COMPOSITIONS FOR INDUCING AN IMMUNE RESPONSE

FIELD OF THE INVENTION

The invention relates to induction of immune responses, suitably protective immune responses, against tumour antigens associated with prostate cancer.

BACKGROUND TO THE INVENTION

Prostate cancer is the most common non-skin cancer and the second leading cause of cancer deaths in men. Approximately 1.1 million men were diagnosed with this disease globally in 2012 thus accounting for 15% of all cancer diagnoses in men. More than 70% of cases of prostate cancer occur in the developed world. For example, in the USA alone in 2014 an estimated 233,000 men were diagnosed with this disease and approximately 30,000 deaths were predicted (Siegel et al (2014). CA Cancer J Clin 64:9–29). While there have been significant advances in prostate cancer treatment, there are few treatments available for advanced stages of the disease and these have demonstrated unsatisfactory effectiveness. Therefore, development of effective therapies remains a high priority for treatment of this disease.

Efforts have intensified to develop active immunotherapies (vaccines) for cancer including prostate cancer. Traditional vaccines have been effective in the induction of protective immunity to pathogens based on recognition of foreign, "non-self" antigens. However, the vast majority of cancer antigens characterized to date are unaltered "self" antigens that are expressed by tumor and normal cells. This poses a challenge in the development of effective active immunotherapies for cancer. Despite this limitation on immune surveillance and clearing of cancer, cancer immunity has been observed clinically in the form of various tumours (Challis & Stam (1990). Acta Oncol. 29:545–550). In addition, histopathology of tumor sections has revealed infiltrating lymphocytes around the tumor bed, and recent studies indicate that ovarian cancer patients with such infiltrates around the tumors have an improved

prognosis, compared with similarly staged patients without lymphocytic infiltrates (Zhang et al (2003). N Engl J Med. 348:203–213). The immune repertoire therefore contains auto-reactive immune cells that may reject tumors, when activated appropriately. These auto-reactive cells, upon recognizing target molecules on normal cells, also have the potential to induce tissue destruction leading to toxic autoimmunity. Accordingly, development of therapies aimed at activating host anti-tumour immunity using appropriate immunological targets remains a promising route to success in treating cancers including prostate cancer.

T cells are known to be important in immune control of cancer, and a significant body of evidence accumulated over the last two decades has shown that prime-boost protocols involving sequential administration of different vectors encoding the same antigen(s) yield considerably higher immune responses with protective capability in several animal models and clinical trials. In fact, a vaccination strategy based on the simian adenovirus prime and MVA boost proved to be the most powerful approach for the induction of polyfunctional protective T cell responses against some human pathogens in clinical trials (Ewer et al (2013). Nat Commun. 4:2836; Antrobus et al (2014). J Am Soc Gene Therapy. 22:668-674; Borthwick et al. (2014). Mol Ther. 22:464-475; Swadling et al (2014). Science translational medicine. 6:261ra153; Hodgson et al (2015). J Inf Dis. 211:1076-1086; and Ewer et al (2016). New Engl J Med. 374:1635-1646).

Although promising, the use of therapeutic vaccination in cancer presents many challenges, with tolerance to self-antigens and active immunosuppressive mechanisms mounted by tumours being two major factors hampering efficacy. The two most advanced prostate cancer immunotherapies, Sipuleucel-T and ProstVac, target two well-defined prostate cancer antigens, prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA), respectively. 5T4, an oncofoetal glycoprotein that belongs to the family of shared tumour antigens, is another promising antigen candidate for a prostate cancer vaccine. It was identified in 1990 by searching for shared surface molecules of human trophoblast and cancer cells, with the rationale

that they may have a function in survival of the foetus as a semi-allograft (Southall et al (1990). Br J Cancer. 61:89-95). 5T4 has been a subject of intensive exploration as a potential target for cancer immunotherapy because of its high expression in a wide range of human solid malignancies (Southall et al. (1990). Br J Cancer. 61:89-95; Starzynska et al (1994). Br J Cancer. 69:899-902; and Amato & Stepankiw (2012). Future Oncol. 8:231–237) and an apparent correlation of its expression with disease progression (Stern et al (2014). Seminars in Cancer Biology. 29:13–20; and Stern & Harrop (2016). Cancer Immunology, Immunotherapy. 2016:1–12).

