment

*Metastatic PCa patients:*

- To assess the magnitude of immune responses in the periphery generated by ChAd-MVA 5T4 vaccine in combination with anti-PD-1 mAbTo evaluate radiographic progression-free survival at 6 months and one year post enrollment
- To evaluate overall survival at 6 months and one year post enrollment

**Exploratory objectives**

- To investigate whether ChAd-MVA 5T4 vaccine in combination with checkpoint blockers will lead to up-regulation of PD-L1 expression in the primary tumour or metastatic lesion
- To determine whether ChAd-MVA 5T4 vaccine in combination with PD-1 mAb will impact on the level of PSA-related kallikreins in the blood
- To assess biochemical (PSA) response rates according to PCWG2 criteria
- To assess whether ChAd-MVA 5T4 vaccine administered in combination with anti-PD-1 mAb will lead to reduction of circulating tumour cells
- To evaluate phenotypic and functional profile of immune cells in the blood secondary to treatment
- To evaluate immunologic antigen spreading to other prostate-associated antigens secondary to treatment

## 4. STUDY DESIGN

### 4.1. Study overview

This is a phase I/II open label non-randomised study to determine the safety and immunogenicity of the viral vectored ChAd-MVA.5T4 vaccines in combination with anti-PD-1 checkpoint blockade in low- or intermediate-risk localised or locally advanced prostate cancer and in metastatic prostate cancer.

This is an adaptive design study. A number of modifications to the protocol may be implemented on review of interim safety and efficacy data, following evaluation by the trial's independent data

and safety monitoring committee. One of the possible modifications may include sample size re-calculation. Subjects with low- or intermediate-risk disease and metastatic prostate cancer enrolled to the study will be evaluated for markers of treatment efficacy using the primary study endpoints (i.e. circulating tumour DNA and PSA kinetics) and vaccine-specific and non-specific immune responses. In the event that interim analysis indicates an early efficacy signal among a particular population of study participants, a sample size re-calculation may be undertaken and recruitment targets adjusted to provide enhanced power on efficacy analyses. Sample size re-calculation and group size adjustments will be made with the oversight of the study independent data and safety monitoring committee and subject to satisfactory interim safety profile of the groups involved. Additionally, if the patients demonstrate a clinical response to the study treatment, the number of vaccinations and/or nivolumab infusions can be increased.
Any changes to the study design will constitute a substantial amendment and, therefore, will be reviewed and approved by the Sponsor, REC/HRA, and MHRA (if appropriate) prior to implementation.

### 4.2. Investigational products

The investigational products to be used in this study are:

- ChAdOx1.5T4
- MVA.5T4
- Nivolumab (anti-PD-1 monoclonal antibody)

#### 4.2.1 Vaccine formulation, storage and accountability

**ChAdOx1.5T4**

ChAdOx1.5T4 vaccine is is an unlicensed medicinal product manufactured by by the University of Oxford Clinical Biomanufacturing Facility (CBF), Churchill Hospital, Oxford. It is formulated in formulation buffer at a nominal concentration of $1.1x10^{11}$ vp/mL. The formulation buffer consists of 10mM Histidine, 7.5% sucrose, 35mM NaCl, 1mM MgCl2, 0.1% PS80, 0.1mM EDTA, 0.5% EtOH, pH 6.6. The fill volume is 0.5-1.0 mL. The dose of ChAdOx1.5T4 to be used in this study is $2.5x10^{10}$ vp. It is manufactured, labelled and released under Good Manufacturing Practice conditions to the clinical sites by the CBF. All movements of the study vaccine between the CBF and the clinical sites will be documented. The vaccine is supplied as liquid in glass vials and is stored at -80C in a locked, alarmed, temperature monitored freezer prior to release to the clinical sites. Following shipment to sites, the vaccine is stored as above, however, short-term storage for up to 3 months at 2-8C is acceptable if required, based on the manufacturer's stability data to date.

**MVA.5T4**

MVA.5T4 vaccine is an unlicensed medicinal product that has not been administered to humans before. VANCE01 trial has made use of the biologically equivalent MVA.5T4 vaccine supplied by Oxford BioMedica (manufactured by Baxter), trade name TroVax®. The new batch of MVA.5T4

vaccine expresses the 5T4 antigen under control of the different poxvirus promoter. It has been manufactured under Good Manufacturing Practice conditions by IDT Biologika GmbH, Germany. MVA.5T4 is supplied as a frozen liquid formulation in 10mM Tris buffer 140 mM NaCl, pH 7.7 in 2.0 mL clear glass vials. The formulation titre is $2x10^9$ pfu/ml, the final drug product titre is $1x10^9$ pfu/ml. The fill volume is 0.55 mL. Further details relating to batch manufacture can be found in the MVA.5T4 IMPD.

To ensure consistency with the VANCE01 clinical trial and all previous University of Oxford trials using MVA vectored vaccines, the dose to be used in this study will be $2x10^8$ pfu. The vaccine will be labelled and released under Good Manufacturing Practice conditions to the clinical sites by the CBF. All movements of the study vaccine between the CBF and the clinical sites will be documented. At CBF it is stored at -80C in a locked, alarmed, temperature monitored freezer prior to release to the clinical sites. Following shipment to sites, the vaccine is stored as above.

**Minimising environmental contamination with Genetically Modified Organisms (GMO)**

In order to minimise dissemination of the recombinant virus into the environment, the inoculation site will be covered with a dressing after immunisation. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site after 60 minutes (± 15 minutes) and will be disposed of as GMO waste by autoclaving as well as unused/expired vaccine vials, in accordance with the relevant Standard Operating Procedure (SOP) and current standard UK practice.

**Checkpoint inhibitor formulation, storage and accountability**

### 4.2.2 Anti-Programmed Death Receptor-1 (PD-1) antibody - nivolumab (Opdivo®)

Nivolumab (Opdivo®) is a human immunoglobulin G4 (IgG4) monoclonal antibody directed against programmed death-1 (PD-1) receptor. It is a licenced pharmaceutical product manufactured and marketed by Bristol Myers Squibb Pharmaceutical Limited. It holds a UK licence for the treatment of unresectable or metastatic advanced melanoma as monotherapy or in combination with ipilimumab, and is also licensed for the treatment of locally advanced or metastatic non-small cell lung cancer after prior chemotherapy, and as monotherapy of advanced renal cell carcinoma. Comprehensive pharmaceutical information for nivolumab is contained in the manufacturer's Summary of Product Characteristics (SmPC). Briefly, nivolumab is a clear to opalescent, colourless to pale yellow solution of pH 6.0 and osmolality of 340 mOsm/kg supplied as a 10 mg/mL sterile concentrate for solution for intravenous infusion. Nivolumab (Opdivo®) will be purchased by the host institution for use in the trial and stored at 2°-8°C in the clinical trials pharmacy until expiry date stated in the SmPC. It will be dispensed for use in ADVANCE from the pharmacy following receipt of a trial-specific prescription and labelled according to the Medicine for Human Use (Clinical Trials) Regulation 2004. All movements of nivolumab between the host institution pharmacy and the clinical sites will be documented.

The recommended dose of nivolumab for current licenced indications is 3 mg/kg administered intravenously every 2 weeks or a flat dose of 480 mg adnministered intravenously every 4 weeks. The new dose of 480 mg 4-weekly was approved by FDA in March 2018 (https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125554s048s049s050s051s052s061s062s064s065s066lbl.pdf).

Therefore, this study will make use of a uniform intravenous dose of nivolumab 480mg per infusion, administered on one or three occasions only, at an interval of 4 weeks apart.

Nivolumab (Opdivo®) for this study will be supplied by a commercial company, Vaccitech Ltd., that will have access to the study results.

### 4.2.3 Administration of study drugs

All study drugs will be administered in the clinical research facility or oncology treatment suite of the respective study centres where trained personel, resuscitation equipment and emergency drugs are immediately available. Vital signs will be measured before and after administration of all investigational products. The doctor/nurse will wear gloves and eye protection. Patients will remain in the unit for 1 hour (+/- 15 min) for observation after each vaccination and 30 min (+/- 15 min) for observation after each checkpoint inhibitor treatment.

**ChAdOx1.5T4 and MVA.5T4 vaccines**

The vaccines will be allowed to thaw to room temperature and will be administered by intramuscular injection within 1 hour of removal from the freezer. The preferred site for vaccination is the vastus lateralis muscle of the thigh but the deltoid muscle of the arm is an acceptable alternative. It is advised to use a different thigh (arm) for each of the two MVA.5T4 immunisations.

**Immune checkpoint inhibitor nivolumab (Opdivo®)**

Nivolumab 480mg, will be prepared from 10mg/ml concentrate, diluted into sodium chloride 9 mg/mL (0.9%) solution for injection and administered intravenously in a volume of 100ml to a final concentration of 4.8mg/ml. Preparation and handling will adopt strict aseptic technique. In line with the manufacturer's instructions, nivolumab will be administered intravenously over a period of 60 minutes using a volumetric pump, an infusion set and an in-line, sterile, non-pyrogenic, low protein binding filter of pore size of 0.2µm to 1.2µm. After administration the line will be flushed with sodium chloride 9 mg/mL (0.9%) solution for injection.

## 4.3. Allocation to study groups and dosing regime

The 36 subjects will be recruited to the study: 12 patients with low- or intermediate-risk prostate cancer (Group 1) and 24 patients with metastatic prostate cancer (Group 2).

**<u>Group 1</u>: Low- or intermediate-risk localised or locally advanced prostate cancer patients scheduled to undergo radical prostatectomy**

12 subjects will be treated with ChAd-MVA.5T4 vaccination plus 1 dose of the anti-PD-1 monoclonal antibody Nivolumab

| | | | |
|---|---|---|---|
| Week 0: | ChAdOx1.5T4 | $2.5 \times 10^{10}$ vp | intramuscular injection |
| Week 1: | MVA.5T4 | $2 \times 10^{8}$ pfu | intramuscular injection |
| | Nivolumab | 480mg | intravenous infusion |

**<u>Group 2</u>: Metastatic prostate cancer patients with disease progression on anti-androgen therapy with either enzalutamide or abiraterone**

24 subjects will be treated with ChAd-MVA.5T4 vaccination plus 3 doses of the anti-PD-1 monoclonal antibody Nivolumab

| | | | |
|---|---|---|---|
| Week 0: | ChAdOx1.5T4 | $2.5 \times 10^{10}$ vp | intramuscular injection |
| Week 4: | MVA.5T4 | $2 \times 10^{8}$ pfu | intramuscular injection |
| | Nivolumab | 480mg | intravenous infusion |
| Week 8: | Nivolumab | 480mg | intravenous infusion |
| Week 12: | ChAdOx1.5T4 | $2.5 \times 10^{10}$ vp | intramuscular injection |
| | Nivolumab | 480mg | intravenous infusion |
| Week 16: | MVA.5T4 | $2 \times 10^{8}$ pfu | intramuscular injection |

**Compliance with Dosing Regimen**

Administration of all vaccines and checkpoint inhibitor therapies will be undertaken by a delegated research nurse or investigator in the clinical research facility or oncology treatment suite of the respective study centres. All study treatments will be recorded in the CRF and the relevant accountability log by the research nurse or investigator. There are no self-administered investigational products involved in this study.

### 4.4. Duration of study

Subjects undergoing radical prostatectomy will all be followed up for a total duration of 6 months from enrolment. Planned follow up for participants with metastatic prostate cancer is 12 months from enrolment.

### 4.5. Definition of the Start and End of the Study

The start of the study is defined as the date of the first administration of a study drug. The end of the study is three months after the last visit of the last subject to allow for sample analysis.

