CANCER VACCINE

The present invention relates to a nucleic acid based cancer vaccine, it use, and methods of treatment or prevention of cancer, particularly oral cancer, and associated combination therapies. The inventors have demonstrated the utility of the nucleic acid based cancer vaccine for several cancer types. Particular combination therapies of interest include immunotherapies, radiotherapy, targeted therapies and chemotherapies.

The present invention relates to nucleic acid vaccines which encode at least a MAGED4B protein, for use in the treatment of cancer in particular. Synergistic combinations with other anti-cancer agents are described, particularly immune checkpoint inhibitors. The cancer vaccine may further comprise an immunologically active fragment to enhance the immune response, and an additional cancer antigen, such as FJX1.

One particular cancer of interest, although not the only cancer suitable for treatment with the vaccine described herein is oral and oropharyngeal cancer. Oral and oropharyngeal cancer (commonly referred to as head and neck cancer; HNSCC), grouped together, is the sixth most common cancer in the world (annual estimated incidence 275,000 for oral (OSCC) and 130,300 for oropharyngeal cancers (OPSCC). In the UK incidence of HNSCC has risen dramatically since the late 1970s (+92%) to over 7500 cases/year ; while the rising UK incidence of OPSCC is related to human papillomavirus (HPV) infection, the cause of the increased incidence of OSCC is unclear, and, it is estimated that rates will continue to rise significantly. Management of patients is often by a costly multidisciplinary approach involving surgery and/or radiotherapy followed by reconstruction and rehabilitation. Treatment results in considerable physical and psychological morbidity and may not prolong life for many of the patients. Surgery and radiotherapy, remain the standard treatments, but despite improvements, are associated with significant morbidity and a relatively static 5-year survival rate of around 50-60%. Immune checkpoint inhibitors against CTLA4 and PD1/PDL1, predicated upon boosting a pre-existing anti-tumour immune response, have shown efficacy across cancer types, and the recent KEYNOTE 012 trial treating HNSCC patients with α-PD1 produced an overall response rate of 18% (J. Clin. Oncol. 34, 3838–3845 (2016). British Journal of Cancer119, 153–159 (2018). However, most patients do not respond to checkpoint inhibitor therapy, likely through lack of a sufficiently strong pre-existing anti-tumour immune response. Experimental approaches to treatment of HPV driven HNSCC targeting HPV antigens are been developed by several companies. These include peptide vaccines, mRNA and DNA vaccines. The majority of oral and oropharyngeal cancers however are HPV-negative; the survival in this patient group is significantly poorer than in those with HPV-positive tumours. 6,000,000 patients annually HPV negative HNSCC require treatment worldwide. So far, a very small number vaccination approaches have been explored in this disease.

While there is intriguing potential for the development of patient-specific vaccines based on an individual's tumour mutanome, the costliness and technical difficulty of such an approach means that, even if successful, it is unlikely to benefit most patients. Identifying common tumour antigens that are shared between patients, to the production of generic cancer vaccines that would provide a cheap and widely available treatment for OSCC. Among the different types of TAA, cancer/testis (CT) antigens are highly promising therapeutic targets; cellular and humoral immune responses to CT antigens are frequently observed in cancer patients, and there is an association between CT antigen

expression and cytolytic activity of tumour immune infiltrates. The immunogenicity and cancer-specificity of CT antigens have made them prioritised targets for cancer immunotherapy, and their therapeutic function has been tested in a variety of clinical settings. CT antigen vaccines are generally well tolerated, and there are presently a large number of ongoing cancer vaccination trials assessing their therapeutic efficacy. A need exists for identifying tumour antigens that can be effectively targeted for treatment or prevention of cancers, such as oral and oropharyngeal cancer.

The inventors have also identified other cancer types which express the cancer antigens described herein, or may be suitable for treatment with the cancer vaccine described herein. Such cancers could include 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. Those skilled in the art may appreciate further cancer types in which the cancer vaccine may be efficacious, based upon mechanism of action of the vaccine. Evidence that the cancer antigens are expressed in various cancer types is included in Figure 29.

The cancer antigen of primary interest herein is the MAGED4B antigen. This may be expressed on the surface of one or more cells associated with cancer, including the tumour cells or cancer associated cells. Another cancer antigen of interest herein is the FJX1 antigen. These may be both expressed in the same cancer, for example be expressed on the surface one or more cell of the cancer or cell associated with the cancer, or in the cancer microenvironment of a tumour. Thus, the vaccine can include one or both cancer antigens.

Previously, it has been identified that MAGED4B and/or FJX1 proteins can be split into small peptides for vaccination (WO2018/169385), by cleaving the protein such that peptides are capable of binding to a MHC class I molecule to induce an anti-cancer immune response in a subject. These peptides are restricted to those that can bind to MHC class I molecules which imposes a limitation on size since the groove of an MHC class I molecule may accept peptides of 8 to 10 amino acids or slightly longer.

However, it is thought that such peptides do not activate a sufficiently sustained immune response in order to help patients induce a durable anti-cancer response.

Since MAGED4B and FJX1 are both self-proteins a major issue with developing a successful cancer vaccine to any one or more of these targets is that patients in need of vaccination are already self-tolerised to these antigens. Further, the targeting of solely MHC class I molecules has been found not to produce a sufficient immune response to produce the anti-cancer response. Such a vaccine approach does not elicit the potent, balanced, and durable CD4 plus CD8 T cell expansion necessary for clinical efficacy. Despite these antigens being identified as good targets for a cancer vaccine, it is regrettable that current technology to date has not produced a vaccine which could be used in a clinical setting.

Further, by providing a vaccine as a small peptide, the vaccine works in a very specific way, and targets a particular type of human leukocyte antigen (HLA) which can limit the usefulness of the vaccine to specific parts of the population. Thus, a vaccine derived from peptides can never provide

a pan-population vaccine, meaning that patients would need to be tested to see if the vaccine was suitable for them, missing parts of the population in need thereof.

Therapeutic cancer vaccines in general have to overcome three major hurdles: low immunogenicity; established disease burden; and the immunosuppressive tumour microenvironment. Aberrantly expressed self-antigens, such as MAGED4B and FJX1 face this hurdle, high-affinity T cells recognising these self-antigens are eliminated by central and peripheral tolerance mechanisms. Thus, the peptide vaccines have, as yet, activated sufficient low affinity T cells. However, high doses of adjuvant needed to stimulate the immune response will have significant side effects for the patient. There is thus still a long-unmet need to be able to help patients with cancers expressing MAGED4B and/or FJX1 to eliminate their tumours.

These issues are such that there was a large risk that a vaccine directed to these antigens in isolation is unlikely to be effective on its own in the treatment of these patients. However, the present inventors have surprisingly found that it is possible to develop a vaccine that demonstrates a good immunological response, providing a CD4 plus CD8 T cell response required for clinical efficacy, and furthermore provides a pan-population effect which means that this can be of general, rather than specific use. Further, they have noted some additional beneficial effects in using the vaccine of the invention that may make the cancer more susceptible in general to additional chemotherapeutic or immunotherapeutic agents, by targeting cells that are associated with the cancer and protecting it from the effects of other anti-cancer agents. This exciting development is of great interest in the treatment of patients in need thereof.

Moreover, there is some evidence that the cancer vaccine described herein may act in a synergistic way with additional anti-cancer agents, which is an exciting development in the treatment of these cancer types, as a combined approach is often clinically recommended to ensure complete remission of the cancer.

In order to provide a successful cancer vaccine, the vaccine must enter the cells and be expressed in vivo, thereby enabling antigen presentation on major histocompatibility molecules (MHC) and T cell recognition. Co-induction of T helper cells (with the surface marker CD4) to cytotoxic T cells (with the surface marker CD8) is critical. Both CD4+ and CD8+ T cells contain several subsets. Therefore, and DNA vaccine must fulfil many requirements before it can be considered to be a good clinical candidate.

The inventors have shown in relevant models and with preclinical data presented here that the desired immunological responses are obtained using the vaccine of the invention. Particularly, the inventors have shown effective tumour infiltration by CD8+ T cells following vaccination, and that circulating T cells exist in human samples that have the potential to be stimulated by the administration of the vaccine, whilst also provoking the formation of newly primed T cells. Data significantly demonstrates good expression and secretion of the protein from the cells transfected with nucleic acid vaccine, which is appropriate, processed to present relevant epitopes thereof to immune surveillance. Further, in mouse tumour models, where the mice had palpable tumours expressing at least one human antigen, the impact of the vaccine on the reduction of tumour size can be seen. Since the tumours associated with at least the MAGED4B protein are known to be particularly aggressive, such mouse model data is hugely encouraging to the inventors.

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 of the present invention

The present invention 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.

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 invention 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 invention 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

vaccine. Suitable helper motifs are discussed herein. One helper motif is an immunogenic fragment of the tetanus toxin, known as DOM.

Thus, the cancer vaccine may comprise nucleic acid encoding MAGED4B protein or a variant thereof together with helper motif. The helper motif may be any suitable helper motif as described here. The helper motif may be DOM. An exemplary vaccine may comprise a sequence described in SEQ ID No. 18 or SEQ ID No. 19, or variants or truncations thereof. An exemplary vaccine may encode a protein sequence described in SEQ ID No. 32 or variants or truncations thereof.

Alternatively, the cancer vaccine may comprise nucleic acid encoding FJX-1 protein or a variant thereof together with helper motif. The helper motif may be any suitable helper motif as described here. The helper motif may be DOM. An exemplary vaccine may comprise a nucleic sequence described in SEQ ID No. 22 or SEQ ID No. 23, or variants or truncations thereof. An exemplary vaccine may encode a protein sequence described in SEQ ID No. 34 or variants or truncations thereof.

Further, the cancer vaccine may comprise nucleic acid encoding MAGED4B protein or a variant thereof and nucleic acid encoding FJX-1 protein or a variant thereof together with helper motif. The helper motif may be any suitable helper motif as described here. The helper motif may be DOM. An exemplary vaccine may comprise a MAGED4B sequence described in SEQ ID No. 18 or SEQ ID No. 19, or variants or truncations thereof. An exemplary vaccine may encode a MAGED4B protein sequence described in SEQ ID No. 32 or variants or truncations thereof. An exemplary vaccine may comprise a nucleic sequence described in SEQ ID No. 22 or SEQ ID No. 23, or variants or truncations thereof. An exemplary vaccine may encode a protein sequence described in SEQ ID No. 34 or variants or truncations thereof.

Where the cancer vaccine as described here includes a fusion protein, a linker may be used between the coding sequences. This fusion may be between cancer antigens MAGED4B and FJX1 or may be between a cancer antigen and helper motif. Any suitable linker sequences may be used. Exemplary linker sequences are given in SEQ ID No. 5, 6, 10 and 11.

The nucleic acid sequence encoding the cancer antigen may include a signal or leader sequence. Such may improve the secretion of the protein from a transfected cell. Exemplary leader sequences are given in SEQ ID No. 1 and 27.

The nucleic acid of the cancer vaccine described here may further comprise a promoter operably linked to the encoding sequences, and optionally further comprising a polyadenylation signal downstream of the encoding sequences. Suitable promoter and polyadenylation signals are described here. Suitable promoters include the sequences described in SEQ ID No. 9 and SEQ ID No. 15.

A cancer vaccine as described here may be a DNA vaccine or an RNA vaccine. The vaccine may also be a non-natural nucleic acid as described here.

The cancer vaccine as described here may be presented as any appropriate nucleic acid construct, including plasmid, minicircle, single stranded circle, closed linear DNA or single stranded RNA. The construct may include any other components in order to promote the expression of the coding sequence, such as enhancers and the like.

Any one of these vaccines is described further below:

A cancer vaccine as described here may be provided as a vaccine composition. A composition may include any suitable excipients or additives which may be supplied in order to stabilise the vaccine composition, making it suitable for storage and transportation, and/or making it suitable for administration, by including buffer solutions and the like to ensure a correct pH.

A cancer vaccine as described here may be used to treat cancer in an animal, including a human. Optionally, the cancer vaccine is for use in treating cancer in a human. Optionally, the cancer vaccine is for use in treating cancer in a non-human animal, such as a domestic, livestock or wild animal. Suitable animals may include cats and dogs, guinea pigs, cattle, horses, sheep, rabbits, and non-human primates. Non-human primates may include chimpanzees and monkeys.

Also envisaged is a method of treating cancer in an animal, including a human, comprising administering the cancer vaccine described here to a patient in need thereof. Suitable animals may include cats and dogs, guinea pigs, cattle, horses, sheep, rabbits, and non-human primates. Non-human primates may include chimpanzees and monkeys.

The cancer vaccine described here may be for use in medicine, optionally for use in treating or preventing cancer.

The cancer vaccine described here may be for use in revealing a tumour to the immune system.

The cancer vaccine described here may be for use in sensitising a tumour to an anticancer agent, optionally an immunotherapy such as a checkpoint inhibitor.

The cancer vaccine described here may be for use in combination with an anti-cancer agent, optionally an immunotherapy such as a checkpoint inhibitor, or chemotherapy.

The cancer vaccine described here may be for a method of sensitising a tumour to infiltration by $CD8^+$ T cells, the method comprising administering the cancer vaccine to a human or animal subject.

The method of treatments described here may further comprise the use of an anti-cancer agent, optionally an immunotherapy such as a checkpoint inhibitor.

As used herein, a checkpoint inhibitor is an 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.

The cancer vaccine described here may be used in a method of increasing the efficacy of immune checkpoint blockade in a patient in need thereof, said method comprising administering a cancer vaccine as described here to a human or animal subject.

The cancer vaccine described herein may be used to co-induce CD4 and CD8 T cells.

The cancer vaccine, uses and methods of treatment described here may be effective for use in the treatment of a multitude of cancer types, including but not limited to 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.

According to a first aspect of the present invention, 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.

Detailed description

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).

The inventors have clearly demonstrated that the cancer vaccine described here increases intra-tumoural T cells (Figure 10B and C). Therefore, the invention extends to a method for increasing the efficacy of immune checkpoint blockade in a patient in need thereof, comprising administering a cancer vaccine described here to a human or animal subject. The cancer vaccine described here may be for use in sensitising a tumour to the immune system or to an anti-cancer agent. Data is presented here that demonstrates, when the cancer vaccine described here is used with an agent capable of causing an immune checkpoint blockade, that a synergistic effect occurs (Figure 9B) which is of great importance.

The vaccine as described here may produce an induced or elicited immune response which may be a cellular immune response. The induced or elicited immune response may include induction or secretion of interferon-gamma (IFN-γ), and/or CD107a/b which is a marker of cytotoxic T cells (Figures 21 and 22).

In particular, where the antigen in the cancer vaccine is a MAGED4B protein antigen, the cancer vaccine can induce an antigen specific response that is additionally directed against cancer-associated fibroblasts (CAF) which have been demonstrated by the inventors to also express this antigen (Figures 27 and 28). A human tumour contains many different types of cell, apart from cancerous cells, including cancer-associated fibroblasts (CAF), endothelial cells, immune cells, adipocytes, and pericytes, which shape the immune microenvironment and promote cancer progression. Thus, the ability to target such cells in the tumour microenvironment is of particular use in the treatment of cancer in general, and may not be limited to the cancer subtypes that themselves express MAGED4B.

All types of solid cancers (tumours) contain CAF-rich subgroups; this ranges from greater than 95% in pancreatic cancers to approximately 50% in head and neck cancers. The proportion of CAF in the tumour is around 25-50% of the tumours, and can be higher in some cancers such as pancreatic cancer where most tumours are CAF-rich. Notably, CAF accumulation in cancers is associated with poor clinical outcome; CAF-rich tumours are clinically aggressive, respond poorly to treatment and are associated with poor survival. This is because CAF promote many of the 'hallmarks of malignancy', promoting tumour growth, invasion, metastasis and angiogenesis. A consistent finding in multiple cancer types has been the inverse correlation between CAF and CD8 T cells suggesting a role for CAF in tumour immune evasion. Recent studies, including those by the applicants, have shown that CAF exclude CD8 T cells from tumours, and thereby promote resistance to anti-PD1/PDL1 checkpoint immunotherapy (and vaccine-based immunotherapy; Ford *et al*., Cancer Res 80, 1846-1860 (2020)). A number of clinical studies have identified CAF gene signatures in patients that do not respond to anti-PD1/PDL1 (Mariathasan, S. *et al*. Nature 554, 544–548 (2018)). CAF-mediated CD8 T cell exclusion is now recognised as a major contributor to checkpoint immunotherapy resistance, and CAF have become an important immunotherapeutic target to improve checkpoint immunotherapy response rates (which currently are around 20% across cancer types). Therapeutic possibilities suggested for targeting CAF include CAF depletion, inhibiting CAF function or CAF normalisation, but previous attempts to specifically target CAF clinically have been unsuccessful.

The fact that the inventors have demonstrated that CAF appear to consistently express MAGED4B is very surprising. Expression is uniformly high across the CAF population, and consistent between

tumours; 70% of HNSCC analysed contained MAGED4B-positive CAF. This CAF MAGED4B expression was confirmed by analysing scRNASeq HNSCC transcriptomic data (Figure 28) (Puram *et al.*, Cell. 2017; 171(7):1611-1624 which also confirmed MAGED4B expression by HNSCC cells).

Generating an immune response that specifically targets CAF is a highly attractive therapeutic, and there have been previous attempts to target CAF by vaccination, for example vaccinating against FAP (fibroblast activated protein). However, FAP is now known to be expressed by other cell types (such as multipotent bone marrow stromal cells) and is not CAF specific. No CAF-specific antigen has yet been identified, but one which the cancer vaccine of the present invention may supply, wherein the antigen the cancer vaccine supplies is a MAGED4B protein or variant thereof.

The present invention is directed to vaccines and methods useful for the restoration of responsiveness to other anti-cancer agents, notably targeted therapies, immunotherapy and chemotherapy, in particular for the restoration of responsiveness to T cell based immunotherapies, including immune checkpoint blockade such as with PD-1 inhibitors, PD-L1 inhibitors, CTLA-4 inhibitors, TIM3 inhibitors, LAG 3 inhibitors, TIGIT inhibitors etc., T cell agonists, such as aCD40, aCD27, OX40 etc., chimeric antigen receptor (CAR) T cells and vaccines.

T cell-mediated elimination of tumours requires signals from the T cell receptor and co-stimulatory molecules to permit effector functions of tumour-antigen specific T cells. There is also an array of immune suppressive mechanisms within the tumour microenvironment that can suppress anti-tumour immunity. The use of monoclonal antibodies to overcome this suppression, in particular targeting co-stimulatory members of the tumour necrosis factor receptor (TNFR) family with agonist Abs enhances T cell function, which has led to encouraging therapeutic results. TNFRs may be important targets for enhancing tumour-specific immune responses and specific TNFRs include OX40, 4-1BB, and CD40.

The cancer vaccines described herein can be administered in therapeutically effective dosages alone or in combination with adjunct cancer therapy such as chemotherapy, radiotherapy, immunotherapy, laser therapy, targeted therapy and/or surgery, all of which may be herein described as an anti-cancer agent. The cancer vaccines described may provide a beneficial effect, such as a reduction in tumour size, slowing rate of tumour growth, inhibiting or slowing metastasis, sensitizing tumours to the immune response or to anti-cancer treatments, or otherwise improving overall clinical condition, without necessarily eradicating the tumour.

Specifically contemplated for combination therapy are cytostatic and cytotoxic agents that target the tumour cells, agents that target angiogenesis (such as angiogenesis inhibitors lenvatinib and sorafenib), agents that target markers that the cancer cells are specifically expressing (i.e. Herceptin that target HER2 positive cells), immune therapies targeting macrophages, immune therapies targeting T cell checkpoint or agonist pathways, adoptive cell therapy (ACT) using T cells engineered to express chimeric antigen receptors (CAR T cells), T -cell receptor (TCR) or *in vitro* expanded T cells and vaccines.

The combination described here can further comprise immune checkpoint inhibitor, for example agents that are active against cytotoxic T-lymphocyte associated protein 4 (CTLA-4), PD-1 and PDL-1, since these may prevent the suppression of elements in the immune system such as MHC class

presentation, T cell presentation and/or differentiation, and cytokine, chemokine or signalling for immune cell proliferation and/or differentiation.

Thus, the cancer vaccine described here may be combined with checkpoint inhibitors such as antibodies directed to CTLA-4, PD-1, PD-L1 and PD-L2 to increase the stimulation of both the cellular and humoral immune responses, but any suitable inhibitor may be used.

