The vaccine of the present invention has therefore been demonstrated to have the capacity to alter the microenvironment surrounding a tumour, making it "visible" to the immune system, permitting T cell infiltration, not only to provoke an immune response to the tumour itself, but further to cancer associated fibroblasts which entirely unexpectedly have been shown to express the MAGED4B protein too. Thus, a vaccine according to the present invention has the capacity to alter cells surrounding the tumour that act to protect such cancer cells from the immune system and anti-cancer agents. Thus, the vaccine of the invention has far-reaching implications for the modulation of the cancer microenvironment, exposing the tumour to the immune system, enhancing the immune response against the tumour and thus greatly assisting tumour cell death.

Notably, the present inventors have found that the vaccine of the invention is very unlikely to cause harm to the normal tissues in the body, since patterns of expression in other tissues is minimal. Thus, this makes the vaccine clinically relevant.

Thus, the present invention provides vaccines that treat and provide protection against tumour growth.

An aim of the present invention is to provide a cancer vaccine that can provide an appropriate immune response against cancer cells, particularly oral and oropharyngeal cancer cells.

Summary

The present disclosure relates to the provision of a nucleic acid vaccine. The vaccine according to any part hereof may be formulated as a vaccine composition. The nucleic acid vaccine is a cancer vaccine, for use in treating cancer. Various alternative vaccines are described. As used herein, either protein may be described as a cancer antigen.

In one aspect, the present disclosure refers to a cancer vaccine comprising a nucleic acid encoding MAGED4B protein or a variant thereof.

In another aspect, the present disclosure refers to the cancer vaccine as disclosed herein for use in therapy.

In another aspect, the present disclosure refers to the use of the cancer vaccine as disclosed herein in the manufacture of a medicament for treating or preventing cancer in a human or animal subject.

In another aspect, the present disclosure refers to the use of the cancer vaccine as disclosed herein in the manufacture of a medicament for revealing a tumour to the immune system.

In another aspect, the present disclosure refers to the use of the cancer vaccine as disclosed herein in the manufacture of a medicament for sensitising a tumour to an anticancer agent, optionally an immune checkpoint inhibitor.

In another aspect, the present disclosure refers to the use of a vaccine as disclosed herein in the manufacture of a medicament for sensitising a tumour to infiltration by CD8+ T cells in a human or animal subject.

In another aspect, the present disclosure refers to a method of selecting a cancer for treatment with the vaccine as disclosed herein, said method comprising determining whether a cancer is associated with the expression of MAGED4B alone, or in combination with FJX1.

In yet another aspect, the present disclosure refers to the use of a cancer vaccine as disclosed herein in the manufacture of a medicament for increasing the efficacy of immune checkpoint blockade in a patient in need thereof.

The cancer vaccine may comprise a nucleic acid which encodes a MAGED4B protein or a variant thereof. This may be the full length protein as described in SEQ ID No. 3 or SEQ ID No. 31, but this can be truncated or modified, such that a sufficient amount of the protein is provided by the nucleic acid, to enable the protein to be processed within the cell and presented to the immune system. Suitable truncations are described in SEQ ID No. 35 to SEQ ID No. 37. Thus, the MAGED4B protein may be an immunogenic fragment of the full length protein as described herein. The sequence MADGED4B protein may further be modified, such that amino acid substitutions are made. Such variants, modifications and truncations are discussed further herein.

Known isoforms (variants) of MAGED4B are described in No. 41-43.

Alternatively defined, the cancer vaccine may include a nucleic acid encoding a MAGED4B protein, said nucleic acid sequence as described in SEQ ID No. 7, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 24, SEQ ID No. 25 or SEQ ID No. 26, or variants and truncated versions thereof. Variants and truncations are as defined herein.

Optionally, the composition may also include a nucleic acid which encodes a FJX1 protein or a variant thereof. The protein may be a full length protein as described in SEQ ID No. 4 or SEQ ID No. 33 or may be truncated or modified. Such variants, modifications and truncations are discussed further herein.