### 4.6. Potential risks to participants

Risks to participants in this Phase II study can be divided as follows:

#### 4.6.1 Phlebotomy

The volume of blood drawn over the study period (up to 616ml) should not compromise patients, as during any one normal blood donation a total volume of 470ml can be drawn and the procedure is allowed for up to 3 times a year according to the NHS regulations.

**Local reactions to vaccine, venepuncture or intravenous cannulation**

Mild tenderness, bruising, light-headedness or, rarely, syncope, may result from venepuncture. Vaccine-related side effects are believed to be related more to the vectors used rather than the specific insert.

#### 4.6.2 Surgery delay

Surgery is expected to take place at least 4 weeks post nivolumab infusion. The risk associated with postponing surgery for low and intermediate risk prostate cancer by a maximum of approximately 6 weeks is difficult to accurately quantify but the researchers consider it to be very small for men with this type of prostate cancer. Indeed, many men with low- and intermediate-risk prostate cancer do not need any immediate treatment at all, and some choose "active surveillance" and potentially delay treatment for many years (or can avoid treatment altogether) in order to avoid treatment-related side effects and potential "over-treatment" of clinically insignificant forms of prostate cancer, rather than undergo immediate treatment with surgery or radiotherapy. Patients with high-risk prostate cancer, where the effect of a 6-week delay might be significant, will not be included in this study. Seventeen studies have investigated the effect of delaying surgery for low and intermediate risk prostate cancer[42]. None of these studies reported lower chance of cure for patients with low risk cancer if surgery delayed for up to several years. Many patients with low risk prostate cancer do not need any treatment at all, but may still opt for surgery rather than active surveillance. One of these studies reported higher risk of recurrence if surgery for intermediate risk cancer was delayed for more than 9 months, but in the other 16 studies no significant negative effect was found among men who had delayed surgery[42]. Patients with high-risk prostate cancer, on the other hand, should be treated without delay and are therefore not eligible for the ADVANCE study. In summary, the researchers therefore believe that a 6-week delay of surgery for men in this study, all of whom have been diagnosed with low or intermediate risk prostate cancer, in order for them to receive their vaccinations poses very little risk with regards a change in the potential of the surgery to cure them of their prostate cancer. All patients will have a mpMRI scan before enrolment that will minimize the risk of any patient with a high-risk cancer being misclassified as low or intermediate risk.

### 4.6.3 Risks associated with study vaccinations

Vaccination may precipitate a local inflammatory reaction. This may include swelling, tenderness or erythema. Systemic reactions that could potentially occur following immunisation with a recombinant ChAdOx1 and MVA vaccines include constitutional manifestations resembling flu such as fever, headache, rash, malaise, fatigue, anorexia, arthralgia, and myalgia. These are typically self-limiting over a period of 48-72 hours following ChAd-MVA.5T4 monotherapy. Allergic and hypersensitivity reactions to the vaccine are a reasonable expectation. Severe hypersensitivity reactions to the vaccine are rare and should be managed in accordance with Advanced Life Support algorithms. Immunological sequaelae following any immunisation are recognised but rare. These are however associated with checkpoint inhibitor therapy as discussed below.

### 4.6.4 Risks associated with checkpoint inhibitor therapy

**Extravascular infiltration**

In the event of inadvertent infiltration into the extravascular compartment subjects may experience localised irritation and discomfort, including swelling and erythema. However, nivolumab (Opdivo®) is a non-vesicant drug and therefore does not confer risk of extravasation tissue necrosis. No specific neutralising or dispersal interventions are indicated. The potential for extravascular infiltration will be minimised by careful selection of the cannulation site and close monitoring of the subject during the infusion. At the earliest appearance of extravascular infiltration the infusion will be stopped, the device removed and the site cared for according to local guidance.

**Infusion reaction**

At the time of licencing the overall incidence of hypersensitivity/infusion reactions associated with nivolumab monotherapy was 5.0%. The majority of infusion reactions are mild in severity and are evident within 2 hours of the infusion. Hypersensitivity reactions may manifest as:

- Flushing or itching or rash
- Nausea or vomiting
- Fever or chills
- Dyspnoea, wheeze or chest discomfort
- Hypotension and changes to vital signs

All infusion reactions will be graded and recorded as adverse events according to NCI CTCAE v4.0 criteria and managed in line with established site-specific treatment algorithms. All treatments will be undertaken by oncology nurses experienced in the management of intravenous therapies and take place in a treatment facility with access to resuscitation equipment and a medical emergency / resuscitation team. Severe infusion reactions and anaphylaxis will be managed according to Advanced Life Support protocols.

**General side effects**

The most frequent side effects of immune checkpoint inhibitor therapy are fatigue, decreased appetite and nausea which each affect between 20 and 50% of cancer patients receiving continuous checkpoint inhibitor treatment in recent Phase III clinical trials. There is limited data on the side effects of short term immune checkpoint inhibitors.

**Immune mediated toxicity**

Checkpoint inhibitor treatment is associated with immune-mediated adverse reactions which may affect any organ system. Current data on the incidence of immune-related toxicities are derived from a number of large phase III clinical trials administering weeks to months of continuous checkpoint inhibitor therapy until disease progression, loss of clinical benefit or unacceptable toxicity, in a range of non-prostate tumour types. This study, however, will make use of **a restricted course of treatment limited to 3 infusions 4 weeks apart for metastatic PCa patients and only 1 infusion for low- or intermediate- risk PCa patients** . The incidence and severity of immune-mediated toxicities following a limited course of 3 infusions four weeks apart is not publically available. However, immune-mediated adverse events may occur at any time from the onset of therapy or after completion of treatment and may not be dose-dependent. Most immune-related adverse reactions, however, are low in severity and resolve with appropriate management, though fatal cases have been reported.

The frequency and median time to onset for the major immune mediated toxicities from pooled phase III datasets are shown in Table 1. These data relate to continuous therapy regimes with repeat nivolumab infusions at 2 week intervals. The incidence of toxicities grade 3 or above is <2% for all organ systems. Importantly, more than 50% of immune-mediated toxicities develop after several weeks of continuous treatment, therefore the frequency and risk of treatment-related adverse events following a restricted course of 3 infusions in this study is anticipated to be considerably lower than during licenced clinical use.

**Table 1. Immune mediated adverse events after continuous monotherapy with Nivolumab**

| Immune-mediated toxicity | Frequency (%) * | | Median (range) time to onset (months) | Corresponding number of doses at median time of onset ** |
|---|---|---|---|---|
| | Any severity | Grade 3 or above | | |
| Rash | 25.6 | 0.9 | 1.4 months( 0.0-113.7) | 3 |
| Diarrhoea / Colitis | 12.6 | 1.3 | 1.4 months (0.0-20.9) | 3 |
| Pneumonitis | 3.0 | 0.8 | 3.3 months (0.0-19.6) | 7 |
| Liver dysfunction/ Hepatitis | 6.4 | 1.8 | 1.9 months (0.0-10.4) | 4 |
| Renal dysfunction | 2.7 | 0.5 | 2.3 months (0.0-18.2) | 5 |
| Endocrinopathy<br>Thyroid disorders | 8.5 | <0.1 | 2.8 months (0.0-14.0) | 6 |

| | | | | |
|---|---|---|---|---|
| Adrenal insufficiency | 0.4 | 0.2 | | |
| Pituitary disorders | 0.4 | 0.2 | | |

** From pooled dataset n = 2227 (CA209066, CA209037, CA209067 trials) nivolumab 3mg/kg across tumour types*

*** Based on standard licenced dosing interval of 2 weeks*

Participants will be under continuous monitoring for checkpoint inhibitor toxicity. Established consensus treatment algorithms for investigation and management are in place and will be adopted according to site-specific clinical practice. All participants will be counselled on recognition and early reporting of potential treatment toxicities. The major immune-mediated toxicities associated with continuous use of nivolumab are:

**Dermatological**

Rash is the most common immune-related toxicity associated with anti-PD-1 therapy. The most frequent manifestation is with maculopapular rash, however, other presentations including urticarial dermatitis, vitiligo, lichenoid or psoriatic lesions are also possible. The majority of cases are grade 1 in severity. Isolated cases of Stevens-Johnson Syndrome and toxic epidermal necrolysis have been observed.

**Diarrhoea**

This presentation incorporates a spectrum of pathology ranging from increased stool frequency to active inflammation of the bowel (colitis) manifesting as abdominal pain, profuse diarrhoea, rectal bleeding and radiographic changes. The majority of cases are grade 1 or 2 in severity. Evaluation of diarrhoea and/or abdominal pain must include assessment for alternative aetiologies, in particular infective enteritis, constipation or obstruction and overflow.

**Respiratory**

Pneumonitis, or diffuse lung inflammation may present with shortness of breath, cough or chest pain. The major differential diagnoses include bacterial pneumonia and pulmonary embolism. The majority of cases are grade 1 or 2 in severity.

**Hepatic and hepatobiliary**

Immune-mediated liver toxicity may manifest with asymptomatic abnormalities in liver transaminases or total bilirubin during monitoring blood tests. Clinically apparent pancreatitis has been reported in ≤1% of patients across clinical trials of continuous monotherapy in multiple tumour types. Laboratory abnormalities or subclinical disorders with elevation in serum amylase and lipase occur more frequently and may not warrant active intervention. Any suspected presentation requires detailed work up to exclude alternative aetiologies such as metabolic or obstructive liver / hepatobiliary pathologies.

**Endocrine**

Immune-mediated endocrinopathy may include thyroiditis with or without deranged thyroid function, adrenal insufficiency, diabetes mellitus or pituitary dysfunction. Presentation may involve non-specific symptoms including fatigue, anorexia, dehydration or headache and requires clinicians to retain a high index of suspicion. Endocrinopathy may require short or long term hormone replacement.

**Renal dysfunction**

Treatment-associated renal dysfunction manifesting as rise in serum creatinine with or without active urinary sediment may represent immune mediated or interstitial nephritis. However, careful attention and investigation are required to exclude alternative causes of renal impairment, including alternative nephrotoxic agents, pre-renal mechanisms and obstructive uropathy, particularly among participants with significant pelvic or retroperitoneal disease.

**Other immune-mediated toxicities**

Immune-mediated toxicity may rarely affect outher organ systems. Immune-mediated neurological toxicity, including peripheral neuropathy, uveitis and ocular disorders, demyelination and myasthenic syndrome have, together been reported in <1% in cancer patients treated with continuous checkpoint inhibitor monotherapy in phase III clinical trials. Haematological disturbances including neutropaenia and thrombocytopaenia may occur.

**4.6.5 Risks associated with exposure to ionising radiation (CT scan and bone scan)**

Exposure to ionising radiation carries a risk of deleterious effect, in this case induction of excess cancers in the exposed population. However, it should be noted that risks relating to cancer induction may not be expressed for many years, and are therefore of reduced significance in some patient groups, e.g. those where life expectancy is reduced, or the elderly. In some such groups the cancer induction risk is unlikely to be expressed during the remaining years of life.

## 4.7. Potential benefits to participants

**Additional medical care**

Through the schedule of study visit and investigations, including study-specific MRI scans and contact with health professionals, participants in this study will benefit from enhanced level of medical care. This enhanced level of care may facilitate the early diagnosis and management of any disease related complications and/or comorbid conditions should they occur.

**Potential anti-tumour activity**

Among the population of men with prostate cancer in this study there is as yet no proven clinical benefit from the therapies under investigation. However, checkpoint inhibitor drugs have demonstrated anti-tumour efficacy and clinically significant benefits as single agent therapies in phase III trials against multiple non-prostatic malignancies, including melanoma, non small cell

lung cancer, bladder cancer and renal cell carcinoma. Moreover, results of a small phase II study of anti-PD-1 antibody treatment in metastatic prostate cancer indicate that a short course of checkpoint inhibitor monotherapy can elicit significant anti-tumour activity and a measurable clinical benefit among some patients.