Clinical testing of the 5T4-targeting vaccine started more than a decade ago, with the 5T4 protein expressed from the modified vaccinia Ankara virus (MVA). This vaccine was administered to late stage colorectal cancer patients as a homologous prime-boost vaccine known under the trade name of TroVax, and it has been given to over 500 patients with colorectal, breast, renal, prostate cancer and mesothelioma to date in the course of phase I–III clinical trials (Kim et al (2010). Human Vaccines. 6:784-791; and Al-Taei et al (2012). Lung Cancer. 77:312-318). TroVax had a good safety profile and was well tolerated with a trend toward improved progression-free survival in those patients with the highest 5T4-specific antibody titres (Harrop et al (2010); however, vaccine-specific cellular immune responses and clinical efficacy were modest, J immunotherapy. 33:999-1005; and Harrop et al (2012). Cancer Immunology, Immunotherapy. 61:2283-2294).

Harrop et al (Cancer Immunology, Immunotherapy 2014, 62(9);1511-1520) disclosed the results of clinical administration of TroVax®, a MVA expressing the 5T4 antigen under control of the mH5 (modified H5) early promoter, with docetaxel in castration-resistant prostate cancer patients. The study demonstrated vaccine tolerance in all patients and greater median progression-free survival for patients receiving TroVax® plus docetaxel compared to those receiving docetaxel alone. However, the measured increase in treatment efficacy was modest.

Cappuccini et al (Oncotarget 2017, 8(29);47474-47489) compared administration of

MVA expressing unmodified 5T4 antigen and the same antigen fused to the MHC class 2-associated invariant chain (Ii) under the control of the p7.5 late promoter as part of a heterologous prime-boost regimen in a mouse model of prostate cancer. This study demonstrated an antibody response to unmodified 5T4, but no measurable T cell response was reported except for the modified antigen. This lack of a T cell immune response to the unmodified "self" antigen indicates that MVA expressing unmodified 5T4 under the control of the p7.5 promoter is unlikely to be an effective anti-tumour vaccine.

Thus, there is no vaccine in the prior art that is demonstrated to deliver effective treatment or protection against prostate cancer either alone or in combination with any other therapeutic agents.

The present seeks to overcome problem(s) associated with the prior art.

SUMMARY OF THE INVENTION

We describe a combination which comprises a modified Vaccinia virus Ankara (MVA) vector expressing the 5T4 protein antigen under the control of the endogenous viral F11 promoter. The present invention is based on the surprising finding by the inventors that expression of 5T4 from the endogenous F11 promoter of MVA was sufficient to break tolerance and induce 5T4-specific T cell immune responses when used as part of a prime-boost regimen following initial immunisation with an adenoviral construct expressing 5T4. Compositions of the invention are therefore useful in breaking tolerance to induce antigen-specific immune responses to treat prostate cancer. Data demonstrating these advantages are provided in the figures and examples below.

In a first aspect, the invention provides composition for inducing a T cell mediated immune response for the treatment or prevention of prostate cancer comprising a

modified Vaccinia virus Ankara (MVA) vector expressing the 5T4 antigen polypeptide under control of a poxvirus F11 promoter.

The compositions of the first aspect can be advantageously used to break immune tolerance to and induce T cell-mediated immune responses against the 5T4 antigen, and this can allow effective treatment or prevention of prostate cancer.

The 5T4 polypeptide expressed by the composition of the first aspect can have the amino acid sequence of SEQ ID NO:1 or it can have an amino acid sequence encoded by the nucleic acid sequence of SEQ ID NO:2.

Advantageously, the composition may further comprise an adjuvant, and the composition may be used for inducing a T cell mediated immune response against the 5T4 antigen polypeptide in a subject and for the treatment or prevention of prostate cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will now be described by way of example, with reference to the accompanying drawings, in which:

Figure 1 shows the amino acid sequence of 5T4 antigen (SEQ ID NO:1).

Figure 2 shows the nucleic acid sequence encoding the full-length 5T4 antigen (SEQ ID NO:2).