Alternatively defined, the cancer vaccine may include a nucleic acid encoding a FJX1 protein, said nucleic acid sequence as described in SEQ ID No. 8, SEQ ID No. 20 or SEQ ID No. 21, or variants and truncated versions thereof. Variants and truncations are as defined herein.

Optionally, if the vaccine comprises a nucleic acid encoding a MAGED4B protein or a variant thereof and a nucleic acid which encodes a FJX1 protein or a variant thereof, these may be provided separately, i.e. as separate nucleic acids, or on the same nucleic acid, either under the control of the same or different promoters, or present as a fusion between the two proteins.

The present disclosure relates to the provision of a nucleic acid vaccine. The nucleic acid encodes a FJX1 protein or a variant thereof. The protein may be the full length protein as described in SEQ ID No. 4 or SEQ ID No. 33, or may be truncated or modified. Such variants, modifications and truncations are discussed further herein. Optionally, the composition may also include a nucleic acid which encodes a MAGED4B protein or variant thereof. The protein may be a full length protein as described in SEQ ID No. 3 or SEQ ID No. 31, or may be truncated (such as described in SEQ ID No. 35 to SEQ ID No. 37) or modified. Such terms are as discussed herein.

The present disclosure relates to the provision of a nucleic acid vaccine composition. The composition may include a nucleic acid that encodes a MAGED4B protein or variant thereof, and a nucleic acid that encodes a FJX1 protein or variant thereof. The nucleic acid may encode both the MADGED4B and the FJX1 protein as a fusion protein. The composition may be two separate nucleic acid constructs, each encoding either a MAGED4B protein or variant thereof or a FJX1 protein or variant thereof, for separate, simultaneous or sequential administration as a vaccine to a patient in need thereof.

Alternatively defined, the cancer vaccine may include a nucleic acid encoding a MAGED4B protein, said nucleic acid sequence as described in SEQ ID No. 7, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 24, SEQ ID No. 25 or SEQ ID No. 26, or variants and truncated versions thereof, together with a nucleic acid encoding a FJX1 protein, said nucleic acid sequence as described in SEQ ID No. 8, SEQ ID No. 20 or SEQ ID No. 21, or variants and truncated versions thereof. Variants and truncations are as defined herein. The nucleic acids may be separate or present on the same nucleic acid construct.

A fusion protein is a protein consisting of at least two domains that are encoded by separate coding sequences that have been joined so that they are transcribed and translated as a single unit, producing a single polypeptide. Thus, the nucleic acid encoding for a MAGED4B protein or variant thereof, and a FJX1 protein or variant thereof may be fused such that they are produced as a single polypeptide when administered. Thus, in a nucleic acid encoding the fusion protein, the fusion protein is expressed under the control of one promoter. The gene order in the fusion may be in either direction, i.e. a FJX1 protein or variant thereof followed by a MAGED4B protein or variant thereof, or vice versa. It may be preferred that the MAGED4B protein or variant thereof precedes the FJX1 protein or variant thereof.

It may be preferred that any of the nucleic acid vaccine compositions described herein further comprises a helper motif. A helper motif may be defined as a nucleic acid sequence that stimulates an immune response either directly (for example the DNA sequence is in itself capable of stimulating an immune response) or the motif encodes an immunogenic protein or fragment thereof. Said helped motif acts to boost the immune response to the proteins encoded by the nucleic acid

melanoma, rectal cancer, thyroid cancer, bladder urothelial carcinoma, renal cancer and stomach adenocarcinoma.

According to a first example of the present disclosure, there is provided a cancer vaccine comprising nucleic acid encoding the proteins MAGED4B and/or FJX1, or variants thereof, and further encoding an immunogenic fragment of tetanus toxin.