Thus, the cancer vaccine described here may be combined with cytostatic and cytotoxic agents that target the tumour cells, agents that target angiogenesis, immune therapies targeting macrophages, immune therapies targeting T cell checkpoint or agonist pathways, adoptive cell therapy (ACT) using T cells engineered to express chimeric antigen receptors (CAR T cells), T cell receptor (TCR) or *in vitro* expanded T cells, T cell agonists and vaccines.

MAGED4B Antigen

MAGED4B is part of a superfamily termed MAGE (melanoma antigen-encoding gene) proteins. MAGE proteins share a conserved domain known as the MAGE homology domain (MHD).

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.

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.

The MAGED4B protein may comprise or consist of the full length MAGED4B protein sequence. The MAGED4B protein may comprise or consist of the sequence of SEQ ID NO: 3 or SEQ ID No. 31, or a variant thereof. In another embodiment, the MAGED4B protein may be encoded by a nucleic acid comprising or consisting of the sequence 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 or a variant thereof.

Advantageously, the full length antigen design can achieve wider population coverage, where it is not focused on targeting individual HLA alleles such as the HLA-A2 allele for example.

Advantageously, the MAGED4B protein may be truncated. The truncation may involve the removal of all or a part or portion of the MAGE homology domain. Two potential regions of homology are found in MAGED4B proteins, and both represent areas which may permit truncation, since they are common across MAGE proteins. The two homology domains have been identified at amino acids 412-500 and amino acids 510-682 in isoform 1 which is represented in SEQ ID No. 3. This sequence is 741 amino acids in length. If both homology domains are removed, this would reduce the length of MAGED4B by 260 amino acids and still provide a functional immunological fragment thereof.

Thus, up to 40% of the full length protein can be removed, optionally up to 50%, and the remaining sequence may still be sufficiently immunogenic to act as a vaccine. Possible truncations are depicted in Figure 16. It may be preferred that known epitopes from the MHD are retained, such as the HLA-2 defined epitope RLSLLLVL. This sits between the two MHDs. As depicted, each of the MHDs have been successfully removed in vaccines, but any portion thereof may be removed. Thus, a truncated version of MAGED4B may include an immunological fragment in which a portion or whole of a MHD is removed. Indeed, in removing the MHD further residues may also be truncated. As a minimum, 450 amino acids of the MAGED4B sequence as described in SEQ ID No. 3 are included. The immunogenic fragment may be 400, 450, 500, 550, 600, 650 or 700 amino acids in length or any number there-between. Various truncations are detailed in SEQ ID No. 35 to SEQ ID No. 37. Other truncations are possible. The inventors postulate that removing the MHD may permit the immune system to recognise the MAGED4B specific epitopes more clearly.

The work here is based on MAGED4B isoform 1 (SEQ ID No. 3). However, 4 isoforms exist, and are included in SEQ ID No. 41-43. Of these, isoform 3 (SEQ ID No. 42) is a very rare alternative splicing, which has a frame shift at position 349, and terminated at position 414. Sequence ID No. 42 has 85% identity sequence to SEQ ID No. 3, due to these alternative splicing and therefor truncations. Thus, in an alternative definition, the truncations may have a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the sequence shown in SEQ ID No. 3.

The cancer vaccine described here may include nucleic acid coding for any one of the MAGED4B proteins described in SEQ ID No. 41-43. These are variants of SEQ ID No. 3.

A variant of MAGED4B may comprise a modified and/or truncated variant of MAGED4B. In particular, the skilled person will understand that some modifications or variants of a sequence may provide the same or substantially similar immunogenic function as the unmodified sequence (i.e. the MAGED4B sequence described herein). Modifications may comprise amino acid residue additions, substitutions, or deletions. In one embodiment, the modification may comprise or consist of no more than 20 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 15 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 10 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 8 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 6 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 5 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 4 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 3 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 2 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than one amino acid residue addition, substitution, or deletion. The amino acid residue additions, substitutions, or deletions may involve consecutive amino acids, multiple groups of amino acids, or non-consecutive amino acid residues, or combinations thereof.

Variants of MAGED4B may comprise or consist of a sequence having at least 80% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 85% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 90% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 95% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 98% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 99% identity with SEQ ID NO: 3. Alternatively, variants of MAGED4B may comprise or consist of a sequence having at least 99.5% identity with SEQ ID NO: 3.

Nucleic acid variations/modifications may comprise conservative substitutions of nucleotides using codon redundancy to encode the same MAGED4B protein, or part thereof, as encoded by SEQ ID NO: 7.

Also included are the nucleic acids disclosed 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 thereof. Variants may have a sequence identity of at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the sequence shown in any one of these sequences. These variants therefore cover the truncations listed above in the protein.

The nucleic acid of the cancer vaccine may be codon optimised, such as the sequence described in SEQ ID No. 17. Codon optimised sequences may code for the same peptide sequence, but is possible because most amino acids are encoded by more than one codon. This may be done to aid synthesis of the nucleic acid, or to prevent a perfect homology with the host genome. As shown in the data, the codon optimised vaccines performed well. Coding sequences can be optimised for stability and high levels of expression.

Variants of the nucleic acid encoding MAGED4B may comprise or consist of a sequence having at least 80% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding MAGED4B may comprise or consist of a sequence having at least 85% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding MAGED4B may comprise or consist of a sequence having at least 90% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding MAGED4B may comprise or consist of a sequence having at least 95% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding the MAGED4B may comprise or consist of a sequence having at least 98% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding the MAGED4B may comprise or consist of a sequence having at least 99% identity with SEQ ID NO: 7. Alternatively, variants of the nucleic acid encoding the MAGED4B may comprise or consist of a sequence having at least 99.5% identity with SEQ ID NO: 7.

The sequence identity may be over at least 600 consecutive nucleotides or amino acid residues. Alternatively, the sequence identity may be over at least 700 consecutive nucleotides or amino acid residues. Alternatively, the sequence identity may be over the whole MAGED4B sequence.

The sequence identity may be over at least 400 consecutive amino acids, at least 450 amino acids, at least 500 amino acids, or at least 550 amino acids.

In another embodiment, variants of MAGED4B may comprise or consist of a truncated sequence of SEQ ID NO: 3. For example, the sequence of SEQ ID NO: 3 herein may be truncated and still provide

immunogenicity. The truncated sequence may comprise at least 200 amino acids of the sequence of SEQ ID NO: 3. The truncated sequence may comprise at least 300 amino acids of the sequence of SEQ ID NO: 3. The truncated sequence may comprise at least 400 amino acids of the sequence of SEQ ID NO: 3. The truncated sequence may comprise at least 500 amino acids of the sequence of SEQ ID NO: 3. Alternatively, the truncated sequence may comprise at least 600 amino acids of the sequence of SEQ ID NO: 3. Alternatively, the truncated sequence may comprise at least 700 amino acids of the sequence of SEQ ID NO: 3. Suitable truncations are depicted in Figure 16.

FJX1 Antigen

The cancer vaccine may comprise or additionally comprise a nucleic acid which encodes a FJX1 (Four-jointed box protein 1) 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 or further 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.

The FJX1 protein may comprise or consist of the full length FJX1 protein sequence. The FJX1 protein may comprise or consist of the sequence of SEQ ID NO: 4, or a variant thereof. In another embodiment, the FJX1 protein may be encoded by a nucleic acid comprising or consisting of the sequence of SEQ ID NO: 8, or a variant thereof.

As previously discussed, the full length antigen design can achieve a wider population coverage, where it is not focused on targeting individual HLA alleles such as HLA-A2 for example.

A variant of FJX1 may comprise a modified and/or truncated variant of FJX1. In particular, the skilled person will understand that some modifications or variants of a sequence may provide the same or substantially similar immunogenic function as the unmodified sequence (i.e. the FJX1 sequence described herein). Modifications may comprise amino acid residue additions, substitutions, or deletions. In one embodiment, the modification may comprise or consist of no more than 20 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 15 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 10 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 8 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 6 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 5 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 4 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 3 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than 2 amino acid residue additions, substitutions, or deletions. In another embodiment, the modification may comprise or consist of no more than one amino acid residue addition, substitution, or deletion. The amino acid residue additions, substitutions, or deletions may involve consecutive amino acids, multiple groups of amino acids, or non-consecutive amino acid residues, or combinations thereof.

Variants of FJX1 may comprise or consist of a sequence having at least 80% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 85% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 90% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 95% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 98% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 99% identity with SEQ ID NO: 4 or SEQ ID No. 33. Alternatively, variants of FJX1 may comprise or consist of a sequence having at least 99.5% identity with SEQ ID NO: 4 or SEQ ID No. 33.

Nucleic acid variations/modifications may comprise conservative substitutions of nucleotides using codon redundancy to encode the same FJX1 protein, or part thereof, as encoded by SEQ ID NO: 8 or SEQ ID No. 20 or SEQ ID No. 21.

The nucleic acid of the cancer vaccine may be codon optimised, such as the sequence described in SEQ ID No. 21. Codon optimised sequences may code for the same peptide sequence, but is possible because most amino acids are encoded by more than one codon. This may be done to aid synthesis of the nucleic acid, or to prevent a perfect homology with the host genome.

Variants of the nucleic acid encoding FJX1 may comprise or consist of a sequence having at least 80% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding FJX1 may comprise or consist of a sequence having at least 85% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding FJX1 may comprise or consist of a sequence having at least 90% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding FJX1 may comprise or consist of a sequence having at least 95% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding the FJX1 may comprise or consist of a sequence having at least 98% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding the FJX1 may comprise or consist of a sequence having at least 99% identity with SEQ ID NO: 8. Alternatively, variants of the nucleic acid encoding the FJX1 may comprise or consist of a sequence having at least 99.5% identity with SEQ ID NO: 8. SED ID No. 8 in this paragraph can be can be substituted with SEQ ID No. 20 or SEQ ID No. 21.

The sequence identity may be over at least 300 consecutive nucleotides or amino acid residues. Alternatively, the sequence identity may be over at least 400 consecutive nucleotides or amino acid residues. Alternatively, the sequence identity may be over the whole FJX1 sequence.

In another embodiment, variants of FJX1 may comprise or consist of a truncated sequence of SEQ ID NO: 4. For example, the sequence of SEQ ID NO: 4 herein may be truncated and still provide immunogenicity. The truncated sequence may comprise at least 200 amino acids of the sequence of SEQ ID NO: 4. The truncated sequence may comprise at least 300 amino acids of the sequence of SEQ ID NO: 4. The truncated sequence may comprise at least 400 amino acids of the sequence of SEQ ID NO: 4. Thus, also encompassed is a immunogenic fragment of FJX1 according to any sequence disclosed herein.

MAGED4B and FJX1 combined

In one embodiment, the nucleic acid encodes both MAGED4B and FJX1, or variants thereof. In one

embodiment, the nucleic acid encodes both MAGED4B and FJX1, or variants thereof with a linker therebetween.

The MAGED4B and FJX1 antigens may be encoded as a single fusion protein, or encoded for separate expression. The MAGED4B may be encoded N-terminal to FJX1.

The combination may extend to a single transcription unit such as MAGED4B-2A peptide-FJX1. 2A self-cleaving peptides, or 2A peptides, are a class of 18–22 aa-long peptides, which can induce ribosomal skipping during translation of a protein in a cell. These peptides share a core sequence motif of DxExNPGP, and are found in a wide range of viral families. They help generating polyproteins by causing the ribosome to fail at making a peptide bond.

Helper motif

The cancer vaccine described here may be provided with a nucleic acid providing a helper motif. A helper motif as used herein is a motif which enhances or improves the immunogenicity of the cancer vaccine. Such may be defined as an adjuvant, a helper epitope, an immunogenic fragment, a cytokine,

The inflammatory signal upon cytosolic nucleic acid recognition may enhance immunogenicity *per se* via the activation of major pro-inflammatory pathways, but this can be further provoked if the nucleic acid vaccine includes a helper motif which is a sequence motif such as CpG motifs. Unmethylated CpG motifs may have an immune stimulating effect by themselves via stimulating the innate immune system through Toll-like receptor (TLR) 9.

Thus, the helper motif may be a nucleic motif known to stimulate the immune system without the need for expression.

The helper motif may encode a protein or polypeptide, or an immunogenic fragment thereof which stimulates the immune system. Thus, the helper motif is expressed in the cell. The helper motif may be present on the same construct as the cancer vaccine antigen, or may be provided on a separate construct. Advantageously, both may be supplied together on the same nucleic acid construct. If both are provided together, they may be under the control of the same or different promoters. Advantageously, these may be provided as a transcriptional or translational fusion. Transcriptional fusions permit the coding sequences to be combined, but does not result in the production of hybrid or fusion proteins. Translational fusions may also be provided, wherein the product of the expression is a hybrid protein. Such may be preferred for vaccination. A linker molecule as described here may be used to link the cancer antigen and the helper motif together in the genetic fusion, and polypeptide fusion.

For example, nucleic acid sequence encoding a cytokine or chemokine can also be delivered directly with the nucleic vaccine, either on the construct or on a separate construct. This enables the appearance of the cytokine or chemokine at the same time and in the same area as the cancer vaccine antigen. Suitable nucleic acids encode cytokines or chemokines such as interleukin (IL)-10, IL-12, dendritic cell-targeting chemokine MIP3α, or IFN-γ, or those discussed further below. Thus, the helper motif may be a nucleic acid encoding a cytokine.

Further, the nucleic acid sequence may encode an immune system stimulator, which can also be delivered directly with the vaccine. Such may include sequences that include trafficking signals (such as MHC class I trafficking signal (MITD)) and the like.

Alternatively, the helper motif may provide immune stimulation by other means, such as by the formation of particles. PVXCP encodes the potato virus X coat protein which provides immune stimulation through the mechanism of linked T-cell help similarly to DOM and permits the formation of particles which enhance the immunogenicity of the expressed polypeptide.

As further example, the helper motif may encode for a protein, polypeptide or an immunogenic fragment thereof which are known to induce a strong immune response. As detailed herein, such includes an immunogenic fragment of the tetanus toxin, in particular DOM. Other suitable helper eptiopes may be derived from the B subunit of *Escherichia coli* heat labile toxin, fragment C of tetanus toxin, diphtheria toxin B subunit, *E. coli* labile toxin fragment, cholera toxin fragment, OVA peptides and/or calreticulin, HIV protein NEF (Negative regulatory factor), HBV surface antigen, promiscuous CD4 epitope PADRE.

Examples are presented herein in which various helper motifs such as CpG motifs, MITD, PVXCP and MIP3α are used in combination with the cancer vaccine.

Suitable helper motifs include nucleic acid sequences encoding: Flt3L, Flt3L-Fc fusion, CD80, CD80-Fc fusion, OX40L, IL-15, 4-1BBL, GM-CSF, CCL21a, IL-23, CCL27, CXCL10, CCL5, CCL3, LAG3, IL-15RA, CXCL10, CpG, E. coli Labile toxin fragment, cholera toxin fragment, calreticulin, HIV NEF, HBV sAg, PADRE, IRF1, CCL25, IL-33 and/or IL-28B.

Particular results have been obtained when the helper motif is an immunogenic fragment of a tetanus toxin, as described herein.

Immunogenic fragment of tetanus toxin DOM

The immunogenic fragment of tetanus toxin may not have the toxic functionality of full length tetanus toxin. In one embodiment, the immunogenic fragment of tetanus toxin may not comprise a neuron binding domain, or may not comprise a functional binding domain. In one embodiment, the immunogenic fragment of tetanus toxin may comprise or consist of the p30 MHC II epitope of tetanus toxin. In one embodiment, the immunogenic fragment of tetanus toxin comprises or consists of DOM. DOM may comprise or consist of the sequence of SEQ ID NO: 2, or a variant thereof.

DOM is an immunogenic fragment of tetanus toxin of *Clostridium tetani* (1), which is safe for human use, because it does not contain a neuron binding domain that is responsible for spastic paralysis. Advantageously, DOM contains a 'promiscuous' p30 MHC II epitope and potentially other CD4 T cell epitopes. P30 is known to be able to bind to a range of mouse and human MHC class II molecules and 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) (2, 3). Further advantageously, DOM has a number of weak CD8 epitopes which are unable to compete with cancer epitopes by the process of immune-dominance, which further provides benefits for inclusion into a DNA vaccine according to the invention, which is intended to induce potent T cell responses against cancer antigens.

In one embodiment, a reference to DOM herein may alternatively be replaced by another immunogenic fragment of tetanus toxin. In particular, the reference to DOM herein may be substituted by p30 or p2 epitopes of tetanus toxin, or other tetanus toxin CD4 helper epitopes alone or in combination.

DOM may be present as the helper motif with any cancer vaccine described herein. It may be described as a helper epitope, since it includes an epitope which my help to induce a strong immune response. Data shown here supports this (Figure 11A).

Various assemblies of DNA vaccines are shown in Figure 7.

DOM, MAGED4B and FJX1 combined

In one embodiment, the nucleic acid encodes DOM with MAGED4B and/or FJX1, or variants thereof. In another embodiment, the nucleic acid encodes DOM with MAGED4B and FJX1, or variants thereof.

In one embodiment DOM, MAGED4B and FJX1are encoded as single fusion protein. In another embodiment DOM and one of MAGED4B or FJX1 are encoded as single fusion protein.

DOM may be encoded N-terminal to MAGED4B and/or FJX1.

Various assemblies of DNA vaccines are shown in Figure 7. Any suitable assembly may be used, including a single transcription unit such as MAGED4B-2A peptide-FJX1.

Other elements

It will be understood that the following section applies to whichever cancer vaccine is of use, including a single antigen or both antigens.

In one embodiment, linker residues may be provided between one or more, or all, of the antigens of DOM, MAGED4B and FJX1. The linker residues may comprise random amino acid sequences, or amino-acids that have been selected to be non-immunogenic based on epitope prediction computer programs or experiments in animal models. For example, a linker may not be considered if it is predicted or known to be an epitope (i.e. in order to avoid an immune response to epitopes, e.g. artificial epitopes, not found in nature). The linker may be flexible. The linker may comprise or consist of K, G, P or S amino acid residues, or combinations thereof. In one embodiment, the linker may comprise or consist of G and/or P amino acid residues. The linker residues may be between 1 and 10 amino acids in length. In another embodiment, the linker residues may be between 2 and 8 residues in length. In another embodiment, the linker residues may be between 1 and 7 residues in length.

The MAGED4B and FJX1 fusion protein may comprise a linker in between the MAGED4B and FJX1 sequences. The linker between MAGED4B and FJX1 may comprise or consist of between about 1 and about 10 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 1 and about 6 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 1 and about 5 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 2 and about 6 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 2 and about 5 amino acids. In another

embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 3 and about 5 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of between about 4 and about 6 amino acids. In another embodiment, the linker between MAGED4B and FJX1 may comprise or consist of about 5 amino acids.

The linker between MAGED4B and FJX1 may comprise or consist of 3-5 amino acids selected from G, S, T, and A. The linker between MAGED4B and FJX1 may comprise or consist of G and/or S residues. In one embodiment, the linker between MAGED4B and FJX1 may comprise or consist of alternating G and/or S residues. In one embodiment, the linker between MAGED4B and FJX1 may comprise or consist of GSGSG (SEQ ID NO: 6 / Linker 2). Alternative linkers may comprise or consist of GGGGG (SEQ ID NO: 10) or SSSSS (SEQ ID NO: 11). Glycine and serine amino-acids are flexible are when included in the linker they allow protein flexibility for efficient expression.

The advantage of such linker is that they are not significantly immunogenic, which will minimise false epitopes (we have used MHC I prediction algorithms to predicts the absence of junctional peptides). The linkers are flexible will not significantly affect the structure of the antigens to allow efficient translation and the proteasomal processing for presentation by MHC.

In an embodiment encoding a DOM with MAGED4B and/or FJX1 fusion protein, the fusion protein may comprise a linker in between the sequence of DOM and the sequence of MAGED4B and/or FJX1. The linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 1 and about 10 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 1 and about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 2 and about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 4 and about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 5 and about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of between about 6 and about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of about 7 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of about 8 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of about 9 amino acids. In another embodiment, the linker between DOM and MAGED4B and/or FJX1 sequences may comprise or consist of about 10 amino acids.