In addition, among men with localised low- and intermediate-risk prostate cancer ChAd-MVA 5T4 vaccination has been shown to elicit immunological responses which are compatible with anti-tumour activity against prostate cancer cells. This effectly forms the basis for the ADVANCE study and supports the possibility that combined checkpoint inhibitor therapy and ChAd-MVA 5T4 vaccination might induce enhanced anti-cancer immunity. Such an affect could potentially reduce the patients' risk of recurrence after surgery for localised and locally advanced prostate cancer and delay, slow or prevent disease progression in patients with metastatic disease.

## 5. RECRUITMENT AND WITHDRAWAL OF STUDY SUBJECTS

### 5.1. Selection of Participants

Participants will be recruited into either of two cohorts within the study - subjects with operable localised or locally advanced prostate cancer who are planned to undergo radical prostatectomy, or subjects with metastatic prostate cancer who demonstrate disease progression on therapy with second generation anti-androgens (enzalutamide or abiraterone).

**Radical prostatectomy group**

The subjects recruited will be men over the age of 18 years, diagnosed with low- or intermediate-risk localised or locally advanced prostate cancer and deemed operable and fit to undergo radical prostatectomy by the treating consultant urological surgeon. The number of subjects newly diagnosed with low- and intermediate-risk prostate cancer and selecting radical prostatectomy as their treatment option at the Oxford Churchill Hospital is approximately 5 per month. At the point when the decision has been made to offer radical prostatectomy as one of the potential treatment options, the trial will be introduced to the patient by the existing medical care team during their routine clinic visit, the PIS will be given and permission will be sought for the research team to approach them.

**Metastatic prostate cancer groups**

Subjects recruited will be men over the age of 18 with a diagnosis of prostate cancer, metastatic to one or more distant sites, who have demonstrated disease progression following anti-androgen treatment with either enzalutamide or abiraterone as a standard of care. Subjects will be identified in the outpatient setting by the existing medical care team during their routine clinic visit, the PIS will be given and permission sought for the research team to approach them. The number of metastatic prostate cancer patients demonstrating evidence of disease progression on enzalutamide is approximately 2 per month at the Oxford Churchill Hospital.

### 5.2. Informed consent

All patients will sign and date the informed consent form before any study-specific procedures are performed. The information sheet will be made available to the patient at least 24 hours prior to the screening visit. The patient will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The patient may withdraw from the study at any time
- The patient is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- The study involves research of an investigational vaccine and checkpoint inhibitor
- The study involves delaying the patient's surgery by approximately 2 months
- There may be no direct benefit for participating

The aims of the study and all tests to be carried out will be explained. The patient will be given the opportunity to ask about details of the study, and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date the consent form, which will also be signed and dated by the investigator. One copy will be given to the partiicpant, one copy will be placed in the study file, and the original will be placed in their medical notes.

### 5.3. Inclusion and Exclusion Criteria

**Inclusion Criteria - Radical Prostatectomy groups**

The participant must satisfy all the following criteria to be eligible for the study:

- Histologically confirmed adenocarcinoma of the prostate
- Clinically localised or locally advanced disease deemed operable by the treating consultant urological surgeon *i.e.:*
    - Gleason score ≤ 7
    - Local tumour stage ≤T3c and deemed operable
    - No evidence of metastases (Nx/N0 and Mx/M0)
    - No evidence of high grade Gleason 5 disease
    - PSA ≤ 20 ng/ml
- Scheduled for and considered fit for radical prostatectomy
- Absence of any indication to perform urgent surgery that would not allow completion of the study interventions prior to radical prostatectomy

**Inclusion Criteria - Metastatic prostate cancer groups**

The participant must satisfy all the following criteria to be eligible for the study:

- Histologically confirmed adenocarcinoma of the prostate cancer. Note, any Gleason grade or primary tumour staging at diagnosis is permitted.
- Evidence of at least one distant metastasis based on MRI, CT, PET or bone scintigraphy.
- Established on and suitable to continue with androgen deprivation therapy (ADT) using any luteinizing hormone releasing hormone (LHRH) agonist. LHRH agonist therapy may include goserelin (Zoladex®), leuprorelin acetate (Prostap®) or any other licenced product in this class.
- On treatment with anti-androgen therapy using either abiraterone (Zytiga®) or enzalutamide (Xtandi®) and demonstrating evidence of disease progression at the time of enrolment, defined according Prostate Cancer Working Group 3 Criteria as either:
    - PSA progression as defined by a minimum of 3 rising PSA levels with an interval of ≥1 week between each assessment where the PSA value at screening should be ≥2 ng/mL o*r*;
    - progressive nodal or visceral disease *or*;
    - progressive bone metastases

  Upon enrolment in the study, the treatment with abiraterone or enzalutamide will be discontinued.
- An archival specimen of tumour tissue should be available; if not, an on-study fresh tumour biopsy must be obtained prior to the first study treatment.
- Patients who have recieved chemotherapy following progression on androgen-targeting therapies are eligibile
- Satisfactory functional status defined as Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 1

**Inclusion Criteria – All participants**

All participants must satisfy all the following criteria to be eligible for the study:

- Males aged over 18 years
- Any antineoplastic therapy, including Radium-223 and limited field radiotherapy must have been completed a minimum of 28 days prior to enrolment.
- Systemic antimicrobial therapy, for any indication, must have been completed a minimum of 7 days prior to enrolment.Baseline laboratory parameters obtained no more than 28 days prior to administration of the first study drug must meet the following criteria:

| Laboratory Index | Eligibility threshold |
|---|---|
| **Haematology** | |
| Haemoglobin | ≥ 80 g/L |
| White cell count | ≥ 2.0 x$10^9$/L |
| Neutrophils | ≥ 1.5 x$10^9$/L * |
| Lymphocytes | ≥ 0.5 x$10^9$/L |
| Platelets | ≥ 100 x$10^9$/L |
| **Clinical Biochemistry** | |
| Renal | |
| Creatinine Clearance | ≥ 40 ml/min by Cockcroft Gault formulation |
| Hepatobiliary | |
| Total Bilirubin | ≤ 1.5 ULN |
| ALT | ≤ 1.5 ULN |
| Amylase | ≤ 1.5 ULN |

* *unless established as stable, benign ethnic neutropaenia*

- On enrolment, the subject, or the subjects partner, must agree to continuously practice a reliable form of contraception during treatment and for a minimum of 5 months following the final administered dose of nivolumab (Opdivo®). Reliable forms of contraception are:
    - Male sterilisation (Vasectomy)
    - Barrier methods of contraception (condom with or without spermicide)
    - Established use of oral, injected or implanted hormonal contraceptives.
    - Placement of an intrauterine device (IUD) or intrauterine system (IUS).
    - Female sterilisation (Tubal occlusion) or Total abdominal hysterectomy
    - Complete abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence, natural family planning and withdrawal are not acceptable methods of contraception.
- Subject is able and willing in the Investigator's opinion to comply with all study requirements
- Subject is able to provide written informed consent

**Exclusion Criteria – All participants**

The patient may not enter the study if any of the following apply:

- Any prior diagnosis or clinical suspicion of autoimmune disease including, but not limited to, systemic lupus erythematosus, inflammatory bowel disease, vasculitis, Grave's disease and type I diabetes mellitus. Note, subjects with known pre-existing clinically silent positive autoantibodies should not be included (although these will not be assayed at screening unless indicated)
- History of allergic disease or reaction likely to be exacerbated by any component of the vaccine, e.g. egg products

- Other prior malignancy with an estimated ≥ 30% chance of relapse within 2 years. Allowed recent cancers include (but are not limited to) non-melanomatous skin cancer and superficial bladder cancer
- Participation in another research study involving an investigational product or investigational surgical procedure in the 30 days preceding enrolment, or planned use during the study period
- Any prior exposure to checkpoint inhibitor drugs including anti-PD-1, anti-PD-L1, or anti-CTLA-4 monoclonal antibodies or any prior treatment with investigational vaccines
- Administration of immunoglobulins and/or any blood products within the one month preceding the planned administration of the study drugs
- Seropositive for hepatitis B surface antigen (HBsAg)
- Seropositive for hepatitis C virus (antibodies to HCV)
- Any confirmed or suspected immunocompromised state, including but not limited to, asplenia, HIV infection, a history of atypical, recurrent or severe infections, or, regular use (> 14 days) of systemic immunosuppressant medication within the past 6 months (except that lower dosage of prednisolone (< 11 mg per day) are allowable)
- Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema
- History of anaphylaxis in relation to vaccination or any clinically significant allergic disease likely to be exacerbated by any component of the vaccine or checkpoint inhibitor preparations, as listed in the Investigator's Brochure and SmPC
- Confirmed or suspected recreational drug use or harmful drinking in the last 5 years
- History of a serious psychiatric condition or other circumstances that may be associated with impaired mental capacity
- Evidence of significant clinical disorder or laboratory finding which in the opinion of the investigating physician increases the level of risk to the patient, limits the ability of the patient to participate in the study or impairs interpretation of the study data

**Exclusion Criteria – Metastatic prostate cancer groups**

The patient may not enter the metastatic prostate cancer arm of the study if:

- The treating oncologist or urological cancer MDT estimates a subject's life expectancy to be ≤ 6 months
- Any active, previously treated, or suspected intracranial or leptomeningeal metastases.

### 5.4. Concurrent therapies

All concomitant medications and interventions at enrolment and during follow up must be recorded in the study CRF.

**Permitted treatments**

There are no restrictions on the use of the following therapies:

- Treatment with alpha blockers (e.g. doxazosin, tamsulosin or terazosin) or anticholinergic preparations (e.g. oxybutynin)) for relief of lower urinary tract symptoms.
- Non-systemic corticosteroids (i.e. topical, ocular, inhaled, intranasal, and intra-articular preparations) for non-autoimmune disorders are permitted throughout the study.
- Palliative (limited-field) radiotherapy for control of pain metastatic bone pain is permitted, as clinically indicated, throughout the study.

**Restricted Treatments**

The following therapies and interventions are restricted during the study:

- Systemic corticosteroids are permitted when in conjunction with abiraterone therapy at a dose ≤ 10mg oral prednisolone daily (or equivalent).
- Systemic corticosteroids >10mg oral prednisolone daily (or equivalent) are permitted in the management of immune-related checkpoint inhibitor toxicity only, according to site-specific treatment algorithms.
- Non-investigational vaccines for the prevention of seasonal or travel-associated infections should be undertaken before enrolment wherever possible. Vaccines within the first 6 months of study therapy should be approved by the Investigators in view of the potentially elevated risk of autoimmune reactivity.

### 5.5. Delay and discontinuation cirteria

**Discontinuation rules**

Treatment with study vaccines and checkpoint inhibitors should be permanently discontinued under the following circumstances:

- On withdrawal of informed consent i.e. the subjects request to discontinue
- Any immune-related checkpoint inhibitor toxicity ≥ Grade 3 in severity in the following organ systems:
    - Dermatological
    - Gastrointestinal, hepatic or hepatobiliary
    - Respiratory
    - Renal
    - Endocrine
    - Neurological (apart from headache)
    - Haematological
- Any infusion reaction of ≥ Grade 3 severity

- Any significant clinical disorder, laboratory finding or adverse event, in the judgment of the Investigator makes it unsuitable for the participant to continue study treatment.