Advantageously, two cancer testis antigens MAGED4B and FJX1 are found to be frequently expressed in OSCC. Overall the two antigens are expressed at 96% of OSCC cases at the RNA level. Furthermore the present study has confirmed the expression of both antigens at protein levels in oral dysplasia and OSCC cases (10/10 were positive; 5 for each condition) with no expression in non-malignant oral mucosa. Examination of expression in healthy tissues at protein levels demonstrated low expression. These expression data have been paralleled by study of pre-existing immunity to the antigens in patients with HPV independent HNSCC using an HLA-A2 tetramer (at present available for MAGED4B only) and overlapping peptide pools (OPP) for the entire amino acid sequence of each antigen. These were measured in both blood and the tumour using expanded tumour infiltrating lymphocytes. Circulating MAGED-4B tetramer positive CD8+ T cells were observed in 5/7 HLA-A2 patients (0.04-0.1% of total CD8+ T cells) with 2/2 expanded TILs also having the tetramer positive at a similar frequency. Higher levels of MAGED4B positive CD8 T cells (5-10 times) was detected in HLA-A2 negative HLA-A1 positive TIL samples using OPP indicating reactivity beyond HLA-A2 restriction. For FJX1 CD8 T cell reactivity has been evaluated in expanded TIL samples using OPP with demonstration of CD8 reactivity in HLA-A1 patients coexisting with MAGED4B CD8 T cells. The patients' data indicate a significant immunogenicity of both antigens more so pronounced for MAGED4B. DNA vaccines encoding full length MAGED4B and FJX1 antigens (e.g. p.Dom-MAGED4BFL and p.Dom-FJX1FL described herein) have been developed and the preclinical data demonstrates the DNA vaccines targeting MAGED4B/FJX1 have a significant potential to suppress the growth of tumour expressing these antigens.

Further advantageously, the provision of an immunogenic fragment of tetanus toxin of *Clostridium tetani* can help to induce strong CD4+ helper T cell responses required for induction of tumour-specific CD4+ and CD8+ T cell responses via activation of dendritic cells (the so called linked T cell mechanisms). Such CD4 and CD8 epitopes are known to be able to bind to a range of mouse and human MHC class II molecules.

<u>Detailed description</u>

The cancer vaccine described here can induce antigen-specific T cell responses, notably a $CD4^+$ and $CD8^+$ T cell response critical for an enduring response, thereby eliciting an immune response that is directed to the tumour expressing the antigen. The cancer vaccine described herein can additionally or alternatively affect the tumour microenvironment, increasing the immune visibility of the tumour and promoting infiltration of CD8 T cells into the tumour mass, thereby making tumour cells more vulnerable to anti-cancer agents, either alone or in combination with said anti-cancer agents. Effectively the vaccine changes the microenvironment of the tumour, making it more susceptible to immune attack.

Lack of intra-tumoural T cells is a major barrier to the efficacy of immune checkpoint inhibitors and other immunotherapies in patients with cancer; this may result from poor tumour immunogenicity (i.e. a lack of T cells) or because T cells fail to infiltrate the tumour (i.e. T cells in the wrong place).

CLAIMS

1. A cancer vaccine comprising a nucleic acid encoding MAGED4B protein or a variant thereof.

2. The cancer vaccine according to claim 1 wherein said MAGED4B protein has at least 85% sequence identity to the sequence as set forth in any one of sequence ID No. 3, 31, 41, 42 or 43 or is an immunologically active truncated version thereof, optionally as set forth in any one of SEQ ID No. 35 to SEQ ID No. 37.

3. The cancer vaccine according to claim 1 or claim 2 wherein said truncated version involves the removal of at least a portion of one or both of the MAGE homology domains.

4. The cancer vaccine according to any preceding claim wherein the nucleic acid sequence has at least 85% sequence identity to the sequence set forth in any one of SEQ ID No. 7, SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 24, SEQ ID No. 25 or SEQ ID No. 26.

5. The cancer vaccine according to any preceding claim further comprising a helper motif, optionally wherein said helper motif encodes a protein which stimulates an immunological response.

6. The cancer vaccine as described in claim 5 wherein said helper motif encodes an immunological fragment of a protein, optionally a helper epitope.

7. The cancer vaccine of claim 5 or claim 6 wherein the helper motif is an immunogenic fragment of tetanus toxin, optionally the p30 MHCII epitope of tetanus toxin.