In one embodiment, the linker between DOM and MAGED4B and/or FJX1 may comprise or consist of AAAGPGP (SEQ ID NO: 5 / Linker 1). The linker between DOM and MAGED4B and/or FJX1 may comprise or consist of 3-10 amino acids selected from G, S, T, and A. Alternatively, the linker between DOM and MAGED4B and/or FJX1 may comprise or consist of 3-5 amino acids selected from G, S, T, and A. In one embodiment, the linker between DOM and MAGED4B and/or FJX1 may comprise or consist of alternating G and/or S residues. In one embodiment, the linker between DOM and MAGED4B and/or FJX1 may comprise or consist of GSGSG (SEQ ID NO: 6 / Linker 2). Alternative linkers may comprise or consist of GGGGG (SEQ ID NO: 10) or SSSSS (SEQ ID NO: 11).

Advantageously, linker AAAGPGP (SEQ ID NO: 5 / Linker 1) minimises the occurrence of false epitopes and inserts a restriction enzyme Not I site to aid cloning. In particular, MHC I prediction algorithms have been utilised to predict the absence of junctional peptides. Furthermore, the linkers are flexible and will not significantly affect the structure of the antigens to allow efficient translation and the proteasomal processing for presentation by MHC.

The nucleic acid may further encode a leader sequence, such as a signal peptide for enhancing the efficacy of secretion. The leader sequence may comprise or consist of an IgH signal peptide, such as a mus IgH signal peptide, or an orthologue thereof. The leader sequence may comprise or consist of the sequence MGWSCIIFFLVATATGVHS (SEQ ID NO: 1), or a functional variant thereof. The leader sequence may be N terminal to the DOM sequence, and forms part of a fusion peptide therewith.

The nucleic acid may comprise one or more promoters. The promoter may comprise a eukaryote promoter, and the nucleic acid may optionally further comprise a prokaryote promoter, such as T7. In one embodiment, the promoter is a dual eukaryote/prokaryote promoter. The promoter may be a strong promoter. In one embodiment, the promoter is a viral promoter. The promoter may be selected from any of the group comprising simian virus 40 early promoter (SV40), cytomegalovirus immediate-early promoter (CMV), T7, human Ubiquitin C promoter (UBC), human elongation factor 1α promoter (EF1A), mouse phosphoglycerate kinase 1 promoter (PGK), and chicken β-Actin promoter coupled with CMV early enhancer (CAGG). In one embodiment, the promoter comprises CMV. In one embodiment, the promoter comprises CMV/T7 dual promoter, for example in accordance with SEQ ID NO: 9. In one embodiment, the promoter comprises CMV/T7 dual promoter comprising or consisting of SEQ ID NO: 9, or a functional variant thereof. The variant of SEQ ID NO: 9 may have at least 80%, 85%, 90%, 95%, 98% or 99% identity to SEQ ID NO: 9.

The promoter may be encoded N-terminal to the antigenic protein(s) to be expressed (e.g. DOM, MAGED4B, and/or FJX1). It will be understood that this means that the promoter is upstream of the coding sequence. The promoter needs simply to be operably linked to the coding sequence.

In an embodiment wherein MAGED4B and FJX1 are expressed as separate polypeptides, the nucleic may comprise a promoter for each polypeptide. A single promoter may be used for one or more, or all of the antigens to be expressed.

The nucleic acid may encode a polyA transcription termination sequence. In one embodiment the polyA transcription termination sequence is a mammalian terminator comprising the sequence motif AAUAAA which promotes both polyadenylation and termination. The mammalian terminator may be any one of SV40, hGH, BGH, and rbGlob. In one embodiment the polyA transcription termination sequence is a bovine growth hormone (BGH) polyA transcription termination sequence. A polyadenylation signal can be downstream of the coding sequence(s) of the cancer antigens. The polyadenylation signal can be a LTR polyadenylation signal, polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β-globin polyadenylation signal. The SV40 polyadenylation signal can be a polyadenylation signal from a pCEP4 vector (Invitrogen, San Diego, CA).

In one embodiment, the nucleic acid is DNA encoding:

a single fusion polypeptide comprising DOM antigen, full length MAGED4B antigen, and full length FJX1 antigen, and with linker residues encoded between each antigen;

a N-terminal CMV/T7 promoter; and
a C-terminal PolyA sequence.

In another embodiment, the nucleic acid is DNA encoding:

a single fusion peptide comprising DOM antigen, and one of full length MAGED4B antigen or full length FJX1 antigen as, and with linker residues encoded between each antigen;

a N-terminal CMV/T7 promoter; and
a C-terminal PolyA sequence.

Ideally, the CMV/T7 promoter may be replaced with a CMV promoter.

The nucleic acid may be a DNA encoding:

a single fusion polypeptide comprising a helper motif and a MAGED4B antigen;

an operably linked promoter; and

a PolyA signal sequence.

The nucleic acid may be a DNA encoding:

a single fusion polypeptide comprising DOM antigen and a MAGED4B antigen;

an operably linked promoter; and

a PolyA signal sequence.

The nucleic acid may comprise sequences encoding SEQ ID NOs: 2-4 described herein, or variants thereof. In another embodiment, the nucleic acid may comprise sequences encoding SEQ ID NOs: 1-4 described herein, or variants thereof. In another embodiment, the nucleic acid may comprise sequences encoding SEQ ID NOs: 2-6 described herein, or variants thereof. In another embodiment, the nucleic acid may comprise sequences encoding SEQ ID NOs: 1-6 described herein, or variants thereof.

The nucleic acid encoding MAGED4B and/or FJX1 may be provided in an appropriate backbone vector for delivery and expression *in vivo*. In one embodiment, the backbone vector comprises pcDNA3.0 vector.

Any suitable nucleic acid construct may be used for the vaccine. For a DNA vaccine, the DNA may be in the form of a plasmid, minicircle, single stranded circle, or closed linear DNA. A closed linear DNA may be preferred as it is possible to include no bacterial sequences and it is a minimal vector designed for uses such as DNA vaccines.

In one embodiment, the nucleic acid may comprise or consist of any one of the vectors selected from pDOM MAGED4B-FJX1; pDOM MAGED4B, and pDOM FJX1 as described herein.

In one embodiment, the nucleic acid may comprise or consist of any one of the constructs selected from DB MAGED4B-FJX1; DBMAGED4B, and DB FJX1. These may further include DOM. Various architectures have been tested in relation to these closed linear DNA structures; these are depicted in Figure 18.

In one embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 12. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 13. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 14.

In one embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 16. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 17. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 18. In one embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 19. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 20. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 21. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 22. In another embodiment, the nucleic acid may comprise or consist of the sequence of SEQ ID NO: 23.

The nucleic acid may comprise or consist of DNA. The nucleic acid may comprise or consist of RNA, such as mRNA or self-replicating RNA. Artificial nucleic acids are also envisioned, as discussed herein.

The nucleic acid may be linear or in a circular form, for example in a plasmid. The nucleic acid may comprise the sequence of a mammalian expression vector, such as pcDNA3.0 vector, or an equivalent thereof. The skilled person will recognise that any appropriate mammalian expression vector may be used to insert the nucleic acid according to the invention herein. It may be preferred that the vector is a closed linear DNA.

The cancer vaccine may comprise a composition. For example, the cancer vaccine may be provided in the form of the nucleic acid in a pharmaceutically acceptable carrier. The MAGED4B and/or FJX1 antigens may be encoded on separate nucleic acids, such as vectors, in the same composition.

Further definitions

As used herein, “coding sequence or "encoding” may mean the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein or a fragment thereof. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered.

As used herein “fragment” or "immunological fragment" with respect to proteins may mean a protein or a polypeptide portion thereof, capable of eliciting an immune response in a mammal that cross reacts with the antigen disclosed herein. The fragments can be polypeptide fragments selected from at least one of the various amino acids sequences described herein. Fragments of proteins can comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% of a protein.

As used herein, "identity" in the context of nucleic acid or protein/polypeptide sequences means that the sequence of one has a specified percentage of residues that are the same over a specified

region to the reference sequence. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. Optionally, where a sequence is referenced herein, sequences which preferably have at least 50% identity, 55%, 60%, 65%, 70%, 75%, 80% sequence identity, 85%, 90%, 93%, 95%, 97%, 98% or 99% sequence identity are also encompassed.

As used herein, nucleic acids can be single stranded or double stranded, or can contain sections of both double stranded and single stranded sequence. The nucleic acid can be DNA (including cDNA), RNA, or a hybrid thereof, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including natural bases (uracil, adenine, thymine, cytosine, guanine) and unnatural bases (such as inosine, xanthine hypoxanthine, isocytosine and isoguanine). The nucleic acid may be composed of any nucleotides. These nucleotides may be natural, modified or artificial. The nucleotides may be polymerised to form RNA, DNA, locked nucleic acid (LNA), peptide nucleic acid (PNA), morpholino nucleic acid, glycol nucleic acid (GNA), threose nucleic acid (TNA), hybrids and mixtures thereof and any other artificial (xeno) nucleic acids. It may be preferred that the polynucleotide is DNA or a modified version thereof (i.e. with modifications in the backbone, sugar residue or nucleobase). ModRNA is considered to be appropriate nucleic acid with which to form the vaccine.

The nucleic acid may be in any appropriate format and include any additional sequences or elements that may be required. The nucleic acid may be a plasmid (double stranded circular), a minicircle, a closed linear DNA, a single stranded circular DNA, or any other nucleic acid construct suitable for delivering the vaccine to a cell. The nucleic acid may be an RNA, such as an mRNA (messenger RNA), self-replicating RNA, or non-replicating mRNA, such as that suitable to transfect dendritic cells *in vitro*.

As used herein, "operably linked" may mean that expression of a coding sequence is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5' (upstream) or 3' (downstream) of a coding sequence under its control.

As used herein "promoter" as used herein means a synthetic or naturally-derived sequence which is capable of conferring, activating or enhancing expression of a coding sequence in a cell. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can regulate the expression of a gene component constitutively or differentially with respect to cell, the tissue or organ in which expression occurs, or in response to external stimuli such as inducing agents. Examples of suitable promoters include lac operator-promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV promoter, or SV40 promoters (e.g. pcDNA3.1, pVAX1, pVIVO2, pCI, pCMV and pSV2).

The terms "signal peptide" and "leader sequence" are used interchangeably herein and refer to an amino acid sequence that can be linked at the N terminus of a protein described here. Signal peptides/leader sequences typically direct localisation of a protein. Signal peptides/leader sequences used herein preferably facilitate secretion of the protein from the cell in which it is produced.

As used herein "immune response" as used herein means the activation of the immune system, e.g., that of a mammal, in response to the introduction of antigen. The immune response can be in the form of a cellular or humoral response, or both. It is preferred that the immune response that is elicited is a CD4+ and CD8+ T cell response.

“Treatment" or "treating" as used herein can mean protecting an animal (including a human) from a disease through means of preventing, suppressing, repressing, or completely eliminating the disease. Preventing the disease involves administering a vaccine of the present invention to an animal (such as a human) prior to onset of the disease. Suppressing the disease involves administering a vaccine of the present invention to an animal (human) after induction of the disease but before clinical appearance. Repressing the disease involves administering a vaccine of the present invention to an animal (human) after appearance of clinical symptoms.

The cancer may be any cancer, including but not limited to head and neck cancer, oral cancer, oropharyngeal cancer, lung cancer, breast cancer, oesophageal cancer, nasopharyngeal cancer stomach cancer, liver cancer, colon cancer, kidney cancer, cholangiocarcinoma, cutaneous melanoma, rectal cancer, thyroid cancer, bladder urothelial carcinoma, renal cancer, and stomach adenocarcinoma.

The cancer vaccine may be used to prevent malignant or cancer cells from developing. In some instances, cellular changes that precede cancer development can be detected, and the cancer vaccine administered or used to prevent the development of cancer. Thus, the cancer vaccine may be used to treat pre-malignant cells. Further the cells may progress through several phenotypes before a cancer phenotype is acquired. Therefore the prevention of cancer may involve the treatment of pre-cancerous but invasive cell types. For example, in cancers of the mouth cells may pass through several phenotypes, including but not limited to oral premalignant lesions (OPML): leukoplakia, erythroplakia, lichen planus, and oral epithelial dysplasia. Further exemplarily, some lung cancers may pass through several phenotypes, including but not limited to squamous metaplasia, atypical adenomatous hyperplasia, and/or squamous carcinoma *in situ* (CIS).

The cancer vaccine can prevent tumour growth. The cancer vaccine can reduce tumour growth. The vaccine can prevent metastasis of tumour cells. The cancer vaccine can reduce immune evasion of a tumour cell. The cancer vaccine can be targeted to treat the cancer antigen of the vaccine induces or eliciting an immune response that is directed to or reactive against the cancer or tumour expressing the antigen. The induced or elicited cellular immune response can include induction or secretion of interferon-gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and/or formation of cytolytic granules containing perforin/ granzymes. In other embodiments, the induced or elicited immune response can reduce or inhibit one or more immune suppression factors that promote growth of the tumour or cancer expressing the antigen, for example, but not limited to, factors that down regulate MHC presentation, factors that up regulate antigen-specific regulatory T cells (Tregs), PD-L1, FasL, cytokines such as IL-10 and TFG-β, tumour associated macrophages, cancer associated fibroblasts, soluble factors produced by immune suppressor cells, CTLA-4, PD-1, MDSCs, MCP-1, and an immune checkpoint molecule.

The vaccine can increase a cellular immune response in a subject administered the vaccine by about 50-fold to about 6000-fold, about 50-fold to about 5500-fold, about 50-fold to about 5000-fold, about 50-fold to about 4500-fold, about 100-fold to about 6000-fold, about 150-fold to about 6000-

fold, about 200-fold to about 6000-fold, about 250-fold to about 6000-fold, or about 300-fold to about 6000-fold as compared to a cellular immune response in a subject not administered the vaccine.

The vaccine can increase interferon gamma (IFN-γ) levels in a subject administered the vaccine by about 50-fold to about 6000-fold, about 50-fold to about 5500-fold, about 50-fold to about 5000-fold, about 50-fold to about 4500-fold, about 100-fold to about 6000-fold, about 150-fold to about 6000-fold, about 200-fold to about 6000-fold, about 250-fold to about 6000-fold, or about 300-fold to about 6000-fold as compared to IFN-γ levels in a subject not administered the vaccine.

The cancer vaccine can be a DNA. A DNA vaccine can further comprise elements or reagents that inhibit it from integrating into the chromosome.

The cancer vaccine can be an RNA of the one or more cancer antigens.

The vaccine of the present invention can have features required of effective vaccines such as being safe so that the vaccine itself does not cause any detriment to normal cells, illness or death; being protective against illness; inducing protective T cell responses; and providing ease of administration, few side effects, biological stability, and low cost per dose, particularly where a closed linear DNA is used.

The cancer vaccine can further comprise one or more inhibitors of one or more immune checkpoint molecules (i.e., an immune checkpoint inhibitor or "checkpoint inhibitor"). The immune checkpoint inhibitor is any nucleic acid or protein that prevents the suppression of any component in the immune system such as MHC class presentation, T cell presentation and/or differentiation, B cell presentation and/or differentiation, any cytokine, chemokine or signalling for immune cell proliferation and/or differentiation. as the cancer vaccine may be combined further with antibodies to checkpoint inhibitors such as CTLA-4, PD-1 and PDL-1 to increase the stimulation of the cellular and immune responses. Using anti-PD-1 or anti-PDL-1 antibodies prevents PD-1 or PDL-1 from suppressing T cell and responses.

Combination Therapy

The cancer vaccine may be used as a vaccine in combination with another therapeutically or prophylactically active ingredient. The cancer vaccine may be used as a vaccine in combination with an adjuvant.

The therapeutically or prophylactically active agent may be any anti-cancer agent.

Suitable anti-cancer agents include chemotherapeutics, including but not limited to alkylating agents, mustard gas derivatives (mechlorethamine, cyclophosphamide, chlorambucil, melphalan, and ifosfamide), ethylenimines, alkylsulfonates, hydrazines and triazines, nitrosureas, metal salts (such as carboplatin, cisplatin, and oxaliplatin), plant alkaloids, vinca alkaloids, taxanes, podophyllotoxins, camptothecan analogues, antitumor antibiotics, anthracyclines, chromomycin, mitomycin, bleomycin, antimetabolites, folic acid antagonist, pyrimidine antagonist, purine antagonist, adenosine deaminase inhibitor, and topoisomerase (I and II) inhibitors.

Suitable anti-cancer agents include immunotherapeutics, including but not limited to immune checkpoint inhibitors, T cell transfer therapy, adoptive cell therapy, adoptive immunotherapy, or

immune cell therapy, antibody therapy, vaccines, or immune system modulators. Further contemplated are immune therapies targeting T cell checkpoint or agonist pathways, adoptive cell therapy (ACT) using T cells engineered to express chimeric antigen receptors (CAR T cells), T cell receptor (TCR) or *in vitro* expanded T cells.

Suitable anti-cancer agents may include radiotherapy or radiomimetics, targeted therapy, surgery or laser therapy.

Suitable anti-cancer agents include targeted therapies. Such therapies depend on the cancer being treated, and may involve an analysis of what genes the cancer cells are expressing or entail a review of what genetic mutations underlie the mutagenesis. Several targeted therapies are described herein, and include cytostatic and cytotoxic agents that target the tumour cells, agents that target angiogenesis (such as angiogenesis inhibitors lenvatinib and sorafenib), agents that target markers that the cancer cells are specifically expressing (i.e. Herceptin that target HER2 positive cells), therapies targeting macrophages, therapies targeting T cell checkpoint or agonist pathways and the like.

Additionally or alternatively, the cancer vaccine according to the invention may be used alone or in combination with a checkpoint inhibitor for prevention or treatment of cancer. The checkpoint inhibitor may comprise an anti-PD1 binding molecule. He binding molecule comprise an anti-PD1 antibody, or fragment thereof. In another embodiment, the checkpoint inhibitor may comprise an anti-CTLA4 binding molecule.

Advantageously, the preclinical data herein demonstrates the DNA vaccines targeting MAGED4B/FJX1 and having a significant potential to suppress the growth of tumour expressing these antigens can be further enhanced by combination with a checkpoint inhibitor, such as an anti-PD1 or anti-CTLA4 binding molecule.

The anti-PD1 binding molecule may comprise an antibody or antibody variant, such as an antibody fragment, or an antibody mimetic. The antibody or variant thereof may be monoclonal. In one embodiment, the antibody or variant thereof may comprise or consist of Nivolumab, or an antibody or variant thereof that competes for binding with Nivolumab. In one embodiment, the antibody or variant thereof may comprise the six heavy and light chain CDRs of Nivolumab. In an alternative embodiment, the antibody or variant thereof may comprise the variable heavy and light chain sequences of Nivolumab.

The anti-CTLA4 binding molecule may comprise an antibody or antibody variant, such as an antibody fragment, or an antibody mimetic. The antibody or variant thereof may be monoclonal. In one embodiment, the antibody or variant thereof may comprise or consist of Ipilimumab, or an antibody or variant thereof that competes for binding with Ipilimumab. In one embodiment, the antibody or variant thereof may comprise the six heavy and light chain CDRs of Ipilimumab. In an alternative embodiment, the antibody or variant thereof may comprise the variable heavy and light chain sequences of Ipilimumab.

In one embodiment, the checkpoint inhibitor, such as anti-PD1 or anti-CTLA4 binding molecules, may be provided by the provision/administration of nucleic acid encoding the checkpoint inhibitor for expression *in vivo*. The checkpoint inhibitor may be encoded on a plasmid. The checkpoint inhibitor

may comprise a DNA-encoded monoclonal antibody (DMAb), for example as described in Perales-Puchalt et al. (Oncotarget. 2019 Jan 1;10(1):13-16. doi: 10.18632/oncotarget.26535), which is herein incorporated by reference.

DNA-encoded monoclonal antibodies (DMAbs) can help to overcome difficulties in production, stability, the requirement of frequent high doses for antibody administration and long intravenous administration are recurring issues. Synthetically designed DMAbs can simplify design and implementation of MAb-based therapies. DMAbs delivered through plasmid DNA injection and electroporation have been used in preclinical models for the treatment or prophylaxis of infectious diseases, cancer and cardiovascular disease. Perales-Puchalt et al. (Oncotarget. 2019 Jan 1;10(1):13-16. doi: 10.18632/oncotarget.26535) and Duperret EK et al. (Cancer Res. 2018;78:6363–70), both of which are incorporated herein by reference, reported that immune checkpoint blockers can be optimised and delivered *in vivo* advancing further DMAb technology by optimisation, expression and in vivo functional characterisation of anti-CTLA4 and anti-PD1 antibodies.