**Dose delay criteria**

Treatment with study vaccine and / or checkpoint inhibitor should be delayed under the following circumstances:

- If the subject has a temperature of > 37.5 °C.
- Any immune-related checkpoint inhibitor toxicity of < Grade 3 in severity, until symptoms have resolved and management with corticosteroids (if indicated) is complete
- Any grade 2 electrolyte disturbance (i.e. abnormalities in serum sodium, potassium, calcium or magnesium), until appropriate correction / replacement has been initiated
- Any significant clinical disorder, laboratory finding, adverse event or intercurrent illness which in the judgment of the Investigator makes it unsuitable for the participant to proceed with treatment.

### 5.6. Withdrawal criteria

Subjects may be withdrawn from the study early:

- By withdrawing voluntarily
- On the decision of the Investigator
- On the advice of the local safety monitor (LSM)

The Investigator may withdraw the subject for any of the following reasons:

- Any adverse event which results in inability to comply with study procedures
- Ineligibility either arising during the study or retrospectively (having been overlooked at screening)
- Evidence of significant change in disease status such that an alternative care pathway becomes indicated, for example the requirement for salvage radiotherapy following prostatectomy or transition to palliative care in participants with metastatic disease.
- Significant protocol deviation
- Subject non-compliance with study requirements
- Loss to follow up (applies to a subject who does not return for protocol study visits, is not reachable by telephone or other means of communication and/or is not able to be located).

The reason for withdrawal will be recorded in the CRF. If the subject is withdrawn due to an AE, the Investigator will arrange for appropriate specialist management or follow up visits or

telephone calls until the AE has resolved or stabilised. Withdrawn participants will not be replaced.

If a subject withdraws from the study, data and blood samples collected before their withdrawal from the trial will be used/stored unless the subject specifically requests otherwise.

### 5.7. Interim monitoring and study stopping rules

An independent Data and Safety Monitoring Committee (DSMC) will be appointed to review the accumulating data and provide advice on whether the accumulating data from the trial justify continuing recruitment of further patients or further follow-up (see also section 8.7). The first review will take place after the first 6 patients (3 patients with intermediate risk disease and 3 metastatic patinets) complete the study treatment and have reached the 24-week timepoint for efficacy assessment, unless there is a need to convene the Committee earlier due to the safety concerns. If a decision is made to continue the study, the DSMC will advise on the frequency of future reviews of the data on the basis of accrual and event rates.

## 6. TREATMENT OF STUDY SUBJECTS

### 6.1. Study visits

The study visits and administration of investigational therapies will be undertaken by an adequately qualified and trained member of the team of investigators. Each visit is assigned a time point and a window period, within which the visit will be conducted. These can be found in the schedule of attendances (Appendix 1).

**Screening visit**

All potential participants will have a screening visit, which may take place up to 4 weeks prior to the first study procedure. Informed consent will be taken at screening prior to any study-specific procedures being carried out, as described in section 5.2. If consent is obtained, the screening procedures indicated in the schedule of attendances will be undertaken. A baseline medical history, physical examination and assessment of performance status will be performed. Vital signs will be recorded and urinalysis will be performed. Inclusion and exclusion criteria will be checked. Blood will be drawn for clinical haematology and biochemistry, HIV, Hepatitis B and Hepatitis C serology, PSA, exploratory biochemistry and ctDNA. Subjects will be counseled by one of the investigators prior to HIV, Hepatitis B and Hepatitis C testing.

Potential subjects will be informed that blood samples, as well as the surgical specimens from radical prostatectomy and archival biopsy samples that are collected as standard of care or newly obtained on-study tumour biopsy samples will be used for research purposes. They will be informed that after study investigations are completed, any surplus samples of blood (and/or surgical or biopsy material) may be stored for possible future ethically approved research,

including for example exploratory immunology, and genotypic testing of genetic polymorphisms potentially relevant to vaccine immunogenicity. Subjects will be able to decide if they permit such future use of any unused or surplus samples. If a subject elects not to permit this, all of that subject's leftover samples will be discarded at the end of the study to meet Good Clinical Practice (GCP) and regulatory requirements.

The eligibility of the subject will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. If eligible, a week 0 enrolment visit will then be scheduled.

**Subsequent Visits**

At each subsequent study visit the ongoing eligibility of the volunteer will be reviewed. Vital signs will be recorded, urinalysis performed, ECOG performance status assessed and if necessary, a medical history and physical examination may be undertaken to determine any relevant interval changes in the participants health. In the event of new clinical or laboratory findings, study procedures involving administration of investigational vaccines or intravenous checkpoint inhibitors may be postponed according to criteria in Section 5.5. All blood tests should be taken prior to administration of any study therapies.

**Group 1**

**Day 0: Enrolment, ChAdOx1.5T4 priming vaccination visit**

Subjects will not be considered enrolled in the study until they have received the first study vaccination with ChAdOx1.5T4 on Day 0. Prior to vaccination, blood tests will be taken as detailed in the schedule of attendances (Appendix 1). Following intramuscular administration of the ChAdOx1.5T4 vaccine, the injection site will be covered with a sterile dressing and the patient will stay under observation, in case of immediate adverse events. Observations will be taken 60±15 min after vaccination and the sterile dressing removed and injection site inspected. A diary card, oral thermometer and tape measure will be given to each participant to solicit the following adverse events:

- Localised adverse events
  - Diameter of redness at vaccination site
  - Diameter of swelling at vaccination site
  - Pain, warmth and erythema at the vaccination site

- Systemic adverse events
  - Recorded temperature and pyrexial episodes
  - Feverishness
  - Headaches
  - Fatigue
  - Nausea
  - Generalised muscle aches

- Generalised joint aches

**Day 7: MVA.5T4 boost vaccination visit and first checkpoint inhibitor therapy**

- Prior to Day 7 study vaccination and checkpoint inhibitor therapy, the subject's diary card entries will be reviewed and any adverse events assessed. Blood tests will be taken as detailed in the schedule of attendances (Appendix 1). The MVA.5T4 vaccine will be administered intramuscularly and the injection site(s) will be covered with a sterile dressing. The participants will remain under observation and vital signs repeated 60±15 min after vaccination when the sterile dressing will be removed removed and injection site inspected. Participants will then undergo intravenous cannulation and intravenous infusion of nivolumab. The infusion will preferably commence a minimum of 60 minutes following administration of the MVA.5T4 vaccine, but if operationally required simply on the same day as immunisation. Participants will remain in the department for a further 30±15 minutes on completion of the infusion and vital signs repeated prior to departure. At the conclusion of this visit, the intravenous canula will be removed.

  Participants will be provided with an alert card, in line with the manufacturer's guidance, outlining the 'red flag' symptoms of immune-mediated checkpoint inhibitor toxicity and emergency contact details for the study medical team. An oral thermometer (if necessary) and new diary card will be given to each participant. In addition to the local and systemic AEs listed above, checkpoint inhibitor associated AEs will also be solicited in the following organ systems:

- Respiratory
- Dermatological
- Gastrointestinal
- Urological

**Group 2**

**Day 0: Enrolment, ChAdOx1.5T4 priming vaccination visit**

Subjects will not be considered enrolled in the study until they have received the first study vaccination with ChAdOx1.5T4 on Day 0. Prior to vaccination, blood tests will be taken as detailed in the schedule of attendances (Appendix 1). Following intramuscular administration of the ChAdOx1.5T4 vaccine, the injection site will be covered with a sterile dressing and the patient will stay under observation, in case of immediate adverse events. Observations will be taken 60±15 min after vaccination and the sterile dressing removed and injection site inspected. A diary card, oral thermometer and tape measure will be given to each participant to solicit the following adverse events:

- Localised adverse events
  - Diameter of redness at vaccination site
  - Diameter of swelling at vaccination site

- Pain, warmth and erythema at the vaccination site

- Systemic adverse events
  - Recorded temperature and pyrexial episodes
  - Feverishness
  - Headaches
  - Fatigue
  - Nausea
  - Generalised muscle aches
  - Generalised joint aches

**Week 4: MVA.5T4 boost vaccination visit and first checkpoint inhibitor therapy**

Prior to Week 4 study vaccination and checkpoint inhibitor therapy, the subject's diary card entries will be reviewed and any adverse events assessed. Blood tests will be taken as detailed in the schedule of attendances (Appendix 1). The MVA.5T4 vaccine will be administered intramuscularly and the injection site(s) will be covered with a sterile dressing. The participants will remain under observation and vital signs repeated 60±15 min after vaccination when the sterile dressing will be removed removed and injection site inspected. Participants will then undergo intravenous cannulation and intravenous infusion of nivolumab. The infusion will preferably commence a minimum of 60 minutes following administration of the MVA.5T4 vaccine, but if operationally required simply on the same day as immunisation. Participants will remain in the department for a further 30±15 minutes on completion of the infusion and vital signs repeated prior to departure. At the conclusion of this visit, the intravenous canula will be removed and participants will be provided with an alert card, in line with the manufacturer's guidance, outlining the 'red flag' symptoms of immune-mediated checkpoint inhibitor toxicity and emergency contact details for the study medical team. In addition to the local and systemic AEs listed above, checkpoint inhibitor associated AEs will also be solicited in the following organ systems:

- Respiratory
- Dermatological
- Gastrointestinal
- Urological

**Week 8: Second checkpoint inhibitor therapy**

Prior to Week 8, blood tests will be taken as detailed in the schedule of attendances (Appendix 1). The results of clinical haematology and biochemistry (excluding serum PSA and thyroid function tests) taken either on arrival or within the preceding 48 hours will be reviewed prior to proceding with checkpoint inhibitor therapy. Participants will undergo intravenous cannulation and intravenous infusion of nivolumab following the same administration and monitoring plan as on Week 4.

**Week 12: Second cycle of ChAd-MVA immunisation – ChAdOx1.5T4 boost and third checkpoint inhibitor therapy**

Prior to week 12, the subject's diary card entries will be reviewed and any adverse events assessed. Blood tests will be taken as detailed in the schedule of attendances (Appendix 1).The results of clinical haematology and biochemistry (excluding serum PSA and thyroid function tests) taken either on arrival or within the preceding 48 hours will be reviewed prior to administration of vaccine. Then the patients will undergo ChAdOx1.5T4 vaccination and nivolumab infusion, following the same administration and monitoring plan as on Week 4.

**Week 16: Second cycle of ChAd-MVA immunisation – MVA.5T4 boost**

Prior to week 16, the subject's diary card entries will be reviewed and any adverse events assessed. Blood tests will be taken as detailed in the schedule of attendances (Appendix 1).The results of clinical haematology and biochemistry (excluding serum PSA and thyroid function tests) taken either on arrival or within the preceding 48 hours will be reviewed prior to administration of vaccine. Then the patients will undergo MVA.5T4 vaccination, following the same administration and monitoring plan as on Day 0.

**All remaining visits – groups 1 and 2**

At all remaining follow up visits, blood tests will be taken as detailed in the schedule of attendances (Appendix 1). Vital signs will be recorded, urinalysis performed, ECOG performance status assessed and if necessary, a medical history and physical examination may be undertaken to determine any relevant interval changes in the participants health. Subjects are recommended to carry their clinical trial alert card, outlining the 'red flag' symptoms of immune-mediated checkpoint inhibitor toxicity and emergency contact details for the study medical team, for a minimum of 6 months after treatment on study.

**Telephone communications**

Patients will be telephoned by a research nurses on day 2-5 (48-120 hours) following vaccination and/or checkpoint inhibitor therapy to enquire about adverse events and provide further advice regarding completion of the diary card.

### 6.2. Additional study procedures

The procedures to be included in each visit are documented in the schedule of attendances in Appendix 1. Additional procedures or laboratory tests may be performed at the discretion of the Investigators, for example in the investigation of potential diease complications or treatment toxicities.