8. The cancer vaccine of claim 7 wherein the helper motif is DOM.

9. The cancer vaccine according to any preceding claim wherein said vaccine further comprises a nucleic acid encoding a FJX1 protein or a variant thereof.

10. The cancer vaccine of claim 9 wherein said FJX1 protein has at least 85% identity to a sequence as set forth in SEQ ID No. 4 or SEQ ID No. 33, or a truncated version thereof, and is optionally encoded by a nucleic acid that has at least 85% identity to a nucleic acid sequence as described in SEQ ID No. 8, SEQ ID No. 20 or SEQ ID No. 21.

11. The cancer vaccine of claim 9 or claim 10 wherein said MAGED4B and FJX1 are encoded by the same nucleic acid as a fusion protein, optionally wherein said fusion protein further comprises an immunological fragment of a protein.

12. The cancer vaccine of claim 11 wherein the fusion protein includes one or more linker proteins between MAGED4B and/or FJX1 and/or the immunological fragment of a protein.

13. The cancer vaccine of any preceding claim wherein at least one nucleic acid encodes a signal peptide.

14. The cancer vaccine as claimed in any preceding claim wherein said nucleic acid is DNA.

15. The cancer vaccine of claim 14 wherein said DNA is a plasmid, a closed linear DNA, a minicircle DNA or a single stranded circular DNA.

16. The cancer vaccine of claim 14 or 15 in which the nucleic acid further comprises a promoter operably linked to the coding sequences, optionally further comprising a polyadenylation signal downstream of the encoding sequences.

17. The cancer vaccine of any one of claims 1 to 13 wherein said nucleic acid is RNA, optionally messenger RNA or self-amplifying RNA

18. A cancer vaccine as claimed in any one of claims 1 to 17 for use in therapy.

19. Use of the cancer vaccine as claimed in any one of claims 1 to 17 in the manufacture of a medicament for treating or preventing cancer in a human or animal subject.

20. Use of the cancer vaccine as claimed in any one of claims 1 to 17 in the manufacture of a medicament for revealing a tumour to the immune system.

21. Use of the cancer vaccine as claimed in any one of claims 1 to 17 in the manufacture of a medicament for sensitising a tumour to an anticancer agent, optionally an immune checkpoint inhibitor.

22. The cancer vaccine for use as claimed in claim 18, or the use of claims 19-21,wherein said vaccine is to be used in combination with an anti-cancer agent, optionally an immune checkpoint inhibitor.

23. Use of a vaccine of any one of claims 1 to 17 in the manufacture of a medicament for sensitising a tumour to infiltration by $CD8^+$ T cells in a human or animal subject.

24. The use of claim 19 or 23, wherein said use further comprises the use of an anti-cancer agent, optionally an immune checkpoint inhibitor.

25. The use of any one of claim 20 to 24 or the cancer vaccine for use of claim 22, wherein said checkpoint inhibitor is agent capable of blocking the action of PD-1, PD-L1, PD-L2 or CTLA-4, optionally wherein said agent is an antibody or aptamer.

26. The cancer vaccine of any one of claims 1-17, the cancer vaccine for use of claim 18 or 22, or the use according to any one of claims 19-25, wherein said cancer is any one or more of head and neck cancer, oral cancer, oropharyngeal cancer, nasopharyngeal cancer, lung cancer, breast cancer, oesophageal cancer, stomach cancer, liver cancer, colon cancer,

kidney cancer, cholangiocarcinoma, cutaneous melanoma, rectal cancer, thyroid cancer, bladder urothelial carcinoma, renal cancer and stomach adenocarcinoma.

27. A method of selecting a cancer for treatment with the vaccine of any one of claims 1-17, said method comprising determining whether a cancer is associated with the expression of MAGED4B alone, or in combination with FJX1.

28. Use of a cancer vaccine of any one of claims 1 to 17 in the manufacture of a medicament for increasing the efficacy of immune checkpoint blockade in a patient in need thereof.