The use may be in a combined formulation. In another embodiment, the use may be concurrent or sequential administration (e.g. formulated separately, but administered together). In one embodiment, the vaccine according to the invention may be administered prior to the checkpoint inhibitor.

According to another aspect of the invention there is provided a fusion peptide encoded by the nucleic acid of the cancer vaccine described herein.

According to another aspect of the invention there is provided a composition comprising the cancer vaccine according to the invention.

The composition may be immunogenic, for example in a mammal, such as a human. The composition may comprise a pharmaceutically acceptable carrier. The composition may be a pharmaceutical composition comprising a pharmaceutically acceptable carrier. The composition may be for use in the prophylaxis or treatment of cancer.

According to another aspect of the invention, there is provided a kit for the treatment or prevention of cancer, the kit comprising

-a cancer vaccine according to the invention herein; and

-a checkpoint inhibitor agent, such as an anti-PD1 binding molecule.

According to another aspect of the invention, there is provided a kit for the treatment or prevention of cancer, the kit comprising

-a first cancer vaccine according to the invention herein, wherein the nucleic acid encodes MAGED4B;

-a second cancer vaccine according to the invention herein, wherein the nucleic acid encodes FJX1; and optionally

-a checkpoint inhibitor agent, such as an anti-PD1 binding molecule.

Accordingly, there may be provided a kit for the treatment or prevention of cancer, the kit comprising

-a cancer vaccine comprising a sequence encoding a MAGED4B antigen or variant thereof; and

-a checkpoint inhibitor agent, such as an anti-PD1 binding molecule.

Accordingly, there may be provided a kit for the treatment or prevention of cancer, the kit comprising

-a first cancer vaccine wherein the nucleic acid encodes MAGED4B or a variant thereof and a

-a checkpoint inhibitor agent, such as an anti-PD1 binding molecule.

The cancer vaccine can further comprise one or more inhibitors of one or more immune checkpoint molecules (i.e., an immune checkpoint inhibitor). The immune checkpoint inhibitor may be any nucleic acid or protein that prevents the suppression of any component in the immune system such as MHC class presentation, T cell presentation and/or differentiation, any cytokine, chemokine or signalling for immune cell proliferation and/or differentiation.

The immune checkpoint inhibitor can be one or more nucleic acid sequences encoding an antibody, a variant thereof, a fragment thereof, or a combination thereof. In other embodiments, the immune check point inhibitor can be an antibody, a variant thereof, a fragment thereof, or a combination thereof.

The immune check point molecule can be a nucleic acid sequence, an amino acid sequence, a small molecule, or a combination thereof.

PD-1 and PD-L1

The immune checkpoint molecule may be programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), a fragment thereof, a variant thereof, or a combination thereof. PD-1 is a cell surface protein encoded by the PDCD1 gene. PD-1 is a member of the immunoglobulin superfamily and is expressed on T cells and pro-B cells, and thus, contributes to the fate and/or differentiation of these cells. In particular, PD-1 is a type 1 membrane protein of the CD28/CTLA-4 family of T cell regulators and negatively regulates T cell receptor (TCR) signals, thereby negatively regulating immune responses. PD-1 can negatively regulated CD8+ T cell responses, and thus inhibit CD8-mediated cytotoxicity and enhance tumour growth.

PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family. PD-L1 is upregulated on macrophages and dendritic cells (DCs) in response to LPS and GM-CSF treatment and on T cells upon TCR receptor signalling. PD-L1 is expressed by several tumour cell lines.

Anti-Immune Checkpoint Molecule Antibody

The immune checkpoint inhibitor can be an antibody. The antibody can bind or react with the immune checkpoint molecule. Accordingly, the antibody may be considered an anti-immune checkpoint molecule antibody or an immune checkpoint molecule antibody. The antibody can be

encoded by a nucleic acid sequence contained in the cancer vaccine, or can be supplied as an antibody.

The antibody can be a polyclonal or monoclonal antibody. The antibody can be a chimeric antibody, a single chain antibody, an affinity matured antibody, a human antibody, a humanised antibody, or a fully human antibody.

Cancer Vaccine Constructs

The cancer vaccine can comprise nucleic acid constructs that encode cancer antigens.
The nucleic acid constructs can include or contain one or more heterologous nucleic acid sequences. The construct can be present in the cell as a functioning extrachromosomal molecule. The construct is preferably a closed linear DNA molecule.

Closed linear DNA is generally understood to be double-stranded DNA covalently closed at each end. The double stranded section of the DNA is therefore complementary. When denatured, closed linear DNA may form a single stranded circle. The DNA may be closed at each end by any suitable structure, including a cruciform, a hairpin or a hairpin loop, depending on preference. The end of the closed linear DNA may be composed of a non-complementary sequence, thus forcing the DNA into a single stranded configuration at the cruciform, hairpin or hairpin loop. Alternatively, the sequence can be complementary. It may be preferred that the end is formed by a portion of a target sequence for a protelomerase enzyme. A protelomerase target sequence is any DNA sequence whose presence in a DNA template allows for the enzymatic activity of protelomerase, which cuts a double stranded section of DNA and re-ligates them, leaving covalently closed ends. In general, a protelomerase target sequence comprises any perfect palindromic sequence i.e. any double-stranded DNA sequence having two-fold rotational symmetry, or a perfect inverted repeat. The closed linear DNA may have a portion of a protelomerase target sequence at one or both ends. This portion is effectively a single strand of the whole double stranded recognition site. The protelomerase target sequence can have the same cognate protelomerase at each end, or require a different protelomerase for each end. Closed linear DNA constructed via the action of various protelomerase enzymes have been previously disclosed in WO2010/086626, WO2012/017210 and WO2016/132129, all of which are incorporated by reference. Closed linear DNA constructed using *in vitro* DNA amplification followed by cleavage with a protelomerase enzyme has the advantage that the closed linear DNA is produced in an *in vitro*, cell-free environment, and can be scaled up for commercial production. These closed linear DNA vectors are known as Doggybone DNA or dbDNA™. It is preferred that the closed linear DNA vectors are made using the prior methods of the applicants, in an *in vitro*, cell-free manner based upon polymerase based amplification of a DNA template with at least one protelomerase target sequence, and processing of the amplified DNA with a protelomerase to produce closed linear DNA.

Closed linear DNA can be constructed by a conversion of a plasmid with the requisite protelomerase target sequences into a closed linear DNA vector, although this is not an efficient method of production.

Other closed linear DNA vectors have been constructed by various *in vitro* strategies including the capping of PCR products, and the “minimalistic immunogenic defined gene expression (MIDGE)” vectors. MIDGE is generated by the digestion of both prokaryotic and eukaryotic backbones after

isolation of plasmid from bacterial cells, followed by ligation of the required DNA sequence into hairpin sequences for end-refilling.

DNA "ministrings", which are produced in an *in vivo* manner in cell culture, based upon the action of protelomerase, are also closed linear DNA vectors that would be suitable for use in the invention.

Other forms of closed linear DNA that may be suitable include those closed at the ends with cruciform structures, which can again be manufactured in cell culture.

It may be preferred that the closed linear DNA is manufactured in a cell-free system, since this ensures purity of product, in the alternative, stringent purification of closed linear DNA made by cellular methods will be required by the regulatory authorities.

The nucleic acid constructs can comprise regulatory elements for gene expression of the coding sequences of the nucleic acid. The regulatory elements can be a promoter, an enhancer, a stop codon, or a polyadenylation signal.

The cancer vaccine as described herein can be capable of expressing the cancer antigens in the cell of an animal in a quantity effective to elicit an immune response in the animal. The cancer vaccine can be useful for transfecting cells with nucleic acid encoding the cancer antigens, wherein expression of the above described antigens takes place. Closed linear DNA is shown to be an effective construct for DNA vaccines.

Methods of Preparing the Vaccine

Closed linear DNA molecules can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using a cell-free synthetic method as described in WO2010/086626, WO2012/017210 and WO2016/132129.

Standard recombinant technologies can be used to prepare the DNA constructs.

Other Aspects

According to another aspect of the present invention, there is provided the cancer vaccine or composition according to the invention herein, for use as a medicament.

According to another aspect of the present invention, there is provided the cancer vaccine or composition according to the invention herein, for use for treating or preventing cancer in a subject.

According to another aspect of the present invention, there is provided a method of treating or preventing cancer in a subject, the method comprising the administration of the cancer vaccine or composition according to the invention herein.

The cancer to be treated or prevented may be oral and/or oropharyngeal cancer. The oral and/or oropharyngeal cancer may be HPV negative or positive oral and/or oropharyngeal cancer. In one embodiment, the cancer is oral cancer. In another embodiment, the cancer is oropharyngeal cancer. In one embodiment the cancer is a squamous cell cancer. In another embodiment, the cancer to be treated may be lung cancer or nasopharyngeal cancer.

The cancer may be characterised by cancer cells expressing or overexpressing MAGED4B and/or FJX1.

The cancer may be characterised by tumour associated cells expressing or overexpressing MAGED4B.

Expression of cancer antigens can be determined by routine methods such as interrogating biopsies or samples with relevant antibodies, and/or probing the RNA sequences present in the cell.

The cancer may additionally or alternatively be characterised by being associated with CAF overexpressing MAGED4B. Such cells may be protecting the cancer cells from the immune system and from other anti-cancer agents.

The expression of the cancer antigen on cancer and cancer associated cells may be determined in comparison with normal cells from the same tissue or organ. Expression or overexpression can therefore be a comparative level of expression relative to normal cells.

In one embodiment, the subject is tested for the presence of MAGED4B and/or FJX1 antigens in their cancerous tissue or cells prior to the treatment or prevention. In another embodiment, the subject is tested for the level of MAGED4B and/or FJX1 antigens in their cancerous tissue or cells prior to the treatment or prevention. The subject may be selected for the treatment or prevention if they have MAGED4B and/or FJX1 antigens in their cancerous tissue or cells, or have overexpression thereof relative to equivalent non-cancerous tissue or cells. The cells around the tumour may also be tested for cancer antigen expression, notably MAGED4B expression.

The skilled person will be familiar with vaccine administration routes and doses. For example the administration may be sub-cutaneous, intra-muscular, or intravenous. A typical dose may be about 4-8mg per patient/subject administered one or more times. For example the dose may be administered multiple times until the therapeutic effect is observed. In one embodiment, *in vivo* electroporation is used to enhance delivery into cells, either *in vivo* or *ex vivo*.

The subject may be mammalian. In one embodiment, the subject is human. In another embodiment, the subject may be a domestic or livestock animal.

According to another aspect of the invention, there is provided a polypeptide comprising a MAGED4B protein or variant or truncated version thereof. The polypeptide may be presented as a fusion with a helper motif. The polypeptide may be presented as a fusion with DOM. Exemplary polypeptide sequences are described here as SEQ ID No. 32, 35, 36 and 37. The MAGED4B sequence may be fused with FJX1 as encoded by SEQ ID No. 12 (with DOM in this instance).

Cancer Vaccine Compositions

The vaccine can be in the form of a composition, optionally pharmaceutical composition. The pharmaceutical compositions can comprise about 5 nanograms to about 10 mg of the vaccine. In some embodiments, pharmaceutical compositions according to the present invention comprise about 25 nanogram to about 5 mg of DNA of the vaccine. In some embodiments, the pharmaceutical compositions contain about 50 nanograms to about 1 mg of DNA of the vaccine.

The composition can further comprise other additives for formulation purposes, which may vary according to the mode of administration. In cases where compositions are injectable, they are sterile, pyrogen free and particulate free. Suitable formulations may include sodium chloride,

dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilisers may include gelatine and albumin.

The vaccine can further comprise an acceptable excipient. The acceptable excipient can be functional molecules as vehicles, adjuvants, carriers, or diluents.

Vaccination

The cancer vaccines disclosed herein are provided for use in methods for treating or prevent cancer. The vaccines described herein can be for use in a method of administration or vaccination, to induce a therapeutic and/or prophylactic immune response. The vaccination process can generate in the animal an immune response against one or more of the cancer antigens. The administration of the vaccine can be the transfection of the one or more cancer antigens as a nucleic acid molecule that is expressed in the cell and thus, delivered to the surface of the cell upon which the immune system recognises and induces an immune response.

The vaccine can be administered to an animal, preferably a mammal in order to elicit an immune response. The mammal can be human, non-human primate (particularly chimpanzee and monkey), cow, pig, sheep, goat, deer, llama, alpaca, dog, cat, guinea pigs, rabbits, mice, rats, and preferably human, dog or cat.

The vaccine dose can be between 1 µg to 10 mg active component/kg body weight/time and can be 20 µg to 10 mg component/kg body weight/time. The vaccine can be administered every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days. The number of vaccine doses for effective treatment can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more doses.

The vaccine may be administered using a “prime boost” strategy. A prime-boost immunisation strategy can be defined as a regimen of immunisation with the same vaccine during the prime and booster doses. There may be one or more booster doses. The treatment strategy used in the Examples in some instances used a prime boost strategy.

The cancer vaccine can be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. The vaccine can be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as tattooing, electroporation ("EP"), "hydrodynamic method", or ultrasound.

The cancer vaccine is a nucleic acid. It may be delivered using any appropriate nucleic acid delivery method used to transfect cells. Naked nucleic acid may be used, particularly where the nucleic acid is in a minimal form (such as a minicircle or a closed linear DNA). However, the nucleic acid may also be “packaged” for administration, for example using different materials. These materials include lipids, linear and branched polymers, and peptides/proteins with either natural or synthetic sources. The complexing products of lipids/nucleic acids lead to lipoplexes with representative lamellar or hexagonal structures. The polymers and nucleic acids may complex into polyplexes with polymer chains tangling together without any ordered internal structure. Peptides/proteins interact with nucleic acids to form either disordered polyplexes or ordered artificial viruses with filamentous or

spherical morphologies, depending on the primary structure of the peptide/protein. Alternatively, the nucleic acid may be packaged in a viral coat, such as a in a virus like particle (VLP) or indeed packaged into any suitable viral vector such as a lentivirus, a retrovirus, and adeno-associated virus (AAV) adenovirus, Modified vaccinia Ankara (MVA) or oncolytic Maraba MG1 rhadovirus.

The cancer vaccine may be used on cells *ex vivo*. Thus, suitable cells can be obtained, which are either autologous or allogenic, transfected in vitro, and then these cells can be supplied to a patient in need thereof. Suitable cells for *ex vivo* transfection include any type of antigen presenting cells including natural killer cells or dendritic cells, autologous or allogeneic tumour cells (usually irradiated). Autologous cells may be transiently transfected with the cancer vaccine in an mRNA or DNA format. The cancer antigen may be codon optimised and contain a helper motif resulting in both MHC-I and MHC-II presentation of cancer antigen peptides to both CD8+ and CD4+ T cells in the patients. As a result, a strong, tumour-specific immune response after reintroduction into the patient may be achieved.

Thus the present application extends to the use of the cancer vaccine to transfect cells *in vitro* or *ex vivo*, prior to the use of those cells for the treatment or prevention of cancer in a patient. Such cells may no longer include the cancer vaccine when administered, since the transfection can be transient.

The data shows that the combination of the cancer vaccine and an immune checkpoint inhibitor induces the immune system more efficiently than a vaccine comprising the cancer antigen alone, and in fact these two work synergistically. This more efficient immune response provides increased efficacy in the treatment of cancer.

Definitions

A Checkpoint inhibitor therapy is a form of cancer immunotherapy. A checkpoint inhibitor targets immune checkpoints, key regulators of the immune system that stimulate or inhibit its actions, which tumours can use to protect themselves from attacks by the immune system. Checkpoint therapy can block inhibitory checkpoints, restoring immune system function.

The term "immunogenic", when applied to the protein or composition of the present invention means capable of eliciting an immune response in a human or animal body. The immune response may be protective.

The term "protective" means prevention of a cancer, a reduced risk of cancer infection, transmission and/or progression, reduced severity of cancer, a cure of a cancer, an alleviation of symptoms, or a reduction in severity of a cancer or cancer symptoms.

The term “treatment”, means a cure of cancer, an alleviation of symptoms, or a reduction in severity of a cancer or cancer symptoms.

The term “prevention” in the context of oral cancer means the prevention of transformation from oral dysplasia to oral cancer.

By “antibody” we include substantially intact antibody molecules, as well as chimeric antibodies, human antibodies, humanised antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bispecific antibodies, antibody heavy

chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same. In particular, the term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen, whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein having a binding domain which is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes (e.g., IgG, IgE, IgM, IgD and IgA) and their isotypic subclasses; fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb, Fd; and diabodies. Antibodies may be polyclonal or monoclonal. A monoclonal antibody may be referred to as a "mAb".

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the CDRs, of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400, incorporated herein by reference. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

As antibodies can be modified in a number of ways, the term "antibody" should be construed as covering any specific binding member or substance having a binding domain with the required specificity. Thus, this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, humanised antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023, incorporated herein by reference. A humanised antibody may be a modified antibody having the variable regions of a non-human, e.g., murine, antibody and the constant region of a human antibody. Methods for making humanised antibodies are described in, for example, US Patent No. 5225539, incorporated herein by reference.

The antibodies of the present disclosure may be intact or engineered For example, the antibody may be fully or partially glycosylated and/or selected for increased or diminished binding to human effector systems such as complement, FcR-bearing effectors, such as macrophages, or to extend or reduce half-life. These modifications can be made to improve effectiveness and potentially also reduce toxic side effects.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding molecules for use in the invention are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments; (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding

site; (viii) bispecific single chain Fv dimers (PCT/US92/09965, incorporated herein by reference) and; (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804, incorporated herein by reference).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (*Proc Natl Acad Sci USA*. 1990 Mar;87(6):2264-8), modified as in Karlin and Altschul (*Proc Natl Acad Sci USA.* 1993 Jun 15;90(12):5873-7). The NBLAST and XBLAST programs of Altschul et al. have incorporated such an algorithm, and may be used under standard parameters.

The skilled person will understand that optional features of one embodiment or aspect of the invention may be applicable, where appropriate, to other embodiments or aspects of the invention.

References to any publications herein shall be taken as incorporation by reference for the purposed of US patent prosecution only.

Embodiments of the invention will now be described in more detail, by way of example only, with reference to the accompanying drawings.

Figure 1. Overexpression of the target antigens in head and neck squamous carcinoma (HNSC) and other types of cancer of potential interest. Both MAGED4B and FJX1 transcripts are expressed at significantly higher levels in Head and Neck tumours, than in adjacent normal tissue. Regarding other cancer types, MAGED4B expression is also significantly higher in lung adeno (LUAD), squamous (LUSC) carcinomas and Cholangiocarcinoma (CHOL), whilst FJX1 is significantly higher in many tumour types compared to their respective adjacent normal tissues. Transcriptional data is from The Cancer Genome Atlas (TCGA), processed by Li et al (2016) and publicly available online at cistrome.shinyapps.io/timer. This data demonstrates that the target cancer antigens are expressed in multiple cancers, depicting the utility of the vaccine as described here.

Figure 2. Both MAGED4B and FJX1 antigens are strongly expressed in HPV negative squamous cell carcinoma (SCC), non-malignant oral dysplasia and nasopharyngeal carcinoma (NPC). MAGED4B (Novus-Bio:NBP1-89594) was stained at 1:200 dilution. FJX1 (Novus-Bio:NBP1-59470) was stained at 1:100 dilution. Staining was performed on a DAKO autostainer. Testis serves as a positive control with strong expression. Oral fibroepithelial polyp (FEP) serves as negative control, indicating low levels of expression in non-dysplastic oral tissue. This data confirms that the target cancer antigens are expressed as proteins.