**Observations**

Vital signs measurements performed on each study visit are, heart rate, blood pressure, oxygen saturations and temperature. Weight and height will additionally be recorded at the screening visit. Vital sign measurements outside the normal range or deviating from the individual's baseline recording will be repeated, clinically evaluated and acted upon as guided by the study nursing and/or medical team.

**Sample collection**

Blood and urine will be collected for the following laboratory tests and processed at the Oxford University Hospitals NHS Foundation Trust and the Christie Hospital NHS Foundation Trust through an accredited NHS pathology service and disposed of after analysis .The following tests will be undertaken:

- **Haematology:** Full Blood Count and differential
- **Biochemistry:** Sodium, potassium, urea, creatinine, albumin, liver function tests (total bilirubin, ALT, ALP), calcium, magnesium, serum amylase, LDH
- **Serum glucose**
- **PSA blood test**
- **Diagnostic serology:** Hepatitis B surface antigen (HBsAg), Hepatitis C Antibody (HCV Ab), and HIV (HIV Ab).
- **Thyroid Function Tests:** Total triiodothyronine (T3) or free T3c, free thyroxine (T4) and thyroid stimulating hormone (TSH)
- **Clinical immunology:** Human Leukocyte Antigen (HLA) typing
- **Urinalysis** for blood, protein and glucose using dipstick tests

**Unprescribed blood tests – at the discretion of the local PI to maintain the welfare of the participant**

Prostate tumour tissue will be collected and processed at the Oxford University Hospitals NHS Foundation Trust and the Christie Hospital NHS Foundation Trust, through an accredited NHS pathology service as standard of care. This tissue will be made available for immunohistochemistry performed at the research laboratories (Oxford).

Research blood will be collected for the following assays performed at the research laboratories (Oxford University and University College London) or a central contract laboratory:

- **Exploratory Immunology***: Ex vivo* ELISPOT assays for interferon gamma; ELISA assays for antibody responses; flow cytometry assays, immunohistochemistry
- **Exploratory Biochemistry:** Four kallikrein peptidases blood test
- **Circulating tumour DNA (ctDNA)** and **circulating tumour cell (CTC)** quantification assays

Other tests, e.g. immunological assays including ELISPOT assays for other cytokines, T cell epitope mapping, RNA and DNA sequencing or other methods of analysis of genetic polymorphism

potentially relevant to the investigational products may also be performed at the discretion of the investigators.

**Multi-parametric Magnetic resonance imaging (mpMRI) – Group 1**

Participants in group 1 who are planned to undergo radical prostatectomy will undergo an mpMRI of the prostate and pelvis prior to enrolment on the study as part of standard care in the prostate cancer diagnostic pathway. The subjects who had a pelvic / prostate mpMRI of adequate quality before screening and enrolment will have a repeat on-study mpMRI to evaluate any post-treatment radiolographic change in the prostate. The on-study mpMRI should take place prior to radical prostatectomy and a minimum of 7 days after final study checkpoint inhibitor therapy, i.e. on or after study week 4 +7 days. Participants, whose pre-enrolment pelvis/prostate mpMRI is, in the opinion of the reporting radiologist, of low diagnostic value (for example, images are significantly degraded by artefact or post-biopsy haemorrhage), are not required to undergo a repeat on-study mpMRI as the baseline scan will not provide adequate comparator for post-treatment changes. Conventional three-planar MRI including T1 and T2 weighted DCE-MRI and diffusion-weighted sequences will be performed on a 1.5 T magnet. Areas of signal abnormality suspicious for malignancy within the prostate will be graded according to PIRADS criteria. Local T-stage disease identified on scans will be reported as per European consensus on prostate MRI[43]. The procedure will be performed at the Oxford University Hospitals NHS Foundation Trust a nd the Christie Hospital NHS Foundation Trust clinical sites.

**Tumour Assement by CT scan, bone scintigraphy, MR scan – Group 2**

Radiological tumour assessments will be performed at baseline (28 day sceening period), at 6 months and 12 months. The assessments will include a CT scan of chest, abdomen and pelvis and bone imaging, e.g. bone scans or body MRI. CT and MR imaging will be performed with contrast unless this is contraindicated for medical reasons.

The response to treatment in patients with measurable disease will be evaluated per Response Evaluation Criteria In Solid Tumours 1.1 (RECIST 1.1) and per immune-related response criteria (iRECIST).

## 7. ASSESSMENT OF SCIENTIFIC OBJECTIVES

### 7.1. Primary endpoints and outcome measures

- Incidence of adverse events, using the National Cancer Common Terminology Criteria (version 4) up to 12 months following completion of the study treatment. Adverse events

will be summarized by type and severity in tabular format. Fisher's exact test will be used to compare adverse events rates between study arms.

- Composite response rate for metatstatic PCa patients defined as one of the following:
  A. PSA response rate:
  - 24-week PSA response rate: Patients who achieve at least a 50% reduction in PSA at 24 weeks compared to baseline.
  - Maximal PSA response rate: Patients who achieve at least a 50% reduction in PSA at any time compared to baseline.

  B. Reduction of circulating tumor DNA following study treatment:
  - 24-week ctDNA response rate: Patients who achieve at least a 50% reduction in ctDNA at 24 weeks compared to baseline.
  - Maximal ctDNA response rate: patients who achieve at least a 50% reduction in ctDNA at any time compared to baseline.

  Changes in ctDNA from pre-treatment (date of screening/randomization) to post-treatment assessments at any time will be evaluated using a paired t-test. ctDNA will be quantified by digital PCR. Changes in ctDNA will be summarized in terms of means and standard deviations.

- For low- and intermediate-risk PCa patients, serum PSA kinetics secondary to study treatment as measured by rate of change before radical prostatectomy. Serum PSA levels will be measured by a standard clinical laboratory test at baseline and at pre-defined timepoints following study treatment as detailed in Appendix 1.

### 7.2. Secondary endpoints and outcome measures

- Development or increase in anti-5T4 cellular responses following study treatment two weeks post last vaccination and at any time-point on the study. Measurement of T cell responses will be done by IFNγ ELISPOT assay to measure T cell responses to tumour and control antigens. Descriptive statistical analysis will be conducted. Immune profile of the prostate (low- or intermediate-risk PCa patients only). Tumour-infiltrating immune cells in surgical specimens and pre-treatment archival diagnostic needle prostate biopsies will be compared. Immune cell density, phenotype and function and will be assessed by immunohistochemistry and digital image analysis of formalin fixed paraffin-embedded tumour tissue samples using a panel of cell surface and intracellular markers. Descriptive statistical analysis will be conducted.
- Radiographic progression-free survival rate as measured by MRI, CT scans or bone scintigraphy (metastatic PCa patients only). Fisher's exact test will be used to compare 6 and 12-month progression-free survival rates between study arms. In order to evaluate progression-free rate as a function of baseline time point, analysis will be conducted using

two different baseline values: date of randomization and one months post last study treatment.

- Overall survival will be assessed for each arm 6 and 12 months post enrolment using the Kaplan-Meier method and log-rank test for statistical significance (metastatic PCa patients only).

### 7.3. Other endpoints and outcome measures

The following exploratory analyses will be performed based on initial immunogenicity assessment and sample availability:

- Detailed analysis of cognate CD4+/CD8+ T cell function and memory phenotype may also be performed by multi-colour flow cytometry to simultaneously analyse surface markers and intracellular production of other cytokines (e.g. IL-2, IFNγ, TNFα). Frequencies of 5T4-specific immune responses will be summarised in tabular format for each study arm and for overall combined study. Descriptive statistical analysis will be conducted, and the continuous variables will be summarised using mean, standard deviation, median, minimum and maximum. Fisher's exact test will be used to compare the 5T4-specific T cell response rates between study arms.
- Up-regulation of PD-L1 expression in a primary tumour or metastatic lesion(s) following study treatment. Density of PD-L1 positive immune cells and epithelial cells will be assessed by immunohistochemistry and digital image analysis of matched tumour tissue specimens before and after study treatment. PD-L1 expression levels will be summarised in terms of means, standard deviations and ranges for each study arm.
- PSA related kallikreins level change secondary to study treatment. The level of kallikreins in the blood will be measured by a 4 kallikrein peptidases test[45]. Descriptive statistical analysis will be conducted.
- Change in number of circulating tumour cells secondary to study treatment may be assessed by CellSearch or Adnatest[44].
- Immunologic antigen spreading to other prostate associated antigens following study treatment (metastatic PCa patients only). The detection of antigen spread to other prostate associated antigens and the identification of specific antigens recognized will be analyzed descriptively by IFNγ ELISPOT assay or ELISA.

**Additional assays may include but are not limited to:**

- Treatment-induced cellular immune responses characterized by cultured ELISPOT assay
- Treatment-induced cellular immune responses characterized by T cell receptor sequencing
- Flow cytometry analysis of immune cells isolated from the blood and surgical specimens

- Measurement of anti-5T4 serum antibody levels by ELISA (using plasma from preparation of above PBMC sample)
- Profiles of gene expression in the blood and surgical specimens by RNA analysis
- DNA or RNA sequencing of blood and tumour specimens for identification of tumour-specific somatic mutations

## 8. SAFETY ASSESSMENT

### 8.1. Specification, timing and recording of safety parameters

At regular intervals (specified in section 6.1), the subjects will be assessed for the following safety parameters:

- General physical examination and vital signs
- Full blood count
- Urea and electrolytes
- Liver function tests
- Kidney function tests

The above will be recorded in the patients' case report form (CRF) by the relevant clinician or their nominee.

Reports of recorded events, along with a narrative from the Chief Investigator, will be submitted to the Safety Monitoring Committee after every 6 completed patients.

### 8.2. Assessment of Adverse Events

**Adverse Event (AE*)***

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

**Adverse Drug Reaction (ADR)**

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

**Unexpected Adverse Reaction (UAR*)***

An adverse reaction the nature and severity of which is not consistent with the product information set out in:

- the summary of product characteristics (SmPC) for products with a marketing authorisation, i.e. nivolumab.
- the Investigator's Brochure (IB) for any other investigational product, i.e. ChAdOx1.5T4, MVA.5T4

**Serious Adverse Event (SAE)**

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the vaccine:

- Death (apart from death where prostate cancer is certified as the underlying cause)
- Life-threatening event (i.e., the volunteer was, in the view of the Investigators, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions)
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation or prolongation of existing hospitalisation
- An important medical event (that may not cause death, be life threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the participant and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.
- Congenital anomaly or birth defect

**Serious Adverse Reaction (SAR)**

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting Investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

**Suspected Unexpected Serious Adverse Reaction (SUSAR)**

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB or SmPC.

### 8.3. Causality assessment

For every AE, an assessment of the relationship of the event to the administration of the study treatments will be undertaken by the CI-delegated clinician. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine or checkpoint inhibitor. Adverse events are considered unrelated if they fall into the category of no relationship or unlikely relationship. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine or checkpoint inhibitor administration; and the known biology of the vaccine and checkpoint inhibitor therapy (Table 2). Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to study treatments will be considered and investigated.