Figure 3 illustrates the magnitude of 5T4-specific T cell responses in blood is significantly higher following the boost with MVA.5T4 expressing 5T4 under the control of F11 promoter compared to the mH5 promoter driven expression. C57BL/6 mice were immunised intramuscularly at three week intervals with 10^{10} VP of

ChAdOx1 vectors expressing the h5T4 antigen followed by 10^7 pfu of MVA vectors expressing the h5T4 under control of p7.5, F11, and mH5 promoters or were given a homologous MVA.h5T4 prime-boost at 10^7 pfu with the antigen expression driven by F11 promoter. Graphs show representative data of ex vivo blood (A) and spleen (B) ELISPOT performed after prime-boost immunisations. X axis: dosing regimens for groups 1-4. Y axis: number of spot forming cells (SFC) per 10^6 PBMCs. Bars represent median. (C=ChAdOx1, M=MVA). Significant p values are shown.

Figure 4 illustrates the flow cytometry analysis of 5T4 specific T cells in blood and spleen from the mice primed with ChAdOx1.5T4 and boosted with MVA.5T4_F11 demonstrates generation of poly-functional CD4+ and CD8+ T cells secreting multiple cytokines. Intracellular cytokine staining (ICS) was performed on PBMCs and splenocytes isolated from mice immunised with ChAdOx1.5T4 following the MVA.5T4 boost with the antigen expression driven by F11 promoter. The graphs show percentage of CD4+ and CD8+ T cells secreting IFN-γ (A), TNF-α (B) and IL-2 (C) in response to overnight in vitro stimulation with h5T4 peptide pool. X axis: CD4+ and CD8+ T cell responses in blood and spleen. Y axis: % of 5T4 specific cytokine secreting T cells. Δ values are calculated by subtracting the background (i.e. percentage of the T cells spontaneously secreting cytokines without specific stimulation) from the percentage of the cytokine secreting T cells following exposure to the h5T4 peptide pool. Bars represent median values.

DETAILED DESCRIPTION

In a first aspect the present invention provides a composition for inducing a T cell mediated immune response for the treatment or prevention of prostate cancer comprising a modified Vaccinia virus Ankara (MVA) vector expressing the 5T4 antigen polypeptide under control of a poxvirus F11 promoter. MVA expressing the 5T4 antigen polypeptide expressed under the control of a poxvirus F11 promoter has not been disclosed previously and is therefore novel. Such compositions can be advantageously used to break immune tolerance to and induce T cell-mediated immune responses against the 5T4 antigen, and this can allow effective treatment or prevention of prostate cancer.

The prior art suggests a prejudice against the use of the unmodified 5T4 antigen in a vaccine for treatment or prevention of prostate cancer. Cappuccini 2017 (*ibid.*) confirmed that antibody responses to 5T4 can be achieved by MVA expression of unmodified 5T4 antigen as part of a homologous or heterologous prime-boost regimens. However, generation of an *in vitro* T cell-mediated immune response to the 5T4 antigen expressed by MVA in a heterologous prime-boost regimen required fusion of the antigen with the MHC class 2-associated invariant chain (Ii). It is an advantage of the present invention that robust cellular immune responses are induced by unmodified 5T4 antigens expressed by MVA under the control of the endogenous F11 promoter.

Prior art prime-boost using MVA-based vaccine candidates produces robust T cell immune responses against a large number of different “non-self” antigens in various indications. It is an advantage of the invention that immune tolerance is broken and a similarly robust T cell-mediated immune response is generated against a “self” antigen. This response was unexpected and provides a number of benefits including more effective treatment and a simpler development and manufacturing scheme because no antigen modification or fusions are necessary.

Preferably the MVA vector expresses the 5T4 antigen polypeptide under the control of the endogenous F11 promoter of MVA. Insertion of polynucleotides encoding antigens in the F11 locus of MVA under the control of the endogenous F11 promoter has been described previously in international publication WO 2011/128704. Such a vector expressing the 5T4 antigen polypeptide under the control of the endogenous F11 promoter has not been disclosed previously and is therefore novel. Advantageously, this conformation simplifies manufacture of the MVA vector. Additionally, Kozac-like sequences in the F11 flanking sequence are believed to aid translation initiation in eukaryotic cells and so boost expression of the 5T4 antigen by the MVA.