Figure 3. MAGED4B (D4B) is immunogenic in patient with HPV negative HNSCC patients. The circulating D4B specific CD8 T cells were detected in 5/7 HLA-A2+ patients HNSC patients (4 shown here but not in HLA-A2+ heathy donor (HD1) $D4B^{501-509}$/HLA-A2-PE tetramer staining in flow cytometry. Staining was performed using peripheral blood mononuclear cells (PBMC) from patients undergoing surgery in Pool, UK. 10^6 PBMC were strained with anti-CD3 (FITC:OKT3), CD4 (APC:OKT4), CD8 (PE-Cy7:SK1) (BioLegend), DAPI (live/dead stain) (Miltenyi), and $MAGED4B^{501-509}$ tetramer (PE). Flow cytometry performed on a FACSCanto, using UltraComp eBeads (Invitrogen) for compensation. Analysis performed using FlowJo; the gating was on live/ dead lymphocytes and CD8 and then tetramer. Tetramer positive CD8 cells are indicated in the gates. This data confirms that in patients

with confirmed HNSCC that there exist a pool of T cells that are available for expansion by vaccination. Further, this is encouraging data which suggests that the presence of such T cells alone is not causing any pathology in these individuals, although they are not fully functional otherwise the individuals would be controlling their tumour using such cells.

Figure 4 (A and B). CD8 T cell specific for both target antigens MAGED4B and FJX1. Figure 4A. D4B specific CD8 T cells were detected in tumour infiltrating lymphocytes (TILs; expanded with 6000IU/ml recombinant human IL-2) in HLA-A2+ HNSCC patient HN337 using $D4B^{501-509}$/HLA-A2 tetramer staining and flow cytometry. 10^6 expanded TILs were strained with anti-CD3 (FITC:OKT3), CD4 (APC:OKT4), CD8 (APC-Cy7) (BioLegend), DAPI (live/dead stain) (Miltenyi) and $MAGED4B^{501-509}$ tetramer (PE). Flow cytometry performed on a FACSCanto, using UltraComp eBeads (Invitrogen) for compensation. Analysis was performed using FlowJo. The gating was on live/ dead lymphocytes, then CD8 plus tetramer. Tetramer positive CD8 cells are indicated in the gates. Figure 4**B.** IFNγ CD8 T cells in expanded TILs from HLA-A2 negative, HLA-A1 positive HN337 tumour sample. Expanded with anti-CD3 (clone OKT3) TILs were stimulated with control (peptide pool MAGED4B HLA-A2 peptides), MAGED4B peptide pool consisting of 15mer peptides with 11aa overlap for the entire sequence of the antigen (183 peptides pooled), FJX1 peptide pool (predicted by NetMHC 4.0, www.iedb.org) to bind HLA-A1 only (3 of 15 mer peptides derived from the FJX1 aa sequence: AR**FADGTRACVRY**GI; DLV**QWTDLILFDY**LT; **WTDLILFDY**LTANFD epitopes are in bold) for 8 h and intracellular IFNγ staining was performed followed by flow cytometry. The antibody panel for flow was anti-CD3 (FITC:OKT3), CD8 (PerCp-Cy5.5: RPA-T8), anti-CD56 (PE: HCD56), IFNγ (APC 4S.B3) (all from BioLegend) and Zombi Aqua (live/dead stain) (Miltenyi) was applied after blocking Fc receptors using HuMan TrueStain (Biolegend). Gates show specific populations of IFNγ CD8 positive T cells after restimulation with the reagent indicated at the top of the plot. The data in these figures again demonstrates that in patients with confirmed HNSCC that there exists a pool of T cells that are available for expansion by vaccination and these cells can be also found in the tumour. Further, this is encouraging data which suggests that the presence of such T cells alone is not causing any pathology in these individuals, although they are not fully functional otherwise the individuals would be controlling their tumour using such cells.

Figure 5. CD8 T cell specific for both target antigens MAGED4B and FJX1. MAGED4B and FJX1-specific CD8 T cells were detected in tumour infiltrating lymphocytes (TILs; expanded with 6000IU/ml recombinant human IL-2) in HLA-A2+ Malaysian OSCC patient 06-0021-18 using $MAGED4B^{501-509}$ and $FJX1^{15-25}$ HLA-A2 tetramer staining and flow cytometry. Expanded TILs were stained with anti-CD3 (FITC; SK7), CD4 (PerCP-Cy5.5; SK3), CD8 (BV510; RPA-T8) (BD Biosciences), FVS780 (viability stain) (BD Biosciences) and $MAGED4B^{501-509}$ tetramer (PE). Flow cytometry performed on a BD LSRFortessa, using BD CompBead for compensation. Analysis was performed using FACS DIVA software. The gating was on live/ dead lymphocytes, then CD8 plus tetramer. Tetramer positive CD8 cells are indicated in the gates. This data demonstrates that antigen-specific T cells have been identified in the tumours. This further suggests that there is a pool of cells available for expansion in addition to *de novo* activation and expansion from the vaccine.

Figure 6. T cells specific for the target antigen MAGED4B express PD1 in HNSC patients and hence can be targeted with anti-PD1. The specific T cells were detected using $MAGED4B^{501-509}$ tetramer in circulation using PBMCs. Half of the tetramer positive population were also PD1+ . This subpopulation is absent in the PBMCs of a HLA*A2 negative HNSC patient (control group). The panel

consisted of CD3 (FITC:OKT3), CD4 (APC:OKT4), CD8 (PE-Cy7:SK1), PD-1 (PerCP-Cy5.5:EH12.2H7), CD19 (Pacific Blue:HIB19.11,) and CD14 (Pacific Blue:HCD14) (BioLegend), Live/Dead Violet (Pacific Blue) (Invitrogen), and MAGED4B$^{501-509}$ tetramer (PE). Flow cytometry performed on a FACSCanto, using UltraComp eBeads (Invitrogen) for compensation. Analysis performed using FlowJo software. This data confirms the specificity of HLA-2 tetramer, since in the HLA-2 negative patient (HN366) it does not work. In the HLA-2 positive patient (HN364) this data confirms the antigen sensitivity of T cells.

Figure 7. Assembly of MAGED4B and FJX1 targeting DNA vaccines to target head and neck cancer. Diagram of vaccine constructs of pDOM (control), vaccines delivering single antigen pDOM-MAGED4B and pDOM-FJX1, and both antigens simultaneously pDOM-MAGED4B-FJX1 vaccines. DOM fragment of tetanus toxin gene and the gene of interest (MAGED4B or FJX1) were linked using a seven amino acid linker 1 (AAAGPGP). The fused gene was inserted between CMV/T7 dual promoter and BGH Poly (A) site. The leader sequence encoding mus IgH signal peptide (MGWSCIIFFLVATATGVHS) was inserted at the N terminus of the construct to enhance the efficacy of secretion. If the gene of interest was encoding the fused MAGED4B and FJX1 antigen, a five amino acids linker 2(GSGSG) was applied to link those two genes. DOM1 was inserted into pcDNA3.0 vector using *Not*I and *Hind*III restriction sites to generate pDOM vector. The genes for MAGED4-B, FJX1 or their fusions of interest were inserted into pDOM vector at *Not*I and *Xho*I restriction enzyme sites to generate the DNA vaccines. As used herein, p in terms of the vector relates to a plasmid vector or construct.

Figure 8. Three groups of 5-6 non-tumour bearing HHD (transgenic for the human HLA-A2 allele) mice were vaccinated with 50 microgram of p.Dom-MAGED4B (A), p.Dom-FJX1 (B) or p.Dom (C) individually on day 1 following by a booster injection of the same DNA vaccine with electroporation on d 22. Their immunogenicity was evaluated by IFNγ ELISpot. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on d 35 and overlapping peptides pool for each target antigen MAGED4B and FJX1 were used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence of each antigen (183 individual peptides were pooled for MAGED4B and 107 peptides for FJX1). P30 peptide was used as a standard for vaccination. Individual peptides were generated by JPT, Germany, to 90% purity. For ELISpot IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were spots were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassberg, Germany). This data confirms the immunogenicity of the antigens in HAL-A2 transgenic mice.

Figure 9 (A-D). DNA vaccines are efficacious as treatment alone and in combination with anti-PD1. Figure 9A depicts the treatment strategy. Individual group of mice (6-10) were challenged with the B16 tumour expressing both MAGED4B and FJX1 and then were treated combined DNA vaccines (DV; p.Dom –MAGED4B and p.Dom FJX1; 100 µg/ mouse in 100 µl of saline injected intra-muscularly 50 µg into each leg) anti-PD-1 antibody (200 µg per injection given i.p. in 0.5 mL) or combination of DV and anti-PD1 as indicated in the diagram. The control group was given control IgG + pDOM (vector backbone). Tumour size was measured every 2-3 days and the tumours sizes for each group are depicted in Figure 9B (mean + s.e.m) Figure 9C. ELISpot assay demonstrated the MAGED4B specific immune responses in a specific fashion in DV and DV + a-PD1 groups but not in a-PD1 or control groups: splenocytes isolated from vaccinated animals are able to secrete IFN-γ upon re-

stimulation with MAGED4B peptide library overlapping the entire antigen sequence (183 peptides 15mer 11 aa overlap pooled together). Figure 9D as in Figure 9C but FJX1 peptide library overlapping the entire antigen was used. This data shows clear impact on tumour progression of the monotherapy (vaccine alone) and the synergistic effect of the combination therapy (vaccine plus anti-PD1). In particular, Figures 9C and 9D show that vaccination expands antigen-specific T cells in tumour bearing mice – these mice have been exposed to the antigens on the tumour but have failed to mount a good immune response. This supports the assertion that the vaccine can improve the immune response and effectively "expose" the tumour to the immune system.

Figure 10 (A-C). DNA vaccine is efficacious in inhibiting tumour growth. Figure 10A depicts the treatment strategy tumour volume reduction and mechanisms of actions. Individual group of mice (8-12) were challenged with the tumour expressing both MAGED4B and FJX1 and then were treated with DNA vaccines (DV; p.Dom –MAGED4B and p.Dom-FJX1; 100 µg/mouse in 100 µl of saline injected intra-muscularly 50 µg into each leg) as indicated in the diagram. The control group was given pDOM vector backbone. Tumour size was measured every 2-3 days and the tumours sizes for each group are depicted in Figure 10A (mean + s.e.m). Figure 10(B) is cell photographs. The staining of the tumour in the right hand panel demonstrates T cell infiltration following vaccination with DNA vaccines, the left hand panel shows poorly infiltrated pDOM control tumours (Hematoxylin & eosin stain, original magnification: x10 objective). Figure 10C depicts the results of flow cytometry analysis, demonstrating increased in CD4+ and CD8+ immune cells in the tumour harvested from the vaccinated animals compared to control animals. Significantly, checkpoint protein PD1 is found to be markedly elevated in both CD4+ and CD8+ immune cells harvested from vaccinated animals. This study has used a higher dose of tumour cells to accelerate the progression of the cancer, and thus is a particularly aggressive tumour model. This again shows that vaccination expands the T cells in tumour bearing mice, in which the mice have failed to raise a good immune response to the tumours despite being exposed to them. This supports the assertion that the vaccine can improve the immune response and effectively "expose" the tumour to the immune system.

Figure 11 (A and B). Demonstration of critical components for the design of MAGED4B and FJX1 targeting DNA vaccines. For both Figures, the treatment strategy is depicted. Four groups of 5 non-tumour bearing C57BL/6 mice were vaccinated with 50 µg of p.Dom-MAGED4B-FJX1, pSP-MAGED4B-FJX1 (no DOM), pDom-FJX1, pnoSPDOM-FJX1 (no Leader/SP) individually on day 1 following by a booster injection of the same DNA vaccine on day 8. Their immunogenicity was evaluated by IFNγ ELISpot. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on d 22 and overlapping peptides pool for each target antigen MAGED4B and FJX1 were used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence of each antigen (183 individual peptides were pooled for MAGED4B and 107 peptides for FJX1). P30 peptide an MHCII peptide from DOM was used as a control for vaccination. Figure 11**A** shows the data for with and without DOM**.** The Dom sequence is shown to be critical for induction of T cells and therefore improved the response for MAGED4B. Figure 11**B** shows the data for with and without a leader sequence**.** A leader sequence that directs the expression of the encoded construct to endoplasmic reticulum for secretion is also essential for induction of T cell immunity. Individual peptides were generated by JPT, Germany, to 90% purity. For ELISpot IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate

reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassberg, Germany). This data shows that generally FJX1 responses are improved by the inclusion of a leader sequence.

Figure 12. DNA vaccine targeting both antigens in tandem as a fusion antigen induces comparable T cell response to DNA vaccine targeting single antigens. The treatment strategy is depicted. Three groups of 5 non-tumour bearing C57BL/6 mice were vaccinated with 50 µg of p.Dom-MAGED4B, pDom-FJX1 or p.Dom-MAGED4B-FJX1 individually on day 1 following by a booster injection of the same DNA vaccine on day 8. Their immunogenicity was evaluated by IFNγ ELISpot. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on day 22 and overlapping peptides pool for each target antigen MAGED4B and FJX1 were used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence of each antigen (183 individual peptides were pooled for MAGED4B and 107 peptides for FJX1). P30 peptide an MHCII peptide from DOM was used as a control for vaccination. DNA vaccine targeting both antigens showed similar capability of inducing specific T cell response. P values were calculated with Mann-Whitney analysis by Graphpad prism 8.0.For ELISpot IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany).

Figure 13. Assembly of alternative MAGED4-B and FJX1 targeting DNA vaccines to target cancer. Diagram of vaccine constructs of pDOM (control), pMAGED4B/FJX1, pMAGED4B/FJX1-MITD, pPVXCP-MAGED4B/FJX1 and pMIP3α-MAGED4B/FJX1. Alternative gene fusion partners includes MITD, PVXCP, and MIP3α. MITD (165bp) encodes MHC I (HLA-A2) trafficking signals. PVXCP (732bp) encodes potato virus X coat protein. MIP3α (252bp) encodes macrophage inflammatory protein 3 alpha. MITD, PVXCP and MIP3α gene were optimised with human codon usage and ordered from GeneArt (Invitrogen). The genes (with or without fusion partner) were inserted between CMV/T7 dual promoter and BGH Poly (A) site. The leader sequence encoding mouse IgH signal peptide (MGWSCIIFFLVATATGVHS) was inserted at the N terminus of the construct to enhance the efficacy of secretion. Fusion partners and the gene of interest (MAGED4B or FJX1) were linked using a seven amino acid linker 1 (AAAGPGP). With the exemption of MITD, all other fusion partners were fused at the upstream of gene of interest. MITD were added at the downstream of gene of interest. The genes for MAGED4-B, FJX1 or their fusions of interest were inserted into pcDNA3 vector at NotI, XhoI and XbaI restriction enzyme sites to generate the DNA vaccines.

Figure 14 MAGED4B specific T cell responses were induced by DNA vaccines on C57BL/6 mice. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM vaccine (as a negative control, 3 mice), 50 µg pSP-MAGED4B (5 mice), 50 µg pSP-MAGED4B-MITD (5 mice), 50 µg pPVXCP-MAGED4B (5 mice), and 50 µg pMIP3a-MAGED4B (5 mice) on day 1 and day 8. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on day 22 and overlapping peptides pool for MAGED4B was used to detect responding specific T cells. The overlapping peptide pools (OPP) consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B). IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to MAGED4B OPP in each group respectively. The values were the responses minus the number of spots without stimulus. Cut off set

as 2x background (Irr OPPs, shown as red-dotted line). P values were calculated by Mann-Whitney test. (Irr = irrelevant). This data shows that MAGED4B responses can be generated from full length protein, and that the response can be improved by the fusion with various helper motifs.

Figure 15 FJX1 specific T cell responses were induced by DNA vaccines on C57BL/6 mice. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM vaccine (as a negative control, 3 mice), 50 µg pSP-FJX1 (5 mice), and 50 µg pSP-FJX1-MITD (5 mice). The vaccinated were administered i.m. (intra-muscularly) on day 1. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on day 14 and overlapping peptides pool for FJX1 was used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence (107 individual peptides were pooled for FJX1). IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to FJX1 OPP in each group respectively. pSP-FJX1-MITD induced the strongest response among all the groups. Median+Interquatile and responses in individual mice are shown. The results were normalised by subtracting the number of spots without stimulus. Cut off set as 2x background (Irr OPPs, shown as red-dotted line). P values were calculated by Mann-Whitney test. Only one experiment was represented, since lab shutdown over the pandemic restricted repeating these experiments.

Figure 16. Amino acid sequence maps of the whole MAGED4B amino acids sequence and three truncated fragments. As a member of melanoma-associated antigen family, MAGED4B contains a MAGE common homology domain MAGED4B 412-682(SEQ ID No. 3). The defined HLA-A2 epitope RLSLLLVIL (MAGED4B501-509) sits inside the homology domain. Three truncated MAGED4B sequences were designed as following: 1) MAGED4B sequence version (v) 1 doesn't contains homology domain but contains RLSLLLVIL; 2) MAGED4B.sv2 retains second half of homology domain (MAGED4B 510-682) including RLSLLLVIL; 3) MAGED4B.sv3 retains first half of homology domain (MAGED4B 412-500) including RLSLLLVIL. These are all, therefore, immunogenic fragments of MAGED4B suitable for use in the vaccine described here.

Figure 17. Fragments of MAGED4B as detailed in Figure 16 are shown to be immunogenic. The treatment strategy is shown: four groups of 5 non-tumour bearing C57BL/6 mice were vaccinated with 50 µg of p.Dom-MAGED4B (full length), p.Dom-MAGED4Bsv1, p.Dom-MAGED4Bsv2, p.Dom-MAGED4Bsv3 individually on day 1 following by a booster injection of the same DNA vaccine on d 22. Their immunogenicity was evaluated by IFNγ ELISpot. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on d 35 and overlapping peptides pool for MAGED4B was used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B). P30 peptide an MHCII peptide from tetanus DOM was used as a control for vaccination. p.Dom-MAGED4Bsv3 induced significantly stronger specific T cell response than p.Dom-MAGED4B (full length), p.Dom-MAGED4Bsv1, and p.Dom-MAGED4Bsv2. Neither p.Dom-MAGED4Bsv1 or p.Dom-MAGED4Bsv2 performed better than p.Dom-MAGED4B. P values were calculated with one-way ANOVA analysis by Graphpad prism 8.0. For ELISpot IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun

Diagnostika GmbH, Strassburg, Germany). Thus, the MAGE homology domain can be removed in whole or in part without affecting the activity of the vaccine.

Figure 18. Vector maps of the closed linear DNA (dbDNA™) used in vaccination experiments. Shown are the sequences at the closed ends of the dbDNA (TelR or TelL from the protelomerase target sequence TelRL) – these are portions of the target sequence that together make a whole sequence. Four construct architectures are shown: Basic 0 (minimal construct architecture – CMV promoter, Dom and antigen fusion, SV40 poly A signal sequence) upon which all other constructs are based. Added to other constructs are: Basic 1 (plus TE – Triple enhancer); SV40 enh (plus TE and SV40 enhancer sequence); CpG (plus TE and a section of sequence with CpG motifs). These are used in the experimental vaccination work described herein.

Figure 19. MAGED4B specific T cell responses were induced by doggy bone (DB) and plasmid DNA vaccines in C57BL/6 mice. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 25 µg DB-MAGED4B-CO (5 mice), and 25 µg pDOM-MAGED4B plasmid (5 mice). The vaccinated were administered i.m. with EP at day 1. Electroporation (EP) was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM) from Ichor EP device. Lymphocytes isolated from mouse spleens were plated to ELISpot plates on day 14 and overlapping peptides pool for MAGED4B was used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B, 107 individual peptides were pooled for FJX1). P30 peptide an MHCII peptide from tetanus DOM was used as a control to valid vaccination. FJX1 OPP served as Irr peptide control in this experiment. IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to p30 peptide and MAGED4B OPP in each group respectively. Median+ Interquatile and responses in individual mice are shown. The values were the responses minus the number of spots without stimulus. Cut off set as 2x background (Irr OPPs, shown as red-dotted line). P values were calculated by Mann-Whitney test.

Figure 20. FJX1 specific T cell responses were induced by doggy bone (DB) and plasmid DNA vaccines. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 25 µg DB-DOM-FJX1 CO (5 mice), and 25 µg pDom-FJX1 plasmid (5 mice). The vaccines were administered i.m. with EP on day 1. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM Ichor Medical System). Lymphocytes isolated from mouse spleens were plated to ELISpot plates on day 14 and overlapping peptides pool for FJX1 was used to detect responding specific T cells. The overlapping peptide pools (OPP) consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B, 107 individual peptides were pooled for FJX1). P30 peptide an MHCII peptide from tetanus DOM was used as a control to valid vaccination. MAGED4B OPP served as Irr peptide control in this experiment. IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to p30 peptide and FJX1OPP in each group respectively. Median+ Interquatile and responses in individual mice are shown. The values

were the responses minus the number of spots without stimulus. Cut off set as 2x background (Irr OPPs, shown as red-dotted line). P values were calculated by Mann-Whitney test.