**Table 2. Guidelines for assessing the relationship of vaccine administration to an AE**

| | | |
|---|---|---|
| 0 | **No Relationship** | No temporal relationship to study product ***and***<br>Alternate aetiology (clinical state, environmental or other interventions); ***and***<br>Does not follow known pattern of response to study product |
| 1 | **Unlikely relationship** | Unlikely temporal relationship to study product; ***and***<br>Alternative aetiology likely (clinical state, environmental or other interventions); ***and***<br>Does not follow known typical or plausible pattern of response to the vaccine |
| 2 | **Possible** | Reasonable temporal relationship to study product; ***or***<br>Event not readily produced by clinical state, environmental or other interventions; ***or***<br>Similar pattern of response to that seen with other vaccines |
| 3 | **Probable** | Reasonable temporal relationship to study product; ***and***<br>Event not readily produced by clinical state, environment, or other interventions ***or***<br>Known pattern of response seen with other vaccines |
| 4 | **Definite** | Reasonable temporal relationship to study product; ***and***<br>Event not readily produced by clinical state, environment, or other interventions; ***and***<br>Known pattern of response seen with other vaccines |

### 8.4. Severity assessment

The severity of adverse events will be assessed according to the NIH Common Terminology Criteria for Adverse Events version 4.0. The generic severity scale is shown in Table 3. NIH CTCAE v4.0 severity scales specific to selected important immune mediated checkpoint inhibitor toxicities are included in Appendix 2.

**Table 3. Generic scale for assessing the severity of AEs (CTCAE v4, NIH NCI)**

| Grade | Description | Definition |
|---|---|---|
| **1** | **Mild** | Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| **2** | **Moderate** | Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*. |
| **3** | **Severe** | Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**. |
| **4** | **Life-threatening** | Urgent intervention indicated |
| **5** | **Death** | Death related to AE |

*Activities of Daily Living (ADL). Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Participants will be asked to record their temperature on a daily basis, as well as the size of any swelling or redness at the vaccination site and the symptoms that can be associated with checkpoint inhibitor therapy. For these solicited AEs participants will be instructed on self assessment of symptom severity based on the NIH CTCAE criteria above. The measured local reaction and daily temperature recording will be numerically graded in severity according to Table 4:

**Table 4. Scale for assessing the severity of solicited AEs with numerical values**

| Adverse Event | Grade | Intensity |
|---|---|---|
| Erythema at injection site | 1 | ≥3 - ≤50 mm |
| | 2 | >50 - ≤100 mm |
| | 3 | >100 mm |
| Swelling at injection site | 1 | >1 - ≤20 mm |
| | 2 | >20 - ≤50 mm |
| | 3 | >50 mm |
| Fever (oral) | 1 | 37.6°C - 38.0°C |
| | 2 | 38.1°C – 39.0°C |
| | 3 | >39.0°C |

Physical observations taken at rest will be graded according to criteria in Table 4 and acted upon based on clinical context and prior history of the individual.

| | Grade 1 (mild) * | Grade 2 (moderate) ** | Grade 3 (severe) *** |
|---|---|---|---|
| Tachycardia (bpm) | 101 – 115 | 116 – 130 | >130 or |
| Bradycardia (bpm) | 50 – 54 | 40 – 49 | <40 |
| Systolic hypertension (mmHg) | 141 - 159 | 160 – 179 | ≥180 |
| Diastolic hypertension (mmHg) | 91 - 99 | 100 – 109 | ≥110 |
| Systolic hypotension (mmHg) | 85 – 89 | 80 – 84 or | <80 or |

### 8.5. Reporting procedures

**Reporting Procedures for All Adverse Events**

After an eligible participant is enroled onto the study on receipt of the first study procedure, all AEs occurred within 28 days of study treatment and only grade 3 AEs outside this time window will be reported in the study case report forms (CRF), whether or not attributed to study procedures. AEs may be observed by the Investigators or reported by the participant either spontaneously or during diary card monitoring. All AEs that result in a subject's withdrawal from the study will be followed up, with their consent until a satisfactory resolution occurs, or until a non-study related causality is assigned.

**Reporting Procedures for Serious Adverse Events**

All serious adverse events (SAEs) occurring after an eligible participant is enrolled onto the study on receipt of the first study intervention, will be reported throughout the trial period. All SAEs will be documented accurately and notification deadlines respected. SAEs will be reported on an SAE Report Form to the Sponsor or the Sponsor's delegated representative (Chief Investigator) and the Study Medical Monitor (SMM), according to the relevant SOP within 24 hours of the Investigator becoming aware of them. SAEs will not normally be reported immediately to the ethical committee(s) and MHRA unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, in the opinion of the Chief Investigator and/or SMM. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report. Any updates to relevant information concerning the SAE that becomes available after the SAE initial report has been sent (outcome, precise history, results of investigations, copy of hospital report, etc) will be forwarded in a timely manner using the SAE Update Report Form. For all deaths, any available post-mortem reports and relevant medical reports will be made available for reporting to the relevant authorities. The Sponsor, or a medically qualified investigator delegated by the

Sponsor, is responsible for commencing, maintaining and completing the SAE Sponsor Report Form. The anonymity of subjects shall be respected when forwarding this information.

**Expectedness**

Expectedness will be determined according to the Investigators' Brochure (ChAOx1.5T4 and MVA.5T4 vaccines) or the SmPC (nivolumab).

**Reporting Procedures for SUSARs**

The Chief Investigator will report all SUSARs to the MHRA and ethical committee(s) within required timelines (15 days for all SUSARs, unless life threatening in which case 7 days, with a final report within a further 8 days (total 15). The Chief Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants. All SUSARs and deaths occurring during the study will be reported to the Sponsor.

### 8.6. Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination, urinalysis or blood tests at any point in the study will be assessed as to their clinical significance. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence and further diagnostic investigations carried out to determine any underlying pathology. If a result or finding is persistent or clinically significant, the subject will be informed and appropriate medical care arranged as appropriate and with the permission of the subject. Decisions to exclude the subject from enrolling in the trial or to withdraw a subject from the trial will be at the discretion of the Investigators, following procedures for adverse events as described above.

### 8.7. Data and Safety Monitoring Committee

A Data and Safety Monitoring Committee (DSMC) will be appointed to provide real-time safety oversight. It will consist of a consultant oncologist, a consultant physican with experience of vectored vaccine trials, a further member with statistical expertise and up to two further members with trial expertise, all of them independent of the study. The DSMC will review all SAEs at the meetings that will be convened following each interim safety report and a narrative from the Chief Investigator at the timepoints as below:

An interim safety report of all recorded events, along with a narrative from the Chief Investigator, will be submitted to the Data and Safety Monitoring Committee for review after the first 6 patients (3 patients with intermediate risk disease and 3 metastatic patients) complete the study treatment and have reached the 24-week timepoint for efficacy assessment. An updated report to the Data and Safety Monitoring Committee, covering all enrolled participants to date will then be triggered when every 6th subsequent participant has reached 24-week timepoint for efficacy assessment.

The DSCM has the power to terminate the study if deemed necessary following a study-related SAE.

The DSMC chair will be notified of SAEs if deemed possibly, probably or definitely related to study-related interventions within 24 hours of the investigators' being aware of their occurrence.

The chair of the DSMC will be contacted for advice and independent review in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to the study vaccine.
- Any other situation where the Investigator feels independent advice or review is important.

### 8.8. Development Safety Update Reports

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee, Host NHS Trust and Sponsor.

## 9. STATISTICS

### 9.1. Sample size

This is a non-randomised multi-centre phase I/II study. The trial will be open label; given the objective nature of the measurements, this is unlikely to introduce significant bias in assessing key outcomes. Study ID will be assigned to participants in the order in which they are enrolled in the trial. The correspondence between the subject study ID and the treatment number/arm allocation will be noted down in the CRF and subsequently entered in the OpenClinica system.

The total number of subjects enrolled to the study will be 36: 12 subjects with low- or intermediate-risk localised or locally advanced prostate cancer and 24 subjects with metastatic disease.

In this adaptive design study, the sample size of each patient cohort is chosen to allow an initial descriptive report of the safety of the vaccine in combination with checkpoint blockade. In this study, we are targeting a 25% response rate to the vaccine in combination with PD-1 blockade in mCRPC patients. The sample size of 24 patients in Group 2 will require that 6 out of 24 treated patients will have a complete response, partial response or stable disease. The patient cohorts recruited to the KEYNOTE-199 study will serve as a control group for ADVANCE and 11% overall response rate to PD-1 blockade as a monotherapy will be compared with the response rate to the vaccine in combination with anti-PD-1 treatment in the ADVANCE patients.

The 12 patients in the surgical group will be compared for response rate to the 26 surgical subjects treated with the same vectored vaccines but no anti-PD1 in the VANCE trial. None of the 26 patients in VANCE showed a significant reduction in PSA levels during that trial. The

sample size of 12 is chosen to allow an initial descriptive comparison of response rates with and without anti-PD1 to help power any potential future trial.

### 9.2. Analysis

A detailed statistical analysis plan (SAP) will be prepared and finalised with the study statistician before the first interim data analysis.

Data analysis of the following endpoints: safety, efficacy and immunogenicity will be assessed as detailed in Section 7. Safety data will be presented according to frequency, severity and duration of adverse events. Efficacy analysis will be primarily based on evaluation of ctDNA reduction, PSA response rate, radiographic progression-free and overall survival between metastaic PCa study arms. Immunogenicity will consist of descriptive analysis of cellular and humoral immune responses to the vaccine transgene in the circulation, focusing on the magnitude and qualities of T cell responses.

Exploratory analyses may also include, but are not limited to, the analyses described below:
Factors predictive of treatment benefit: baseline demographic, tumour status, haematological or immunological variables may be included as interactions with treatment in logistic, longitudinal or proportional hazards models of efficacy endpoints in order to assess their value as factors predictive of treatment benefit.

Factors predictive of immune response: baseline demographic, tumour status, haematological or immunological variables may be included as explanatory variables in models of qualitative and quantitative immunological endpoints in order to assess their value as factors predictive of immune response.

## 10. DATA HANDLING AND RECORD KEEPING

### 10.1. Data Handling

The Chief Investigator will have overall responsibility for receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study, but these tasks may be delegated to other Investigators. The data will be entered into the participants' CRFs which will be in a paper and/or electronic format using the OpenClinica™ database. This includes safety data, laboratory data (both clinical and immunological) and outcome data. Data are entered in a web browser on PCs and then transferred to the OpenClinica Database by encrypted (https) transfer. Electronic data will be stored on secure servers which are outsourced by OpenClinica™.

### 10.2. Record Keeping and Access to Data

The Investigators will maintain appropriate medical and research records for this trial, in compliance with the requirements of the Medicines for Human Use (Clinical Trial) Regulations 2004, ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of participants. Participants will only be referred to by their ID number on all study data. The Principal Investigator, co-investigators and clinical research nurses will have

access to records. The Investigators will permit authorized representatives of the sponsor, relevant NHS Trust, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. All source data and subject CRFs in paper and electronic form will be stored securely at the clinical sites in locked cupboards and secure servers respectively, and anonymised prior to archiving. The study data will be stored for a minimum of 10 years after publication or public release.

### 10.3. Source Data and Case Report Forms (CRFs)

All protocol required information will be collected in CRFs designed by the Investigators. All source documents will be filed in the patient medical records at the relevant site. Source documents are original documents, data, and records from which the subject's CRF data are obtained. For this study, these will include, but are not limited to, subject consent form, blood results, clinician response letters, laboratory records, diary cards and alert cards. In the majority of cases, CRF entries will be considered source data where the CRF is the site of the original recording.

### 10.4. Data Protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

## 11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

**Investigator procedures**

Approved SOPs will be used at all clinical and laboratory sites.

**Monitoring**

Regular Monitoring will be performed according to ICH Good Clinical Practice (GCP). Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities

**Modification to protocol**

No substantial amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any substantial amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for a substantial amendment, it will be produced

in writing by the Chief Investigator and will be made a formal part of the protocol following ethical and regulatory approval.