The present inventors provide a vaccine for treatment or prevention of prostate cancer comprising a MVA viral vector containing a nucleic acid sequence encoding the full-length, unmodified human 5T4 antigen polypeptide having the amino acid sequence of SEQ ID NO:1. The MVA construct is made such that there is no marker gene present in the recombinant virus.

The MVA vaccine construct of the present invention ((F11)5T4) was compared to MVA constructs expressing the 5T4 antigen under the control of the modified H5 early promoter ((mH5)5T4) or under the control of the p7.5 early/late promoter ((p7.5)5T4) in a mouse model to measure T cell-mediated immune responses. When administered as part of a heterologous prime-boost regimen the MVA(F11)5T4 construct induced robust 5T4-specific T-cell responses, as measured using IFNγ ELISPOT assays in peripheral blood mononuclear cells (PBMCs) and in splenocytes (Figure 3). This response in PBMCs was more than 3-fold greater than that induced by MVA(p7.5)5T4 while no detectable response in PBMCs was induced using MVA(mH5)5T4 (Figure 3A). The same MVA(F11)5T4 construct failed to induce the same 5T4 specific response when administered alone in a homologous prime-boost regimen. Advantageously, MVA(F11)5T4 was effective in breaking tolerance to induce a robust T cell response against 5T4 and is therefore expected to be effective in treating or preventing prostate cancer.

In certain embodiments the poxvirus F11 promoter is the endogenous MVA F11 promoter. Endogenous enhancer sequences and Kozac-like sequences in the region of the MVA F11 promoter serve to enhance transcription of the 5T4 antigen in human cells.

In a particular embodiment the 5T4 antigen polypeptide has the amino acid sequence provided in SEQ ID NO:1.

In another particular embodiment the 5T4 antigen polypeptide has the amino acid sequence encoded by the nucleic acid sequence provided in SEQ ID NO:2. The use of such a codon-optimised sequence encoding the 5T4 antigen polypeptide improves expression of the antigen polypeptide in the subject after administration of the composition.

In certain embodiments the composition further comprises an adjuvant. Inclusion of an adjuvant can improve the immune response generated on administration of the composition to a subject.

The invention also provides the use of the composition as defined above in the induction of a T cell-mediated immune response to the 5T4 antigen polypeptide. The inventors have found that administration of the composition is effective in inducing such an immune response against 5T4, a “self” antigen. The composition is preferably used to induce a $CD8^+$ T cell response.

Advantageously, the composition may be usefully administered in the treatment or prevention of prostate cancer in a subject.

In another aspect the invention provides a method of inducing a T cell-mediated immune response against the 5T4 antigen polypeptide and inducing a T cell-mediated immune response for the treatment or prevention of prostate cancer comprising the administration of a composition of the first aspect to a subject in need of such a T cell-mediated immune response.

In preferred embodiments the composition of the invention is administered in the method at a dose between 1 x 10^6 and 5 x 10^8 plaque forming units (pfu). In the most preferred embodiment the composition is administered in the method at a dose of 1 x 10^7 pfu. Such doses provide robust immune responses while minimizing unnecessary administration and wastage of the composition.

In certain embodiments the T cell-mediated immune response induced by the method comprises a $CD8^+$ T cell response. Such a cytolytic T cell response is suitable for the effective removal of cells expressing the 5T4 antigen by the subject.

In preferred embodiments the method is a prime-boost method in which the composition of the first aspect is administered to the subject to induce a primary T cell mediated immune response or to boost an existing T cell mediated immune response. In a particularly preferred embodiment the composition of the first aspect is administered as the boost to a previously administered prime vaccination. Such schedules of administration have been shown to advantageously break tolerance and allow induction of robust anti-5T4 T cell responses.

Preferred prime vaccinations of the method are provided by administration of an adenovirus expressing the 5T4 antigen polypeptide, and in the most preferred embodiments the adenovirus used is ChAdOx1.