Figure 21. dbDNA DOM-MAGED4B and pDOM-MAGED4B DNA vaccines induce specific CD4 and CD8 T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated with 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 10 µg DB-MAGED4B-CO (5 mice), 10 µg DB-MAGED4B-CO basic 1 (5 mice), 10 µg DB-MAGED4B-CO SV40 (5 mice), 10 µg DB-MAGED4B-CO CpG (5 mice), and 10 µg pDOM-MAGED4B plasmid (5 mice). The vaccines were administered i.m. with EP at day 1 and day 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). FACS was performed following *in vitro* stimulation 50 µl blood samples taken on day 12, bleeds were treated using red blood lysis buffer (RBC lysis buffer, Biolegend) first. White blood cells were stimulated with 1µM MAGED4B overlapping peptide pool (OPP; in 96-well plate, added 1 µl of anti-CD107a-FITC and anti-CD107b-FITC (both from Biolegend) and incubated at 37°C 5% CO2 overnight. The following day the cells were wash and stained with anti-CD3-PE, anti-CD4-PEcy7, anti-CD8-APCcy7, anti-CD137 (4-1BB)-APC, anti-PD1-PerCPcy5 (all from Biolegend), and live/dead-violet (Invitrogen). The OPP consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B). Flow cytometry was performed on a FACSCanto II. Analysis was performed using FlowJo software. The responses were evaluated using FACS. CD107a/b+ PD-1+ double positive T cells and 4-1BB+ PD-1+ double positive T cells indicate cytotoxic and activated population specific to MAGED4B OPP respectively. The responses to MAGED4B OPP from groups vaccinated with pDOM, DB-MAGED4B-CO, DB-MAGED4B-CO basic 1, DB-MAGED4B-CO SV40, DB-MAGED4B-CO CpG and pDOM-MAGED4B plasmid respectively were summarised in this column graph. Median+ Interquatile and individual mice responses are shown. P values were calculated by Kruskal-Wallis test. This data shows that the MAGED4B antigen can be delivered in different architectures and still induce antigen-experiences CD4 and CD8 T cells. CO is a codon optimised sequence.

Figure 22. Doggybone DOM-FJX1 and pDOM-FJX1 DNA vaccine induces specific CD4 and CD8 T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated with 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 10 µg DB-FJX1-CO (5 mice), 10 µg DB-FJX1-CO basic 1 (5 mice), 10 µg DB-FJX1-CO SV40 (5 mice), 10 µg DB-FJX1-CO CpG (5 mice), and 10 µg pDOM-FJX1 plasmid (5 mice). The vaccines were administered i.m. with EP at day 1 and day 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). FACS was performed following In vitro stimulation 50 µl bleed blood samples taken on day 12, (50 µl bleeds were used which were treated using red blood lysis buffer (RBC lysis buffer, Biolegend) first. White blood cells were stimulated with 1µM FJX1 overlapping peptide pool (OPP; in 96-well plate, whilst added 1 µl of anti-CD107a-FITC and anti-CD107b-FITC (both from Biolegend) and incubated at 37°C 5%CO2 overnight. The following day the cells were wash and stained with anti-CD3-PE, anti-CD4-PEcy7, anti-CD8-APCcy7, anti-CD137 (4-1BB)-APC, anti-PD1-PerCPcy5 (all from Biolegend), and live/dead-violet (Invitrogen). The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence (107 individual peptides were pooled for FJX1). Flow cytometry was performed on a FACSCanto II. Analysis was performed using FlowJo software. The responses were evaluated using FACS. CD107a/b+ PD-1+ double positive T cells and 4-1BB+ PD-1+ double positive T cells indicate cytotoxic and activated population specific FJX 1 OPP respectively. T cell responses to FJX1 OPP from groups

vaccinated with pDOM, DB-FJX1-CO, DB-FJX1-CO basic 1, DB-FJX1-CO SV40, DB-FJX1-CO CpG, and pDOM-FJX1 plasmid respectively were summarised in this column graph. Median+ Interquatile and individual mice responses are shown. P values were calculated by Kruskal-Wallis test. This data shows that the FJX1 antigen can be delivered in different architectures and still induce antigen-experiences CD4 and CD8 T cells. CO is a codon optimised sequence.

Figure 23. Doggybone DOM-MAGED4B and pDOM-MAGED4B DNA vaccines induce specific CD4 and CD8 T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 10 µg DB-MAGED4B-CO (5 mice), 10 µg DB-MAGED4B-CO basic 1 (5 mice), 10 µg DB-MAGED4B-CO SV40 (5 mice), 10 µg DB-MAGED4B-CO CpG (5 mice), and 10 µg pDOM-MAGED4B plasmid (5 mice). The vaccines were administered i.m. with EP at day 1 and day 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). On d 35 lymphocytes were isolated from mouse spleens and were plated to ELISpot plates and overlapping peptides pool (OPP) for MAGED4B was used to stimulate responding specific T cells. The OPP consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B). P30 peptide an MHCII peptide from tetanus DOM sequence was used to assess induction of CD4 responses against Dom helper sequence. IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to p30 peptides, Irr OPP (FJX1 OPP), and MAGED4B OPP. DB-MAGED4B-CO basic 0 induced the strongest response among all the groups. Median+Interquatile and responses in individual mice are shown. Cut off set as 2x background (Irr OPPs), shown as a dotted line. The values were the responses minus the number of spots without stimulus. The number of spots/10^6 cells is shown. P values were calculated by Mann-Whitney test. This data shows that the MAGED4B antigen can be delivered in different architectures and still induce antigen-experiences CD4 and CD8 T cells. CO is a codon optimised sequence.

Figure 24. dbDNA (DB) DOM-FJX1 and plasmid (p) DOM-FJX1 DNA vaccines induce specific CD4 and CD8 T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 10 µg DB-FJX1-CO (5 mice), 10 µg DB-FJX1-CO basic 1 (5 mice), 10 µg DB-FJX1-CO SV40 (5 mice), 10 µg DB-FJX1-CO CpG (5 mice), and 10 µg pDOM-FJX1 plasmid (5 mice). The vaccines were administered i.m. with EP at day 1 and day 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). Lymphocytes isolated from mouse spleens were plated to ELISpot plates on d 35 and overlapping peptides pool for FJX1 was used to detect responding specific T cells. The overlapping peptide pools (OPP) consisted of 15 mer peptides with 11aa overlap for the entire sequence (107 individual peptides were pooled for FJX1). P30 peptide an MHCII peptide from tetanus DOM sequence was used to assess induction of CD4 responses against Dom helper sequence. IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graph shows the responses to p30 peptides, Irr OPP (MAGED4B OPP), and FJX1 OPP. All groups performed with similarities. Median+ Interquatile and responses in individual mice are shown. Cut off set as 2x background (Irr OPPs), shown as a dotted line. Cut off set

as 2x background (Irr OPPs), shown as a dotted line. The values were the responses minus the number of spots without stimulus. The number of spots/10^6 cells is shown. P values were calculated by Mann-Whitney test. This data shows that FJX1 can be delivered by multiple architectures can induce antigen-specific CD4 and CD8 T cells.

Figure 25. dbDNA (DB) DOM-MAGED4B and plasmid (p) DOM-MAGED4B DNA vaccines induce specific T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 25 µg DB-MAGED4B-CO (6 mice), 25 µg DB-MAGED4B-CO basic 1 (6 mice), 25 µg DB-MAGED4B-CO SV40 (6 mice), and 25 µg pDOM-MAGED4B plasmid (6 mice). The vaccines were administered i.m. with EP at day 1 and day 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). On day 35 lymphocytes were isolated from mouse spleens and were plated to ELISpot plates and overlapping peptides pool (OPP) for MAGED4B was used to stimulate responding specific T cells. The OPP consisted of 15 mer peptides with 11aa overlap for the entire sequence (183 individual peptides were pooled for MAGED4B). IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). Response to MAGED4B OPP is shown. Median+Interquatile and individual mice are shown. The values were the responses minus the number of spots without stimulus. P values were calculated by Mann-Whitney test.

Figure 26. dbDNA (DB) DOM-FJX1 and plasmid (p) DOM-FJX1 DNA vaccines induce specific CD4 and CD8 T cell responses. The treatment strategy is depicted. Non-tumour bearing C57BL/6 mice were vaccinated 50 µg pDOM plasmid vaccine (as a negative control, 3 mice), 10 µg DB-FJX1-CO (5 mice), 10 µg DB-FJX1-CO basic 1 (5 mice), 10 µg DB-FJX1-CO sv40 (5 mice), 10 µg DB-FJX1-CO CpG (5 mice), and 10 µg pDOM-FJX1 plasmid (5 mice) on day 1 and d 21. EP was carried out on mice anaesthetised by isofluorane, using intramuscular TriGrid Delivery System (TDS-IM; Ichor Medical System). Lymphocytes isolated from mouse spleens were plated to ELISpot plates on d 35 and overlapping peptides pool for FJX1 was used to detect responding specific T cells. The overlapping peptide pools consisted of 15 mer peptides with 11aa overlap for the entire sequence (107 individual peptides were pooled for FJX1). IFNγ ELISpot kit from BD Bioscience was used according to manufacturer's protocol. Spots corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The values were the responses minus the number of spots without stimulus. The graph shows the responses to FJX1 OPP. Median+Interquatile and responses in individual mice shown.

Figure 27. Cancer Associated Fibroblasts (CAF) express MAGED4B. Immunohistochemical analysis of HNSCC cases, demonstrating MAGED4B expression in cancer cells and cancer associated fibroblast, the latter demonstrating strong expression. Anti-MAGED4B monoclonal antibody (Santa Cruz, G12; sc-393059) was used on HNSCC tissues at 1:50 dilution. Staining was performed on a DAKO autostainer (K4065). Six individual HNSCC cases which were available in Pathology lab in Southampton General hospital (Southampton, UK) are presented.

Figure 28 (A and B) Cancer Associated Fibroblasts (CAF) express MAGED4B. Figure 28A: Publicly available single-cell RNA-sequencing (scRNA-seq) data, generated from Smart-Seq analysis of HNSCC

patient samples was used to assess MAGED4B expression across different cell-types. Cell lineages were identified as described by Puram *et al* [Cell]. 2017 Dec 14;171(7):1611-1624. Figure 28B: Fibroblast subpopulations were identified by unsupervised hierarchical clustering as implemented in the Seurat R package (v3.2) (Butler A *et al*, Nat Biotechnol. 2018 Jun;36(5):411-420). Fibroblast subpopulation markers MCAM, ACTA2 and POSTIN were identified using a Wilcox-test and compared with those previously identified for lung cancer fibroblast subpopulations to annotate different subpopulations (CJ Hanley *et al*, bioRxiv 2020.06.08.134270).

Figure 29 Both MAGED4B and FJX1 antigens are strongly expressed in colon, prostate, rectal, breast, lung and nasopharyngeal carcinoma. Expression levels of the target proteins were detected by immunohistochemistry (IHC) using anti-MAGED4B (1:100; Sigma Aldrich, Cat. #HPA003554) and anti-FJX1 (1:200; Sigma Aldrich, Cat. #HPA059220) antibodies, and processed using Dakocytomation Envision+Dual Link System HRP (DAB+) kit (Dako, Cat. #K4065) as described previously.

SEQUENCES

Sequences, or encoded sequences, of the potential DNA vaccine components are described below. The DNA vaccine of the invention may comprise any one of the nucleic acid sequences provided below, or variants thereof. Alternatively, or additionally, the DNA vaccine of the invention may comprise nucleic acid encoding any one of the amino acid sequences provided below, or variants thereof.

Amino acid sequences of the genes in the DNA vaccine assembled as in Figure 7:

Leader sequence (SEQ ID NO: 1)

MGWSCIIFFLVATATGVHS

DOM (SEQ ID NO: 2)

KNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDIEYNDMFNNFTVSFWLRVPK
VSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGS
AEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGN

MAGED4B (SEQ ID NO: 3)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERA
TYTLEKKFGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDLRKLITDDFVK
QKYLEYKKIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHARAQMRAQMNIGDEALI
GRWSWDDIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTRNAARAGASF
FSWIQHR

FJX1 (SEQ ID NO: 4)

MGRRMRGAAATAGLWLLALGSLLALWGGLLPPRTELPASRPPEDRLPRRPARSGGPAPAPRFPLPPPLAWDARGGSLKTFRALLTLAAGAD
GPPRQSRSEPRWHVSARQPRPEESAAVHGGVFWSRGLEEQVPPGFSEAQAAAWLEAARGARMVALERGGCGRSSNRLARFADGTRACVR
YGINPEQIQGEALSYYLARLLGLQRHVPPLALARVEARGAQWAQVQEELRAAHWTEGSVVSLTRWLPNLTDVVVPAPWRSEDGRLRPLRDA
GGELANLSQAELVDLVQWTDLILFDYLTANFDRLVSNLFSLQWDPRVMQRATSNLHRGPGGALVFLDNEAGLVHGYRVAGMWDKYNEPLL
QSVCVFRERTARRVLELHRGQDAAARLLRLYRRHEPRFPELAALADPHAQLLQRRLDFLAKHILHCKAKYGRRSGT

Linker 1 (SEQ ID NO: 5)

AAAGPGP

Linker 2 (SEQ ID NO: 6)

GSGSG

MAGED4B Nucleotide Sequence (SEQ ID NO: 7):

>NG_029896.1:5001-12446 Homo sapiens MAGE family member D4B (MAGED4B), RefSeqGene on chromosome X

GCATGCGCAGGCTACCCAGCCGCGGGGGGTGCACGGAGAAAAGGGGCGGGGTGGTCCGGGCTGCTGTGCT
GGCAGCAGTAGGCGAGGGCGCGGCTGCGGGGTTCCTGGTGCTGAGGACGGACGCCATTGGAGTTCCCGAG
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TCCAACACAAGTTTGCTGAGCATGTTTTCACTCCACGTAGTCCCTACCCTCAGGACTGGTGGGAGAAGAG
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GGGCCCAGATGAATATCGGGGATGAAGCGCTGATTGGACGGTGGAGCTGGGATGACATACAAGTCGAGCT
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FJX1 Nucleotide Sequence (SEQ ID NO: 8):

>NC_000011.10:35618460-35620865 Homo sapiens chromosome 11, GRCh38.p13 Primary Assembly

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CMV/T7 Dual promoter sequence (SEQ ID NO: 9):

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SEQ ID NO: 10

GGGGG

SEQ ID NO: 11

SSSSS

pDOM.MAGED4B-FJX1 (SEQ ID NO: 12)

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DOM1 = underlined

MAGED4B = double underlined

FJX1 = dotted underlined

pDOM.MAGED4B (SEQ ID NO: 13)

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DOM1 = underlined; MAGED4B = double underlined

pDOM.FJX1 (SEQ ID NO: 14)

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DOM1 = underlined; FJX1 = dotted underlined

CMV promoter (SEQ ID NO:15)

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MAGED4B DNA wild type (SEQ ID NO: 16)

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MAGED4B DNA codon optimised (SEQ ID NO: 17)

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CCTCGGCTGAGCCTGCTGCTCGTGATCCTGGGCGTGATTTTCATGAACGGCAACAGAGCTTCTGAGGCAGTGCTGTGGGAAGCCCTCAG
AAAGATGGGCCTGAGACCCGGAGTCAGACATCCTTTCCTGGGCGACCTGAGAAAGCTGATCACCGACGACTTCGTGAAGCAGAAGTAC
CTGGAATACAAGAAGATCCCTAATAGCAATCCTCCAGAGTACGAGTTCCTGTGGGGCCTGCGGGCCCGCCACGAGACATCCAAGATGA
GAGTGCTGAGGTTCATCGCCCAGAACCAGAACCGCGACCCCAGAGAGTGGAAGGCCCACTTCCTGGAAGCCGTGGATGACGCTTTTAA
GACAATGGATGTGGACATGGCCGAGGAACACGCCCGAGCTCAGATGCGGGCCCAAATGAACATCGGCGACGAGGCCCTGATCGGCAG
ATGGTCCTGGGACGATATCCAGGTGGAACTGCTGACCTGGGATGAGGACGGCGATTTCGGCGACGCCTGGGCCCGAATCCCATTCGCC
TTCTGGGCTAGATACCACCAGTACATCCTGAACAGCAACAGAGCTAACCGTAGAGCCACCTGGCGGGCCGGCGTGTCCAGCGGCACAA
ACGGCGGCGCCTCTACAAGCGTGCTGGACGGCCCAAGCACAAGCAGCACCATCAGAACCAGAAACGCCGCTAGAGCCGGCGCCAGCTT
CTTCAGCTGGATCCAGCATAGATGA

DOM-MAGED4B DNA sequence (SEQ ID NO: 18)

ATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACCTTGATTGTTGGGTCGACAACGAAGA
AGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAACAACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCT
GTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCAACGGCAAAGCTATCCACCTGGTTAACAACGAATCTTCTGAAGTTATCGTG
CACAAGGCCATGGACATCGAATACAACGACATGTTCAACAACTTCACCGTTAGCTTCTGGCTGCGCGTTCCGAAAGTTTCTGCTTCCCAC
CTGGAACAGTACGGCACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTG
AAGGGTAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTCAGATCACTTTCCGCGACCTGCCGGACAAGTTCAACGC
GTACCTGGCTAACAAATGGGTTTTCATCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATCAACGGCGTTCTGATGGGCTCC
GCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCTGGACCGTTGCAACAACAACAACCAGTACGTATC
CATCGACAAGTTCCGTATCTTCTGCAAAGCACTGAACCCGAAAGAGATCGAAAAACTGTATACCAGCTACCTGTCTATCACCTTCCTGCG
TGACTTCTGGGGTAACGCGGCCGCTGGACCCGGACCTATGGCTGAGGGAAGCTTCAGCGTGCAATCGGAAAGCTACAGTGTTGAAGAC
ATGGATGAGGGTAGCGACGAAGTCGGGGAGGAAGAGATGGTTGAAGGCAACGACTATGAAGAATTCGGTGCGTTTGGTGGCTATGGC
ACCCTCACCAGCTTTGACATCCATATCCTCAGAGCCTTCGGAAGCTTGGGTCCAGGCCTTCGCATCTTATCGAATGAGCCCTGGGAACTG
GAAAACCCTGTGCTGGCCCAGACCCTGGTGGAGGCATTGCAGCTGGATCCGGAAACACTTGCCAATGAGACGGCCGCCCGTGCTGCCA
ACGTAGCCCGCGCCGCCGCCTCCAACCGTGCGGCTCGGGCCGCTGCCGCCGCTGCCCGTACCGCCTTCAGTCAGGTGGTCGCTAGCCAC
CGGGTGGCCACGCCGCAGGTCTCAGGAGAGGATACCCAGCCCACGACCTACGCCGCCGAGGCTCAGGGGCCCACCCCTGAGCCACCCC
TTGCTTCTCCGCAGACCTCCCAGATGTTAGTCACCAGTAAGATGGCTGCCCCCGAGGCTCCGGCAACCTCCGCACAGTCCCAGACAGGCT
CCCCGGCCCAGGAGGCTGCTACTGAGGGCCCTAGTAGCGCCTGTGCTTTCTCTCAGGCTCCGTGTGCCAGGGAGGTGGACGCCAACCG