A non-substantial amendment is one that modifies administrative and logistical aspects of a protocol but does not affect the subjects' safety, the objectives of the trial and its progress. A non-substantial amendment does not require ethical or regulatory approval.

**Protocol deviation**

Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

**Audit & inspection**

The sponsor may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004. The sponsor will assist in any inspections and will formally respond to the MHRA as part of the inspection procedure.

**Serious Breaches**

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach.

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

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

In the event that a serious breach is suspected, the sponsor must be contacted within 1 working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory Authority and the NHS host organisation within 7 calendar days.

**Trial Progress**

The progress of the trial will be overseen by the Chief Investigator or a nominated person.

## 12. ETHICAL AND REGULATORY CONSIDERATIONS

### 12.1.Good Clinical Practice

This trial will be conducted in accordance with the principles of the Declaration of Helsinki as agreed by the World Medical Association General Assembly (2013), ICH Good Clinical Practice

(GCP), the requirements of the Medicines for Human Use (Clinical Trial) Regulations 2004, and local regulatory requirements.

### 12.2. Ethical Review

A copy of the protocol, proposed informed consent form and other written subject information will be submitted to an independent EC for written approval. The Investigators will submit and, where necessary, obtain approval from the EC for all subsequent substantial amendments to the protocol and informed consent document (and previously approved by the sponsor). The Investigators will notify serious deviations from the protocol or SAEs occurring at the site to the Sponsor and will notify the EC of these in accordance with local procedures.

### 12.3. Informed Consent

Written informed consent will be obtained at screening as detailed in section 5.2.

### 12.4 . Surgery delay

The risk associated with postponing surgery by approximately 6 weeks is considered very small as detailed in section 4.6.2. The investigators will discuss this issue with the potential participants in detail. The patients who require an operation without delay are not eligible for this study.

### 12.5. Approvals

The protocol and related documents will be submitted to the Sponsor, an appropriate Research Ethics Committee (REC), the Health Research Authority (HRA) and host institution(s) for approval and the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

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

### 12.6. Reporting

The CI shall submit an Annual Progress report to the REC Committee, host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

### 12.7. Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s), with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only

accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

## 13. FINANCE AND INSURANCE

### 13.1. Financing

The study will be funded by the IMPROVE EC FP7 grant.

### 13.2 Insurance

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

### 13.3. Compensation

Subjects will be compensated for their time and for the inconvenience caused by procedures. The amount of compensation will be stated in the subject's information sheet. If subjects withdraw without completing the study then they are entitled to receive a pro-rata amount of compensation. If subjects need to have additional visits (e.g. for repeats of safety bloods) then additional compensation can be given. Where participants incur higher travel costs due to additional unanticipated visits then appropriate compensation will be provided.

## 14. PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the EC FP7 grant. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

## APPENDIX 1: SCHEDULE OF ATTENDANCE AND STUDY PROCEDURES

### Group 1: Participants scheduled for Radical Prostatectomy

| Visit/Telephone Call Number | S | V1 | *T1* | V2 | *T2* | V3 | V4 | | V5 | V6 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time from enrolment (weeks +days)** | **≤ -4** | **0** | *0+2* | **1** | *1+2* | **2** | **5** | **≥5** | **16** | **24** |
| **Window (days) *** | **0** | | *=3* | **+3** | *+3* | **+3** | **±7** | **+14** | **±7** | **±7** |
| Informed consent | X | | | | | | | | | |
| Review of inclusion criteria | X | X | | | | | | | | |
| Review of exclusion criteria | X | X | | X | | X | X | | X | X |
| Medical history | X | (X) | | (X) | | (X) | (X) | | (X) | (X) |
| Physical examination | X | | | | | | | | | |
| Targeted physical examination | | (X) | | (X) | | (X) | (X) | | (X) | (X) |
| Retrieval of archival diagnostic biopsy | | X** | | | | | | | | |
| Fresh biopsy (if required) | | X** | | | | | | | | |
| Telephone call | | | X | | X | | | | | |
| Recording vital signs | X | X | | X | | X | X | | X | X |
| ECOG Status | X | | | | | | | | X | X |
| ChAdOx1.5T4 vaccination | | **X** | | | | | | | | |
| MVA.5T4 vaccination | | | | **X** | | | | | | |
| Nivolumab infusion | | | | **X** | | | | | | |
| Radical Prostatectomy (mpMRI – optional) | | | | | | | | X | | |
| Assessment & recording of adverse events | | X | X | X | X | X | X | | X | X |
| Patient alert card provided | | | | X | | | | | | |
| Diary card provided | | A | | C | | | | | | |
| Diary card returned | | | | X | | | X | | | |
| Haematology[1] | 2 | 2 | | 2 | | 2 | 2 | | 2 | 2 |
| Biochemistry[1] | 4 | 4 | | 4 | | 4 | 4 | | 4 | 4 |
| Serum glucose[1] | 2 | 2 | | 2 | | 2 | 2 | | 2 | 2 |
| Thyroid function tests[1,2] | 2 | 2 | | | | | 2 | | | 2 |
| PSA[1] | *5* | 5 | | 5 | | 5 | 5 | | 5 | 5 |
| Urinalysis | X | X | | X | | X | X | | X | X |
| HBVsAg, HIV &HCV Ab[1] | 5 | | | | | | | | | |
| Human leukocyte antigen typing (ml) | | 5 | | | | | | | | |
| Exploratory immunology (ml) | | 50 | | 50 | | 50 | 50 | | 50 | 50 |
| Exploratory biochemistry (ml) | | 2 | | 2 | | 2 | 2 | | 2 | 2 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Total blood volume per visit (ml)[1] | 20 | 70 | | 65 | | 65 | 67 | | 67 | 67 |
| **Cumulative blood volume ≈ 421ml[1]** | | | | | | | | | | |

* Window is based on preceeding IMP administration

** Window is –28 days

[1] Volume can vary by site according to local practices

[2] Total triiodothyronine (T3) or free T3c, free thyroxine (T4) and thyroid stimulating hormone (TSH)

**Group 2: Participants with Metastatic Prostate Cancer**

**Table of events (part 1 of 2)**

| Visit/Telephone Call Number | S | V1 | *T1* | V2 | V3 | *T2* | V4 | V5 | *T3* | V6 |
|---|---|---|---|---|---|---|---|---|---|---|
| Time from enrolment (weeks+days) | ≤ -4 | 0 | *0+2* | 2 | 4 | *4+2* | 5 | 8 | *8+2* | 9 |
| Window (days)* | | | *+3* | ±2 | ±2 | *+3* | +3 | ±2 | *+3* | +3 |
| Informed consent | X | | | | | | | | | |
| Review of inclusion/exclusion criteria | X | X | | (X) | X | | (X) | X | | (X) |
| Medical history | X | (X) | | (X) | (X) | | (X) | (X) | | (X) |
| Physical examination (if required) | X | (X) | | (X) | (X) | | (X) | (X) | | (X) |
| Retrieval of archival diagnostic biopsy | | X[1] | | | | | | | | |
| Fresh biopsy (if required) | | X[1] | | | | | | | | |
| Telephone Call | | | X | | | X | | | X | |
| Recording vital signs | X | X | | X | X | | X | X | | X |
| ECOG Status | X | | | | X | | | X | | |
| ChAdOx1.5T4 vaccination | | X | | | | | | | | |
| MVA.5T4 vaccination | | | | | X | | | | | |
| Nivolumab infusion | | | | | X | | | X | | |
| Assessment & recording of adverse events | | X | X | X | X | X | X | X | X | X |
| Radiological assessment (bone scintigraphy, CT scan, MR scan) | | X[1] | | | | | | | | |
| Patient alert card provided | | X | | | | | | | | |
| Diary card provided | | B | | | C | | | D | | |
| Diary card returned | | | | X | | | | X | | |
| Haematology[2] | 2 | 2 | | 2 | 2 | | 2 | 2 | | 2 |
| Biochemistry[2] | 4 | 4 | | 4 | 4 | | 4 | 4 | | 4 |
| Serum glucose[2] | 2 | 2 | | 2 | 2 | | 2 | 2 | | 2 |
| Thyroid function tests[2-3] | 2 | 2 | | | | | | 2 | | |
| PSA[1] | 5 | 5 | | 5 | 5 | | 5 | 5 | | 5 |
| Urinalysis | X | X | | X | X | | X | X | | X |
| HBVsAg, HIV &HCV Ab[1] | 5 | | | | | | | | | |
| Human leukocyte antigen typing (ml) | | | | 5 | | | | | | |
| Exploratory immunology (ml) | | 30 | | 40 | | | 40 | | | 40 |
| CTC (ml) | | 8 | | | | | | 8 | | |
| ctDNA (ml) | | 8 | | | 8 | | | 8 | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Total blood volume per visit (ml)[2] | 20 | 61 | | 58 | 21 | | 53 | 31 | | 53 |

* Window is based on preceeding IMP administration

[1] Window is –28 days

[2] Volume can vary by site according to local practices

[3] Total triiodothyronine (T3) or free T3c, free thyroxine (T4) and thyroid stimulating hormone (TSH)

## Group 2: Participants with Metastatic Prostate Cancer
## Table of events (part 2 of 2)

| Visit/Telephone Call Number | V7 | *T4* | V8 | V9 | *T5* | V10 | V11 | V12 | V13 |
|---|---|---|---|---|---|---|---|---|---|
| Time from enrolment (weeks+days) | 12 | *12+2* | 13 | 16 | *16+2* | 17 | 24 | 36 | 48 |
| Window (days)* | ±7 | *+3* | +3 | ±2 | *+3* | +3 | ±7 | ±14 | ±14 |
| Informed consent | | | | | | | | | |
| Review of inclusion/exclusion criteria | X | | (X) | X | | (X) | (X) | (X) | (X) |
| Medical history | (X) | | (X) | (X) | | (X) | (X) | (X) | (X) |
| Physical examination (if required) | (X) | | (X) | (X) | | (X) | (X) | (X) | (X) |
| Telephone Call | | X | | | X | | | | |
| Recording vital signs | X | | X | X | | X | X | X | X |
| ECOG Status | X | | | X | | | X | X | X |
| ChAdOx1.5T4 vaccination | X | | | | | | | | |
| MVA.5T4 vaccination | | | | X | | | | | |
| Nivolumab infusion | X | | | | | | | | |
| Assessment & recording of adverse events | X | X | X | X | X | X | X | X | X |
| Radiological assessment (bone scintigraphy, CT MR scan) | | | | | | | X | | X |
| Diary card provided | C | | | B | | | | | |
| Diary card returned | X | | | X | | X | | | |
| Haematology[1] | 2 | | 2 | 2 | | 2 | 2 | 2 | 2 |
| Biochemistry[1] | 4 | | 4 | 4 | | 4 | 4 | 4 | 4 |
| Serum glucose[1] | 2 | | 2 | 2 | | 2 | 2 | 2 | 2 |
| Thyroid function tests[1, 2] | 2 | | | 2 | | | 2 | 2 | 2 |
| PSA[1] | 5 | | 5 | 5 | | 5 | 5 | 5 | 5 |
| Urinalysis | X | | X | X | | X | X | X | X |
| Exploratory immunology (ml) | | | 40 | | | 40 | 30 | 40 | 40 |
| CTC (ml) | | | | | | | 8 | | |
| ctDNA (ml) | 8 | | | | | | 8 | | |
| Total blood volume per visit (ml)[1] | 23 | | 53 | 15 | | 53 | 61 | 55 | 55 |

| Cumulative blood volume = 612ml[1] |
|---|

* Window is based on preceeding IMP administration

[1] Volume can vary by site according to local practices

[2] Total triiodothyronine (T3) or free T3c, free thyroxine (T4) and thyroid stimulating hormone (TSH)