In preferred embodiments the adenovirus expressing the 5T4 antigen polypeptide is administered in the method as a dose between 1 x 10^8 and 1 x 10^{12} virus particles (VP), and more preferably it is between 1 x 10^9 and 1 x 10^{11} VP. In the most preferred embodiment the adenovirus is administered in the method at a dose of 1 x 10^{10} VP. Such doses provide robust immune responses while minimizing unnecessary administration and wastage of the composition.

In additional embodiments the methods of the invention further comprise administration of a composition of the first aspect of the invention in combination with an immune checkpoint inhibitor compound. In preferred such embodiments the immune checkpoint inhibitor compound is an anti-PD1 monoclonal antibody.

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

EXAMPLES

MVA construction

A codon optimised polynucleotide encoding the 5T4 antigen polypeptide ((NCBI Reference Sequence: NM_006670.4) was synthesised by GeneArt Gene Synthesis (Thermo Fisher Scientific). The 5T4 transgene was then cloned into a shuttle plasmid vector designed to have the upstream and downstream (flanks) of the *F11L* ORF as homologous sequence arms. Inserting the 5T4 transgene within these arms enabled the utilisation of the endogenous F11 promoter, which is part of the right homologous arm, while deleting the native F11L ORF. This resulted in the shuttle vector for generation of MVA.(F11)5T4 (F11 shuttle vector).

MVA.(mH5)5T4 and MVA.(p7.5)5T4 were constructed as previously described in Harrop et al (2010) and Cappuccini et al (2017) respectively.

The MVA constructs were made such that there is no marker gene is present in the recombinant virus.

5T4 immunogenicity

Groups of 6 male C57BL/6 mice (Harlan, UK) received a prime immunization on day 0

that consisted of intramuscular (i.m.) administration of 1 x 10^{10} VP ChAdOx1.5T4 (Groups 1 to 3) or 1 x 10^{7} pfu MVA.(F11)5T4 (Group 4).

The same animals received a boost immunization on day 21 consisting of i.m administration of 1 x 10^{7} pfu MVA.(p7.5)5T4 (Group 1), 1 x 10^{7} pfu MVA.(F11)5T4 (Group 2), 1 x 10^{7} pfu MVA.(mH5)5T4 (Group 3) or 1 x 10^{7} pfu MVA.(F11)5T4 (Group 4).

Blood and spleen from each mouse were collected 3 weeks after the boost (day 42) and PBMCs and splenocytes were tested for the presence of 5T4 specific T cells by IFNg ELISPOT. Results of ELISPOT analysis are provided in Figure 3.

Flow cytometry analysis of 5T4 specific T cells was also performed on PBMCs and splenocytes from the mice primed with ChAdOx1.5T4 and boosted with MVA. (F11)5T4. Results of flow cytometry analysis are provided in Figure 4.

All animal procedures were performed in accordance with the terms of the UK Animals (Scientific Procedures) Act (ASPA) for the project license 30/2947 and were approved by the University of Oxford Animal Care and Ethical Review Committee. All mice were housed for at least 7 days for settlement prior to any procedure in the University animal facility, Oxford, UK under Specific Pathogen Free (SPF) conditions.

MVA-(F11)5T4 safety and immunogenicity in human subjects

MVA-(F11)5T4 has been administered to human subjects in clinical trials to treat late stage metastatic prostate cancer.

These prostate cancer patients received a priming immunization on week 0 that consisted of intramuscular (i.m.) administration of a simian adenoviral vector ChAdOx1 encoding 5T4 at a dose of 2 x 10^{10} vp and a booster intramuscular dose of MVA-(F11)5T4 at week 4 together with an intravenous dose of the checkpoint inhibitor anti-PD1. The same patients are receiving a second round of immunizations at 12 and 16 weeks and further standard i.v. doses of anti-PD1 at 8 and 12 weeks. Blood samples are collected at weeks 0, 2, 5, 9, 13, 17, 24 and 36 to measure immune responses, and any adverse events (AEs) are being documented and investigated.

11 patients have been administered the MVA-(F11)5T4, and the safety profile has been very good. 50% of patients reported pain or tenderness at the injection site the day following vaccination. There have been three (3) serious adverse events (SAEs), but investigation has concluded that none of them were due to MVA-(F11)5T4.