## APPENDIX 2: SEVERITY GRADING FOR IMPORTANT IMMUNE-MEDIATED AEs

| Adverse Event | Grade | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| **Rash** | Cutaneous disorder composite of erythema or lesions of maculopapular, pruritic, pustular or other character | | | |
| | Lesions covering <10% BSA<br>Symptomatic or asymptomatic | Lesions covering 10-30% BSA<br>Symptomatic or asymptomatic<br>Limiting instrumental ADLs | Lesions covering > 30% BSA<br>Symptomatic or asymptomatic<br>Exfoliative, ulcerated or bullous<br>Localised superinfection | Life-threatening consequences; urgent intervention indicated |
| **Hepatitis or Transaminitis** | Disorder characterised by deranged liver function tests or inflammation of the liver | | | |
| | Asymptomatic<br>ALT or AST ≤ 2.5 x ULN<br>Total bilirubin ≤ 1.5 x ULN | Symptomatic<br>ALT or AST > 2.5x ULN and ≤ 5x ULN<br>Total bilirubin ≤ 3x ULN | Symptomatic<br>ALT or AST > 5x ULN<br>Total Bilirubin > 3x ULN | Decompensated liver function<br>(e.g., ascites, oedema coagulopathy,<br>encephalopathy) |
| **Diarrhoea and Colitis** | Disorder frequent and watery bowel movements and/or inflammation of the colon | | | |
| | Increase of less than four stools per day over baseline; | Increase of four to six stools per day over baseline;<br>Abdominal pain | Increase of seven or more stools per day over baseline; incontinence; hospitalization | Life-threatening consequences; urgent intervention indicated |

| | | | | |
|---|---|---|---|---|
| | | Blood or mucus per rectum | indicated; limiting self-care activities of daily living<br>Ileus<br>Fever<br>Peritonism | |
| **Pneumonitis** | Disorder characterized by inflammation focally or diffusely affecting the lung parenchyma | | | |
| | Asymptomatic; clinical or diagnostic observations only; intervention not indicated | Symptomatic; medical intervention indicated; limiting instrumental ADL | Severe symptoms; limiting self care ADLs; oxygen indicated | Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation) |
| **Nephritis / Renal dysfunction** | Disorder characterised by immune mediated acute kidney injury | | | |
| **[urological causes of acute kidney injury must be excluded]** | Asymptomatic<br>Serum creatinine > ULN and ≤ 1.5 baseline<br>1+ proteinuria; urinary protein <1.0 g/24 hrs | Serum creatinine > 1.5x baseline and ≤ 3x baseline<br>Active urinary sediment<br>2+ proteinuria; urinary protein 1.0 - 3.4 g/24 hrs; | Serum creatinine > 3x baseline and ≤ 6x baseline<br>Active urinary sediment<br>3+ proteinuria; urinary protein >=3.5 g/24 hrs; | Serum creatinine > 6 x baseline |

**REFERENCES**


1. Dunn, G.P., Bruce, A.T., Ikeda, H., Old, L.J. & Schreiber, R.D. Cancer immunoediting: from immunosurveillance to tumor escape. *Nature immunology* **3**, 991-998 (2002).
2. Engels, E.A.*, et al.* Spectrum of cancer risk among US solid organ transplant recipients. *JAMA* **306**, 1891-1901 (2011).
3. Kantoff PW, H.C., Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, Xu Y, Frohlich MW, Schellhammer PF. IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med.* **363**, 411-422. (2010).
4. Sheikh, N.A.*, et al.* Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. *Cancer immunology, immunotherapy : CII* **62**, 137-147 (2013).
5. Gulley, J.L.*, et al.* Immune Impact Induced by PROSTVAC (PSA-TRICOM), a Therapeutic Vaccine for Prostate Cancer. *Cancer Immunology Research* **2**, 133141 (2013).
6. Kyi, C. & Postow, M.A. Checkpoint blocking antibodies in cancer immunotherapy. *FEBS Lett* **588**, 368-376 (2014).
7. Alme, A.K., Karir, B.S., Faltas, B.M. & Drake, C.G. Blocking immune checkpoints in prostate, kidney, and urothelial cancer: An overview. *Urologic oncology* **34**, 171-181 (2016).
8. Brahmer, J.R.*, et al.* Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* **28**, 3167-3175 (2010).
9. Topalian, S.L.*, et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *The New England journal of medicine* **366**, 2443-2454 (2012).
10. Casimiro, D.R.*, et al.* Heterologous human immunodeficiency virus type 1 priming-boosting immunization strategies involving replication-defective adenovirus and poxvirus vaccine vectors. *J Virol* **78**, 11434-11438 (2004).
11. Gilbert, S.C.*, et al.* Synergistic DNA-MVA prime-boost vaccination regimes for malaria and tuberculosis. *Vaccine* **24**, 4554-4561 (2006).
12. Hill, A.V.*, et al.* Prime-boost vectored malaria vaccines: progress and prospects. *Hum Vaccin* **6**, 78-83 (2010).
13. Antrobus, R.D.*, et al.* Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved Influenza A antigens. *Mol Ther* **22**, 668-674 (2014).
14. Harrop, R.*, et al.* Vaccination of Colorectal Cancer Patients with Modified Vaccinia Ankara Delivering the Tumor Antigen 5T4 (TroVax) Induces Immune Responses which Correlate with Disease Control: A Phase I/II Trial. *Clinical Cancer Research* **12**, 3416-3424 (2006).
15. Harrop, R.*, et al.* Vaccination of colorectal cancer patients with TroVax given alongside chemotherapy (5-fluorouracil, leukovorin and irinotecan) is safe and induces potent immune responses. *Cancer immunology, immunotherapy : CII* **57**, 977-986 (2008).
16. Amato, R.J. & Stepankiw, M. Evaluation of MVA-5T4 as a novel immunotherapeutic vaccine in colorectal, renal and prostate cancer. *Future Oncol* **8**, 231-237 (2012).

17. Carsberg CJ, M.K., Stern PL. Metastasis-associated 5T4 antigen disrupts cell-cell contacts and induces cellular motility in epithelial cells. *International journal of cancer. Journal international du cancer* **68**, 84-92. (1996).
18. Starzynska T, M.P., Schofield PF et al. . Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. . *Br J Cancer* **69**, 899-902 (1994).
19. Carbognin, L*., et al.* Differential Activity of Nivolumab, Pembrolizumab and MPDL3280A according to the Tumor Expression of Programmed Death-Ligand-1 (PD-L1): Sensitivity Analysis of Trials in Melanoma, Lung and Genitourinary Cancers. *PloS one* **10**(2015).
20. Janis, M.T*., et al.* Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti–PD-1 Therapy. *Clinical Cancer Research* **20**, 5064-5074 (2014).
21. Gerlinger, M*., et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *The New England journal of medicine* **366**, 883-892 (2012).
22. Brown, J.A*., et al.* Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *Journal of immunology* **170**, 1257-1266 (2003).
23. Keir, M.E., Butte, M.J., Freeman, G.J. & Sharpe, A.H. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* **26**, 677-704 (2008).
24. Linh, T.N. & Pamela, S.O. Clinical blockade of PD1 and LAG3 — potential mechanisms of action. *Nature Reviews Immunology* **15**, 45-56 (2015).
25. Kinter, A.L*., et al.* The common gamma-chain cytokines IL-2, IL-7, IL-15, and IL-21 induce the expression of programmed death-1 and its ligands. *Journal of immunology* **181**, 6738-6746 (2008).
26. Bishop, J.L., Sio, A., Angeles, A., Roberts, M.E. & Azad, A.A. PD-L1 is highly expressed in Enzalutamide resistant prostate cancer. *Oncotarget* (2015).
27. Ness, N*., et al.* The prognostic role of immune checkpoint markers programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) in a large, multicenter prostate cancer cohort. *Oncotarget* **8**, 26789-26801 (2017).
28. Gevensleben, H*., et al.* The Immune Checkpoint Regulator PD-L1 Is Highly Expressed in Aggressive Primary Prostate Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **22**, 1969-1977 (2016).
29. Brahmer, J.R*., et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *The New England journal of medicine* **366**, 2455-2465 (2012).
30. Kwon, E.D*., et al.* Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* **15**, 700-712 (2014).
31. Beer, T.M*., et al.* Randomized, Double-Blind, Phase III Trial of Ipilimumab Versus Placebo in Asymptomatic or Minimally Symptomatic Patients With Metastatic Chemotherapy-Naive Castration-Resistant Prostate Cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **35**, 40-47 (2017).
32. Graff, J.N., Drake, C.G. & Beer, T.M. Complete biochemical (prostate-specific antigen) response to sipuleucel-T with enzalutamide in castration-resistant prostate cancer: a case report with implications for future research. *Urology* **81**, 381-383 (2013).
33. Graff, J.N*., et al.* Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. *Oncotarget* **7**, 52810-52817 (2016).
34. Cappuccini, F., Stribbling, S., Pollock, E., Hill, A.V. & Redchenko, I. Immunogenicity and efficacy of the novel cancer vaccine based on simian adenovirus and MVA vectors alone

and in combination with PD-1 mAb in a mouse model of prostate cancer. *Cancer immunology, immunotherapy : CII* **65**, 701-713 (2016).
35. Cappuccini, F., Pollock, E., Stribbling, S., Hill, A.V.S. & Redchenko, I. 5T4 oncofoetal glycoprotein: an old target for a novel prostate cancer immunotherapy. *Oncotarget* (2017).
36. Elkord, E.*, et al.* An MVA-based vaccine targeting the oncofetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases. *Journal of immunotherapy* **31**, 820-829 (2008).
37. Amato RJ, D.N., Naylor S et al. . Vaccination of Prostate Cancer Patients With Modified Vaccinia Ankara Delivering the Tumour Antigen 5T4 (TroVax): A Phase 2 Trial. . *Journal of immunotherapy* **31**, 577-585 (2008).
38. Kaufman, H.L.*, et al.* Phase II trial of Modified Vaccinia Ankara (MVA) virus expressing 5T4 and high dose Interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma. *Journal of translational medicine* **7**, 2 (2009).
39. Hawkins, R.E.*, et al.* Vaccination of patients with metastatic renal cancer with modified vaccinia Ankara encoding the tumor antigen 5T4 (TroVax) given alongside interferon-alpha. *Journal of immunotherapy* **32**, 424-429 (2009).
40. Amato, R.J. & Stepankiw, M. Clinical Efficacy of TroVax in the Treatment of Progressive Castration-resistant Prostate Cancer. *Clinical Medicine Insights. Oncology* **6**, 67-73 (2012).
41. Harrop, R.*, et al.* Vaccination of castration-resistant prostate cancer patients with TroVax (MVA-5T4) in combination with docetaxel: a randomized phase II trial. *Cancer immunology, immunotherapy : CII* **62**, 1511-1520 (2013).
42. van den Bergh, R.C.*, et al.* Timing of curative treatment for prostate cancer: a systematic review. *European urology* **64**, 204-215 (2013).
43. Barentsz, J.O.*, et al.* ESUR prostate MR guidelines 2012. *European radiology* **22**, 746-757 (2012).
44. Todenhofer, T.*, et al.* Preliminary experience on the use of the Adnatest system for detection of circulating tumor cells in prostate cancer patients. *Anticancer research* **32**, 3507-3513 (2012).
45. Vickers, A.J.*, et al.* A four-kallikrein panel predicts prostate cancer in men with recent screening: data from the European Randomized Study of Screening for Prostate Cancer, Rotterdam. *Clinical cancer research : an official journal of the American Association for Cancer Research* **16**, 3232-3239 (2010).