GCCCAGCACAGCCTTCCTGGGCCAGAATGATGTCTTCGATTTCACTCAGCCGGCAGGTGTCAGTGGCATGGCCTTCCCGCGCCCCAAGA
GACCTGCCCCAGCCCAAGAGGCTGCCACAGAGGGCCCCAGTGCTGCCTCTGGTGTGCCCCAGACGGGACCTGGCAGGGAGGTGGCAG
CCACCCGGCCCAAGACCACCAAGTCGGGGAAGGCGCTGGCCAAGACTCGGTGGGTGGAGCCTCAGAATGTTGTGGCAGCAGCTGCTG
CCAAGGCCAAGATGGCCACGAGCATCCCTGAGCCGGAGGGTGCAGCTGCTGCCACTGCTCAGCACAGTGCTGAGCCCTGGGCCAGGAT
GGGAGGCAAGAGGACCAAGAAGTCCAAGCACCTGGATGATGAGTATGAGAGCAGCGAGGAGGAGAGAGAGACTCCCGCGGTCCCAC
CCACCTGGAGAGCATCACAGCCCTCATTGACGGTGCGGGCTCAGTTGGCCCCTCGGCCCCCGATGGCCCCGAGGTCCCAGATACCCTCA
AGGCACGTACTGTGCCTGCCCCCCCGCAACGTGACCCTTCTGCAGGAGAGGGCAAATAAGTTGGTGAAATACCTGATGATTAAGGACTA
CAAGAAGATCCCCATCAAGCGCGCAGACATGCTGAAGGATGTCATCAGAGAATATGATGAACATTTCCCTGAGATCATTGAACGAGCAA
CGTACACCCTGGAAAAGAAGTTTGGGATCCACCTGAAGGAGATCGACAAGGAAGAACACCTGTATATTCTTGTCTGCACACGGGACTCC
TCAGCTCGCCTCCTTGGAAAAACCAAGGACACTCCCAGGCTGAGTCTCCTCTTGGTGATTCTGGGCGTCATCTTCATGAATGGCAACCGT
GCCAGCGAGGCTGTCCTCTGGGAGGCACTACGCAAGATGGGACTGCGCCCTGGGGTGAGGCACCCATTCCTCGGCGATCTGAGGAAGC
TCATCACAGATGACTTTGTGAAGCAGAAGTACCTGGAATACAAGAAGATCCCCAACAGCAACCCACCTGAGTATGAATTCCTCTGGGGC
CTGCGAGCCCGCCATGAGACCAGCAAGATGAGGGTCCTGAGATTCATCGCCCAGAATCAGAACCGAGACCCCCGGGAATGGAAGGCTC
ATTTCTTGGAGGCTGTGGATGATGCTTTCAAGACAATGGATGTGGATATGGCCGAGGAACATGCCAGGGCCCAGATGAGGGCCCAGAT
GAATATCGGGGATGAAGCGCTGATTGGACGGTGGAGCTGGGATGACATACAAGTCGAGCTCCTGACCTGGGATGAGGACGGAGATTT
TGGCGATGCCTGGGCCAGGATCCCCTTTGCTTTCTGGGCCAGATACCATCAGTACATTCTGAATAGCAACCGTGCCAACAGGAGGGCCA
CGTGGAGAGCTGGCGTCAGCAGTGGCACCAATGGAGGGGCCAGCACCAGCGTCCTAGATGGCCCCAGCACCAGCTCCACCATCCGGAC
CAGAAATGCTGCCAGAGCTGGCGCCAGCTTCTTCTCCTGGATCCAGCACCGTTGA

DOM-MAGED4B Codon optimised DNA sequence (SEQ ID NO: 19)

ATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACCTTGATTGTTGGGTCGACAACGAAGA
AGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAACAACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCT
GTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCAACGGCAAAGCTATCCACCTGGTTAACAACGAATCTTCTGAAGTTATCGTG
CACAAGGCCATGGACATCGAATACAACGACATGTTCAACAACTTCACCGTTAGCTTCTGGCTGCGCGTTCCGAAAGTTTCTGCTTCCCAC
CTGGAACAGTACGGCACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTG
AAGGGTAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTCAGATCACTTTCCGCGACCTGCCGGACAAGTTCAACGC
GTACCTGGCTAACAAATGGGTTTTCATCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATCAACGGCGTTCTGATGGGCTCC
GCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCTGGACCGTTGCAACAACAACAACCAGTACGTATC
CATCGACAAGTTCCGTATCTTCTGCAAAGCACTGAACCCGAAAGAGATCGAAAAACTGTATACCAGCTACCTGTCTATCACCTTCCTGCG
TGACTTCTGGGGTAACGCGGCCGCTGGACCCGGACCTATGGCCGAGGGATCTTTTTCTGTGCAGAGCGAAAGCTACAGCGTCGAGGAC
ATGGACGAGGGTTCTGATGAAGTTGGCGAAGAAGAAATGGTGGAAGGAAATGACTACGAGGAGTTCGGCGCCTTCGGCGGCTACGGC
ACCCTGACATCCTTCGACATCCACATCCTAAGAGCCTTCGGCTCTCTGGGCCCTGGTCTTCGGATCCTGTCTAATGAGCCTTGGGAGCTG
GAAAACCCCGTGCTGGCTCAAACCCTGGTGGAAGCACTCCAGCTGGATCCTGAAACCCTGGCCAACGAGACAGCTGCGCGTGCTGCCA
ATGTGGCCAGAGCTGCTGCAAGCAACAGAGCTGCTCGCGCCGCCGCTGCTGCCGCCCGGACAGCCTTTAGCCAGGTGGTGGCCAGCCA
CAGAGTGGCCACTCCTCAGGTTAGCGGCGAGGATACACAGCCTACCACCTACGCCGCCGAAGCCCAGGGCCCCACCCCTGAACCCCCTC
TGGCCTCCCCTCAGACCTCCCAGATGCTGGTGACAAGCAAAATGGCCGCACCTGAGGCCCCTGCCACATCAGCCCAAAGCCAGACAGGC
AGCCCTGCTCAGGAGGCCGCTACTGAGGGCCCTAGCTCAGCTTGTGCCTTCAGCCAGGCCCCGTGCGCCAGAGAGGTGGACGCCAACA
GACCTAGCACCGCCTTCCTGGGCCAGAACGACGTCTTTGATTTCACCCAGCCAGCCGGAGTGTCCGGCATGGCCTTTCCTAGACCCAAGA
GACCTGCCCCTGCCCAGGAGGCCGCCACCGAGGGCCCTAGCGCCGCCAGCGGAGTTCCACAGACCGGCCCCGGCAGAGAAGTGGCCG
CCACGAGACCTAAGACCACAAAGAGCGGCAAAGCCCTGGCTAAGACAAGATGGGTCGAACCGCAAAACGTGGTGGCCGCCGCTGCCG
CCAAGGCCAAAATGGCTACAAGTATCCCTGAGCCTGAGGGCGCTGCCGCGGCCACCGCCCAGCACAGCGCCGAGCCCTGGGCCCGGAT
GGGCGGAAAGAGAACCAAAAAAAGCAAGCACCTCGATGATGAGTACGAGAGCTCTGAGGAAGAGCGGGAAACACCTGCCGTGCCCCC
CACCTGGAGAGCCAGCCAGCCTAGCCTGACCGTGCGGGCCCAGCTGGCCCCTCGCCCACCTATGGCCCCTAGAAGCCAGATCCCTAGCA
GACACGTGCTGTGCCTGCCTCCCCGGAACGTGACCCTGCTGCAGGAGAGAGCCAACAAGCTGGTGAAGTACCTGATGATCAAGGACTA
TAAGAAGATCCCCATCAAGCGGGCCGACATGCTGAAGGATGTGATTAGAGAGTACGACGAGCACTTCCCCGAGATCATCGAGCGGGCC
ACGTACACCCTGGAAAAGAAATTCGGCATCCACCTGAAAGAGATCGACAAGGAAGAACACCTGTACATCCTGGTGTGCACCAGAGACA
GCAGCGCTCGGCTGCTGGGAAAAACCAAGGACACCCCTCGGCTGAGCCTGCTGCTCGTGATCCTGGGCGTGATTTTCATGAACGGCAAC
AGAGCTTCTGAGGCAGTGCTGTGGGAAGCCCTCAGAAAGATGGGCCTGAGACCCGGAGTCAGACATCCTTTCCTGGGCGACCTGAGAA
AGCTGATCACCGACGACTTCGTGAAGCAGAAGTACCTGGAATACAAGAAGATCCCTAATAGCAATCCTCCAGAGTACGAGTTCCTGTGG
GGCCTGCGGGCCCGCCACGAGACATCCAAGATGAGAGTGCTGAGGTTCATCGCCCAGAACCAGAACCGCGACCCCAGAGAGTGGAAG
GCCCACTTCCTGGAAGCCGTGGATGACGCTTTTAAGACAATGGATGTGGACATGGCCGAGGAACACGCCCGAGCTCAGATGCGGGCCC
AAATGAACATCGGCGACGAGGCCCTGATCGGCAGATGGTCCTGGGACGATATCCAGGTGGAACTGCTGACCTGGGATGAGGACGGCG
ATTTCGGCGACGCCTGGGCCCGAATCCCATTCGCCTTCTGGGCTAGATACCACCAGTACATCCTGAACAGCAACAGAGCTAACCGTAGA

GCCACCTGGCGGGCCGGCGTGTCCAGCGGCACAAACGGCGGCGCCTCTACAAGCGTGCTGGACGGCCCAAGCACAAGCAGCACCATC
AGAACCAGAAACGCCGCTAGAGCCGGCGCCAGCTTCTTCAGCTGGATCCAGCATAGATGA

FJX1 wild type DNA sequence (SEQ ID NO: 20)

ATGGGCAGGAGGATGCGGGGCGCCGCCGCCACCGCGGGGCTCTGGCTGCTGGCGCTGGGCTCGCTGCTGGCGCTGTGGGGAGGGCT
CCTGCCGCCGCGGACCGAGCTGCCCGCCTCCCGGCCGCCCGAAGACCGACTCCCACGGCGCCCGGCCCGGAGCGGCGGCCCCGCGCCC
GCGCCTCGCTTCCCTCTGCCCCCGCCCCTGGCGTGGGACGCCCGCGGCGGCTCCCTGAAAACTTTCCGGGCGCTGCTCACCCTGGCGGC
CGGCGCGGACGGCCCGCCCCGGCAGTCCCGGAGCGAGCCCAGGTGGCACGTGTCAGCCAGGCAGCCCCGGCCGGAGGAGAGCGCCG
CGGTGCACGGGGGCGTCTTCTGGAGCCGCGGCCTGGAGGAGCAGGTGCCCCCGGGCTTTTCGGAGGCCCAGGCGGCGGCGTGGCTGG
AGGCGGCTCGCGGCGCCCGGATGGTGGCCCTGGAGCGCGGGGGTTGCGGGCGCAGCTCCAACCGACTGGCCCGTTTTGCCGACGGCA
CCCGCGCCTGCGTGCGCTACGGCATCAACCCGGAGCAGATTCAGGGCGAGGCCCTGTCTTACTATCTGGCGCGCCTGCTGGGCCTCCAG
CGCCACGTGCCGCCGCTGGCACTGGCTCGGGTGGAGGCTCGGGGCGCGCAGTGGGCGCAGGTGCAGGAGGAGCTGCGCGCTGCGCA
CTGGACCGAGGGCAGCGTGGTGAGCCTGACACGCTGGCTGCCCAACCTCACGGACGTGGTGGTGCCCGCGCCCTGGCGCTCGGAGGA
CGGCCGTCTGCGCCCCCTCCGGGATGCCGGGGGTGAGCTGGCCAACCTCAGCCAGGCGGAGCTGGTGGACCTAGTACAATGGACCGAC
TTAATCCTTTTCGACTACCTGACGGCCAACTTCGACCGGCTCGTAAGCAACCTCTTCAGCCTGCAGTGGGACCCGCGCGTCATGCAGCGT
GCCACCAGCAACCTGCACCGCGGTCCGGGCGGGGCGCTGGTCTTTCTGGACAATGAGGCGGGCTTGGTGCACGGCTACCGGGTAGCA
GGCATGTGGGACAAGTATAACGAGCCGCTGTTGCAGTCAGTGTGCGTGTTCCGCGAGCGGACCGCGCGGCGCGTCCTGGAGCTGCACC
GCGGACAGGACGCCGCGGCCCGGCTGCTGCGCCTCTACCGGCGCCACGAGCCTCGCTTCCCCGAGCTGGCCGCCCTTGCAGACCCCCAC
GCTCAGCTGCTACAGCGCCGCCTCGACTTCCTCGCCAAGCACATTTTGCACTGTAAGGCCAAGTACGGCCGCCGGTCTGGGACTTAG

FJX1 Codon optimised DNA sequence (SEQ ID NO: 21)

ATGGGCAGAAGAATGAGAGGCGCCGCTGCCACCGCCGGACTCTGGCTACTGGCTCTGGGCAGCCTGCTGGCTCTGTGGGGCGGCCTGC
TGCCTCCACGAACAGAGCTGCCCGCTAGCAGACCTCCAGAAGATAGACTGCCTCGGCGGCCTGCCAGAAGCGGCGGACCTGCACCAGC
CCCTAGATTCCCCCTGCCTCCTCCTCTTGCCTGGGATGCCAGAGGCGGAAGCCTGAAGACCTTCAGAGCCCTGCTCACCCTGGCAGCTGG
AGCCGACGGCCCTCCTAGACAGAGCAGATCAGAGCCTCGGTGGCACGTGTCCGCCCGGCAGCCTCGGCCCGAGGAAAGCGCCGCCGT
GCACGGCGGCGTGTTCTGGTCCAGAGGCCTGGAAGAACAGGTGCCTCCCGGCTTCTCAGAGGCCCAGGCCGCTGCCTGGCTGGAAGCT
GCTAGAGGCGCCAGAATGGTGGCCCTCGAGCGGGGCGGTTGTGGCAGAAGCAGCAATAGACTGGCTCGGTTCGCCGATGGCACCAGA
GCCTGCGTGCGGTACGGCATCAACCCCGAGCAGATCCAGGGCGAGGCCCTCAGCTACTACCTGGCCAGACTGCTGGGACTGCAAAGAC
ACGTGCCACCTCTGGCCCTCGCCAGGGTGGAAGCCAGAGGGGCCCAGTGGGCCCAAGTGCAGGAGGAACTGAGAGCCGCCCACTGGA
CCGAGGGCAGCGTGGTCAGCCTGACCAGATGGCTGCCCAACCTGACCGACGTGGTGGTTCCTGCCCCTTGGCGGTCTGAAGATGGAAG
ACTGAGACCCCTGCGCGATGCCGGCGGCGAGCTGGCCAATCTGAGCCAGGCCGAGCTGGTCGACCTGGTGCAGTGGACAGACCTGATC
CTGTTTGATTACCTGACCGCCAACTTCGACCGGCTGGTGTCCAACCTGTTCAGCCTGCAGTGGGACCCTAGAGTGATGCAGCGGGCCAC
AAGCAACCTCCACCGGGGTCCTGGCGGCGCCCTCGTGTTTCTGGACAACGAGGCCGGACTGGTTCATGGCTACAGAGTGGCCGGCATG
TGGGACAAGTACAACGAGCCCCTGCTTCAAAGCGTGTGCGTGTTCCGCGAGAGAACCGCCAGAAGAGTGCTGGAACTGCACAGAGGA
CAGGACGCCGCCGCCAGACTGCTGCGGCTGTACCGGCGGCACGAGCCTAGATTCCCTGAACTGGCCGCTCTGGCCGACCCCCACGCCCA
GCTGCTGCAGAGAAGGCTCGACTTCCTGGCTAAGCACATCCTGCACTGCAAGGCCAAGTACGGCAGACGGAGCGGAACATGA

DOM-FJX1 DNA sequence (SEQ ID NO: 22)

ATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACCTTGATTGTTGGGTCGACAACGAAGA
AGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAACAACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCT
GTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCAACGGCAAAGCTATCCACCTGGTTAACAACGAATCTTCTGAAGTTATCGTG
CACAAGGCCATGGACATCGAATACAACGACATGTTCAACAACTTCACCGTTAGCTTCTGGCTGCGCGTTCCGAAAGTTTCTGCTTCCCAC
CTGGAACAGTACGGCACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTG
AAGGGTAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTCAGATCACTTTCCGCGACCTGCCGGACAAGTTCAACGC
GTACCTGGCTAACAAATGGGTTTTCATCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATCAACGGCGTTCTGATGGGCTCC
GCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCTGGACCGTTGCAACAACAACAACCAGTACGTATC
CATCGACAAGTTCCGTATCTTCTGCAAAGCACTGAACCCGAAAGAGATCGAAAAACTGTATACCAGCTACCTGTCTATCACCTTCCTGCG
TGACTTCTGGGGTAACGCGGCCGCTGGACCCGGACCTATGGGCAGGAGGATGCGGGGCGCCGCCGCCACCGCGGGGCTCTGGCTGCT
GGCGCTGGGCTCGCTGCTGGCGCTGTGGGGAGGGCTCCTGCCGCCGCGGACCGAGCTGCCCGCCTCCCGGCCGCCCGAAGACCGACTC
CCACGGCGCCCGGCCCGGAGCGGCGGCCCCGCGCCCGCGCCTCGCTTCCCTCTGCCCCCGCCCCTGGCGTGGGACGCCCGCGGCGGCT
CCCTGAAAACTTTCCGGGCGCTGCTCACCCTGGCGGCCGGCGCGGACGGCCCGCCCCGGCAGTCCCGGAGCGAGCCCAGGTGGCACGT
GTCAGCCAGGCAGCCCCGGCCGGAGGAGAGCGCCGCGGTGCACGGGGGCGTCTTCTGGAGCCGCGGCCTGGAGGAGCAGGTGCCCC
CGGGCTTTTCGGAGGCCCAGGCGGCGGCGTGGCTGGAGGCGGCTCGCGGCGCCCGGATGGTGGCCCTGGAGCGCGGGGGTTGCGGG

CGCAGCTCCAACCGACTGGCCCGTTTTGCCGACGGCACCCGCGCCTGCGTGCGCTACGGCATCAACCCGGAGCAGATTCAGGGCGAGG
CCCTGTCTTACTATCTGGCGCGCCTGCTGGGCCTCCAGCGCCACGTGCCGCCGCTGGCACTGGCTCGGGTGGAGGCTCGGGGCGCGCA
GTGGGCGCAGGTGCAGGAGGAGCTGCGCGCTGCGCACTGGACCGAGGGCAGCGTGGTGAGCCTGACACGCTGGCTGCCCAACCTCAC
GGACGTGGTGGTGCCCGCGCCCTGGCGCTCGGAGGACGGCCGTCTGCGCCCCCTCCGGGATGCCGGGGGTGAGCTGGCCAACCTCAG
CCAGGCGGAGCTGGTGGACCTAGTACAATGGACCGACTTAATCCTTTTCGACTACCTGACGGCCAACTTCGACCGGCTCGTAAGCAACC
TCTTCAGCCTGCAGTGGGACCCGCGCGTCATGCAGCGTGCCACCAGCAACCTGCACCGCGGTCCGGGCGGGGCGCTGGTCTTTCTGGA
CAATGAGGCGGGCTTGGTGCACGGCTACCGGGTAGCAGGCATGTGGGACAAGTATAACGAGCCGCTGTTGCAGTCAGTGTGCGTGTTC
CGCGAGCGGACCGCGCGGCGCGTCCTGGAGCTGCACCGCGGACAGGACGCCGCGGCCCGGCTGCTGCGCCTCTACCGGCGCCACGAG
CCTCGCTTCCCCGAGCTGGCCGCCCTTGCAGACCCCCACGCTCAGCTGCTACAGCGCCGCCTCGACTTCCTCGCCAAGCACATTTTGCACT
GTAAGGCCAAGTACGGCCGCCGGTCTGGGACTTAG

DOM-FJX1 Codon optimised DNA sequence (SEQ ID NO: 23)

ATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCCAAAAACCTTGATTGTTGGGTCGACAACGAAGA
AGACATCGATGTTATCCTGAAAAAGTCTACCATTCTGAACTTGGACATCAACAACGATATTATCTCCGACATCTCTGGTTTCAACTCCTCT
GTTATCACATATCCAGATGCTCAATTGGTGCCGGGCATCAACGGCAAAGCTATCCACCTGGTTAACAACGAATCTTCTGAAGTTATCGTG
CACAAGGCCATGGACATCGAATACAACGACATGTTCAACAACTTCACCGTTAGCTTCTGGCTGCGCGTTCCGAAAGTTTCTGCTTCCCAC
CTGGAACAGTACGGCACTAACGAGTACTCCATCATCAGCTCTATGAAGAAACACTCCCTGTCCATCGGCTCTGGTTGGTCTGTTTCCCTG
AAGGGTAACAACCTGATCTGGACTCTGAAAGACTCCGCGGGCGAAGTTCGTCAGATCACTTTCCGCGACCTGCCGGACAAGTTCAACGC
GTACCTGGCTAACAAATGGGTTTTCATCACTATCACTAACGATCGTCTGTCTTCTGCTAACCTGTACATCAACGGCGTTCTGATGGGCTCC
GCTGAAATCACTGGTCTGGGCGCTATCCGTGAGGACAACAACATCACTCTTAAGCTGGACCGTTGCAACAACAACAACCAGTACGTATC
CATCGACAAGTTCCGTATCTTCTGCAAAGCACTGAACCCGAAAGAGATCGAAAAACTGTATACCAGCTACCTGTCTATCACCTTCCTGCG
TGACTTCTGGGGTAACGCGGCCGCTGGACCCGGACCTATGGGCAGAAGAATGAGAGGCGCCGCTGCCACCGCCGGACTCTGGCTACTG
GCTCTGGGCAGCCTGCTGGCTCTGTGGGGCGGCCTGCTGCCTCCACGAACAGAGCTGCCCGCTAGCAGACCTCCAGAAGATAGACTGC
CTCGGCGGCCTGCCAGAAGCGGCGGACCTGCACCAGCCCCTAGATTCCCCCTGCCTCCTCCTCTTGCCTGGGATGCCAGAGGCGGAAGC
CTGAAGACCTTCAGAGCCCTGCTCACCCTGGCAGCTGGAGCCGACGGCCCTCCTAGACAGAGCAGATCAGAGCCTCGGTGGCACGTGT
CCGCCCGGCAGCCTCGGCCCGAGGAAAGCGCCGCCGTGCACGGCGGCGTGTTCTGGTCCAGAGGCCTGGAAGAACAGGTGCCTCCCG
GCTTCTCAGAGGCCCAGGCCGCTGCCTGGCTGGAAGCTGCTAGAGGCGCCAGAATGGTGGCCCTCGAGCGGGGCGGTTGTGGCAGAA
GCAGCAATAGACTGGCTCGGTTCGCCGATGGCACCAGAGCCTGCGTGCGGTACGGCATCAACCCCGAGCAGATCCAGGGCGAGGCCCT
CAGCTACTACCTGGCCAGACTGCTGGGACTGCAAAGACACGTGCCACCTCTGGCCCTCGCCAGGGTGGAAGCCAGAGGGGCCCAGTGG
GCCCAAGTGCAGGAGGAACTGAGAGCCGCCCACTGGACCGAGGGCAGCGTGGTCAGCCTGACCAGATGGCTGCCCAACCTGACCGAC
GTGGTGGTTCCTGCCCCTTGGCGGTCTGAAGATGGAAGACTGAGACCCCTGCGCGATGCCGGCGGCGAGCTGGCCAATCTGAGCCAGG
CCGAGCTGGTCGACCTGGTGCAGTGGACAGACCTGATCCTGTTTGATTACCTGACCGCCAACTTCGACCGGCTGGTGTCCAACCTGTTCA
GCCTGCAGTGGGACCCTAGAGTGATGCAGCGGGCCACAAGCAACCTCCACCGGGGTCCTGGCGGCGCCCTCGTGTTTCTGGACAACGA
GGCCGGACTGGTTCATGGCTACAGAGTGGCCGGCATGTGGGACAAGTACAACGAGCCCCTGCTTCAAAGCGTGTGCGTGTTCCGCGAG
AGAACCGCCAGAAGAGTGCTGGAACTGCACAGAGGACAGGACGCCGCCGCCAGACTGCTGCGGCTGTACCGGCGGCACGAGCCTAGA
TTCCCTGAACTGGCCGCTCTGGCCGACCCCCACGCCCAGCTGCTGCAGAGAAGGCTCGACTTCCTGGCTAAGCACATCCTGCACTGCAA
GGCCAAGTACGGCAGACGGAGCGGAACATGA

MAGED4B truncations

MAGED4B sv1 (SEQ ID NO: 24)

ATGGCTGAGGGAAGCTTCAGCGTGCAATCGGAAAGCTACAGTGTTGAAGACATGGATGAGGGTAGCGACGAAGTCGGGGAGGAAGA
GATGGTTGAAGGCAACGACTATGAAGAATTCGGTGCGTTTGGTGGCTATGGCACCCTCACCAGCTTTGACATCCATATCCTCAGAGCCTT
CGGAAGCTTGGGTCCAGGCCTTCGCATCTTATCGAATGAGCCCTGGGAACTGGAAAACCCTGTGCTGGCCCAGACCCTGGTGGAGGCA
TTGCAGCTGGATCCGGAAACACTTGCCAATGAGACGGCCGCCCGTGCTGCCAACGTAGCCCGCGCCGCCGCCTCCAACCGTGCGGCTCG
GGCCGCTGCCGCCGCTGCCCGTACCGCCTTCAGTCAGGTGGTCGCTAGCCACCGGGTGGCCACGCCGCAGGTCTCAGGAGAGGATACC
CAGCCCACGACCTACGCCGCCGAGGCTCAGGGGCCCACCCCTGAGCCACCCCTTGCTTCTCCGCAGACCTCCCAGATGTTAGTCACCAGT
AAGATGGCTGCCCCCGAGGCTCCGGCAACCTCCGCACAGTCCCAGACAGGCTCCCCGGCCCAGGAGGCTGCTACTGAGGGCCCTAGTA
GCGCCTGTGCTTTCTCTCAGGCTCCGTGTGCCAGGGAGGTGGACGCCAACCGGCCCAGCACAGCCTTCCTGGGCCAGAATGATGTCTTC
GATTTCACTCAGCCGGCAGGTGTCAGTGGCATGGCCTTCCCGCGCCCCAAGAGACCTGCCCCAGCCCAAGAGGCTGCCACAGAGGGCC
CCAGTGCTGCCTCTGGTGTGCCCCAGACGGGACCTGGCAGGGAGGTGGCAGCCACCCGGCCCAAGACCACCAAGTCGGGGAAGGCGC
TGGCCAAGACTCGGTGGGTGGAGCCTCAGAATGTTGTGGCAGCAGCTGCTGCCAAGGCCAAGATGGCCACGAGCATCCCTGAGCCGG
AGGGTGCAGCTGCTGCCACTGCTCAGCACAGTGCTGAGCCCTGGGCCAGGATGGGAGGCAAGAGGACCAAGAAGTCCAAGCACCTGG
ATGATGAGTATGAGAGCAGCGAGGAGGAGAGAGAGACTCCCGCGGTCCCACCCACCTGGAGAGCATCACAGCCCTCATTGACGGTGC

GGGCTCAGTTGGCCCCTCGGCCCCCGATGGCCCCGAGGTCCCAGATACCCTCAAGGCACGTACTGTGCCTGCCCCCCCGCAACGTGACC
AGACTGTCTCTGCTGCTGGTCATCCTGTACATTCTGAATAGCAACCGTGCCAACAGGAGGGCCACGTGGAGAGCTGGCGTCAGCAGTG
GCACCAATGGAGGGGCCAGCACCAGCGTCCTAGATGGCCCCAGCACCAGCTCCACCATCCGGACCAGAAATGCTGCCAGAGCTGGCGC
CAGCTTCTTCTCCTGGATCCAGCACCGT

MAGED4B sv2 (SEQ ID NO: 25)

ATGGCTGAGGGAAGCTTCAGCGTGCAATCGGAAAGCTACAGTGTTGAAGACATGGATGAGGGTAGCGACGAAGTCGGGGAGGAAGA
GATGGTTGAAGGCAACGACTATGAAGAATTCGGTGCGTTTGGTGGCTATGGCACCCTCACCAGCTTTGACATCCATATCCTCAGAGCCTT
CGGAAGCTTGGGTCCAGGCCTTCGCATCTTATCGAATGAGCCCTGGGAACTGGAAAACCCTGTGCTGGCCCAGACCCTGGTGGAGGCA
TTGCAGCTGGATCCGGAAACACTTGCCAATGAGACGGCCGCCCGTGCTGCCAACGTAGCCCGCGCCGCCGCCTCCAACCGTGCGGCTCG
GGCCGCTGCCGCCGCTGCCCGTACCGCCTTCAGTCAGGTGGTCGCTAGCCACCGGGTGGCCACGCCGCAGGTCTCAGGAGAGGATACC
CAGCCCACGACCTACGCCGCCGAGGCTCAGGGGCCCACCCCTGAGCCACCCCTTGCTTCTCCGCAGACCTCCCAGATGTTAGTCACCAGT
AAGATGGCTGCCCCCGAGGCTCCGGCAACCTCCGCACAGTCCCAGACAGGCTCCCCGGCCCAGGAGGCTGCTACTGAGGGCCCTAGTA
GCGCCTGTGCTTTCTCTCAGGCTCCGTGTGCCAGGGAGGTGGACGCCAACCGGCCCAGCACAGCCTTCCTGGGCCAGAATGATGTCTTC
GATTTCACTCAGCCGGCAGGTGTCAGTGGCATGGCCTTCCCGCGCCCCAAGAGACCTGCCCCAGCCCAAGAGGCTGCCACAGAGGGCC
CCAGTGCTGCCTCTGGTGTGCCCCAGACGGGACCTGGCAGGGAGGTGGCAGCCACCCGGCCCAAGACCACCAAGTCGGGGAAGGCGC
TGGCCAAGACTCGGTGGGTGGAGCCTCAGAATGTTGTGGCAGCAGCTGCTGCCAAGGCCAAGATGGCCACGAGCATCCCTGAGCCGG
AGGGTGCAGCTGCTGCCACTGCTCAGCACAGTGCTGAGCCCTGGGCCAGGATGGGAGGCAAGAGGACCAAGAAGTCCAAGCACCTGG
ATGATGAGTATGAGAGCAGCGAGGAGGAGAGAGAGACTCCCGCGGTCCCACCCACCTGGAGAGCATCACAGCCCTCATTGACGGTGC
GGGCTCAGTTGGCCCCTCGGCCCCCGATGGCCCCGAGGTCCCAGATACCCTCAAGGCACGTACTGTGCCTGCCCCCCCGCAACGTGACC
AGGCTGAGTCTCCTCTTGGTGATTCTGGGCGTCATCTTCATGAATGGCAACCGTGCCAGCGAGGCTGTCCTCTGGGAGGCACTACGCAA
GATGGGACTGCGCCCTGGGGTGAGGCACCCATTCCTCGGCGATCTGAGGAAGCTCATCACAGATGACTTTGTGAAGCAGAAGTACCTG
GAATACAAGAAGATCCCCAACAGCAACCCACCTGAGTATGAATTCCTCTGGGGCCTGCGAGCCCGCCATGAGACCAGCAAGATGAGGG
TCCTGAGATTCATCGCCCAGAATCAGAACCGAGACCCCCGGGAATGGAAGGCTCATTTCTTGGAGGCTGTGGATGATGCTTTCAAGACA
ATGGATGTGGATATGGCCGAGGAACATGCCAGGGCCCAGATGAGGGCCCAGATGAATATCGGGGATGAAGCGCTGATTGGACGGTGG
AGCTGGGATGACATACAAGTCGAGCTCCTGACCTGGGATGAGGACGGAGATTTTGGCGATGCCTGGGCCAGGATCCCCTTTGCTTTCTG
GGCCAGATACCATCAGTACATTCTGAATAGCAACCGTGCCAACAGGAGGGCCACGTGGAGAGCTGGCGTCAGCAGTGGCACCAATGGA
GGGGCCAGCACCAGCGTCCTAGATGGCCCCAGCACCAGCTCCACCATCCGGACCAGAAATGCTGCCAGAGCTGGCGCCAGCTTCTTCTC
CTGGATCCAGCACCGT

MAGED4B sv3 (SEQ ID NO: 26)

ATGGCTGAGGGAAGCTTCAGCGTGCAATCGGAAAGCTACAGTGTTGAAGACATGGATGAGGGTAGCGACGAAGTCGGGGAGGAAGA
GATGGTTGAAGGCAACGACTATGAAGAATTCGGTGCGTTTGGTGGCTATGGCACCCTCACCAGCTTTGACATCCATATCCTCAGAGCCTT
CGGAAGCTTGGGTCCAGGCCTTCGCATCTTATCGAATGAGCCCTGGGAACTGGAAAACCCTGTGCTGGCCCAGACCCTGGTGGAGGCA
TTGCAGCTGGATCCGGAAACACTTGCCAATGAGACGGCCGCCCGTGCTGCCAACGTAGCCCGCGCCGCCGCCTCCAACCGTGCGGCTCG
GGCCGCTGCCGCCGCTGCCCGTACCGCCTTCAGTCAGGTGGTCGCTAGCCACCGGGTGGCCACGCCGCAGGTCTCAGGAGAGGATACC
CAGCCCACGACCTACGCCGCCGAGGCTCAGGGGCCCACCCCTGAGCCACCCCTTGCTTCTCCGCAGACCTCCCAGATGTTAGTCACCAGT
AAGATGGCTGCCCCCGAGGCTCCGGCAACCTCCGCACAGTCCCAGACAGGCTCCCCGGCCCAGGAGGCTGCTACTGAGGGCCCTAGTA
GCGCCTGTGCTTTCTCTCAGGCTCCGTGTGCCAGGGAGGTGGACGCCAACCGGCCCAGCACAGCCTTCCTGGGCCAGAATGATGTCTTC
GATTTCACTCAGCCGGCAGGTGTCAGTGGCATGGCCTTCCCGCGCCCCAAGAGACCTGCCCCAGCCCAAGAGGCTGCCACAGAGGGCC
CCAGTGCTGCCTCTGGTGTGCCCCAGACGGGACCTGGCAGGGAGGTGGCAGCCACCCGGCCCAAGACCACCAAGTCGGGGAAGGCGC
TGGCCAAGACTCGGTGGGTGGAGCCTCAGAATGTTGTGGCAGCAGCTGCTGCCAAGGCCAAGATGGCCACGAGCATCCCTGAGCCGG
AGGGTGCAGCTGCTGCCACTGCTCAGCACAGTGCTGAGCCCTGGGCCAGGATGGGAGGCAAGAGGACCAAGAAGTCCAAGCACCTGG
ATGATGAGTATGAGAGCAGCGAGGAGGAGAGAGAGACTCCCGCGGTCCCACCCACCTGGAGAGCATCACAGCCCTCATTGACGGTGC
GGGCTCAGTTGGCCCCTCGGCCCCCGATGGCCCCGAGGTCCCAGATACCCTCAAGGCACGTACTGTGCCTGCCCCCCCGCAACGTGACC
CTTCTGCAGGAGAGGGCAAATAAGTTGGTGAAATACCTGATGATTAAGGACTACAAGAAGATCCCCATCAAGCGCGCAGACATGCTGA
AGGATGTCATCAGAGAATATGATGAACATTTCCCTGAGATCATTGAACGAGCAACGTACACCCTGGAAAAGAAGTTTGGGATCCACCTG
AAGGAGATCGACAAGGAAGAACACCTGTATATTCTTGTCTGCACACGGGACTCCTCAGCTCGCCTCCTTGGAAAAACCAAGGACACTCC
CAGGCTGAGTCTCCTCTTGGTGATTCTGTACATTCTGAATAGCAACCGTGCCAACAGGAGGGCCACGTGGAGAGCTGGCGTCAGCAGTG
GCACCAATGGAGGGGCCAGCACCAGCGTCCTAGATGGCCCCAGCACCAGCTCCACCATCCGGACCAGAAATGCTGCCAGAGCTGGCGC
CAGCTTCTTCTCCTGGATCCAGCACCGT

Leader/signal peptide

mIGH SP (SEQ ID NO: 27)

ATGGGTTGGAGCTGTATCATCTTCTTTCTGGTAGCAACAGCTACAGGTGTGCACTCC

Alternative fusions – Helper Motifs

PVXCP (SEQ ID NO: 28)

ATGAGCGCCCCTGCCTCTACAACACAGCCTATCGGCAGCACCACCTCCACCACCACAAAAACAGCTGGCGCTACCCCTGCCACAGCCTCT
GGCCTGTTTACAATCCCTGACGGCGACTTCTTCAGCACCGCCAGAGCTATCGTGGCCTCTAACGCCGTGGCCACAAACGAGGACCTGAG
CAAGATCGAGGCCATCTGGAAGGACATGAAGGTGCCCACCGACACAATGGCCCAGGCTGCTTGGGATCTCGTCAGACACTGTGCCGAT
GTGGGCAGCTCTGCCCAGACAGAGATGATCGACACAGGCCCCTACAGCAACGGCATCAGCAGAGCTAGACTGGCCGCTGCCATCAAAG
AAGTGTGCACCCTGAGACAGTTCTGCATGAAGTACGCCCCTGTCGTGTGGAACTGGATGCTGACCAACAACAGCCCTCCTGCCAACTGG
CAGGCTCAGGGCTTTAAGCCAGAGCACAAGTTCGCCGCCTTCGATTTCTTCAACGGCGTGACAAACCCTGCCGCCATCATGCCTAAAGA
GGGCCTGATCAGACCTCCTAGCGAGGCCGAGATGAACGCCGCTCAGACTGCTGCCTTCGTGAAGATCACCAAGGCCAGGGCTCAGAGC
AACGACTTCGCCTCTCTTGATGCCGCCGTGACCAGAGGCAGAATCACCGGAACCACAACAGCCGAGGCTGTCGTGACATTGCCTCCTCC
A

MIP3a (SEQ ID NO: 29)

ATGTGCTGTACCAAGTCTCTGCTGCTGGCCGCTCTGATGTCTGTGCTGCTGCTGCATCTGTGTGGCGAGTCTGAGGCCGCCAGCAACTTC
GACTGTTGTCTGGGCTACACCGACAGAATCCTGCATCCTAAGTTCATCGTGGGCTTCACCAGACAGCTGGCCAACGAGGGCTGTGACAT
CAACGCCATCATCTTCCACACCAAGAAGAAGCTGAGCGTCTGCGCTAACCCCAAGCAGACCTGGGTCAAGTACATCGTGCGGCTGCTGA
GCAAGAAAGTGAAGAACATG

MITD: HLA-A2 MITD (SEQ ID NO: 30)

ATCGTGGGAATTGTGGCTGGACTGGCCCTGTTTGGCGCCGTGATTACAGGTGCTGTGGTGGCCGCTGTTATGTGGCGGAGAAAGAGCA
GCGACAGAAAAGGCGGCAGCTACTCTCAGGCCGCCAGCTCTGATTCTGCCCAGGGCTCTGATGTGTCCCTGACAGCT

MAGED4B protein (SEQ ID NO: 31)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERA
TYTLEKKFGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDLRKLITDDFVK
QKYLEYKKIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHARAQMRAQMNIGDEALI
GRWSWDDIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTRNAARAGASF
FSWIQHR

DOM-MAGED4B protein (SEQ ID NO: 32)

MGWSCIIFFLVATATGVHSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDIE
YNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITI
TNDRLSSANLYINGVLMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNAAAGPGPMAEG
SFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDPETLAN
ETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPEAPATS
AQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVPQTGPG
REVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEERETPAVP
PTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERATYTLEKK
FGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDLRKLITDDFVKQKYLEYK
KIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHARAQMRAQMNIGDEALIGRWSWD
DIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTRNAARAGASFFSWIQHR

FJX1 protein sequence (SEQ ID NO: 33)

MGRRMRGAAATAGLWLLALGSLLALWGGLLPPRTELPASRPPEDRLPRRPARSGGPAPAPRFPLPPPLAWDARGGSLKTFRALLTLAAGAD
GPPRQSRSEPRWHVSARQPRPEESAAVHGGVFWSRGLEEQVPPGFSEAQAAAWLEAARGARMVALERGGCGRSSNRLARFADGTRACVR

YGINPEQIQGEALSYYLARLLGLQRHVPPLALARVEARGAQWAQVQEELRAAHWTEGSVVSLTRWLPNLTDVVVPAPWRSEDGRLRPLRDA
GGELANLSQAELVDLVQWTDLILFDYLTANFDRLVSNLFSLQWDPRVMQRATSNLHRGPGGALVFLDNEAGLVHGYRVAGMWDKYNEPLL
QSVCVFRERTARRVLELHRGQDAAARLLRLYRRHEPRFPELAALADPHAQLLQRRLDFLAKHILHCKAKYGRRSGT

DOM-FJX1 protein sequence (SEQ ID NO: 34)

MGWSCIIFFLVATATGVHSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVITYPDAQLVPGINGKAIHLVNNESSEVIVHKAMDIE
YNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITI
TNDRLSSANLYINGVLMGSAEITGLGAIREDNNITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNAAAGPGPMGRR
MRGAAATAGLWLLALGSLLALWGGLLPPRTELPASRPPEDRLPRRPARSGGPAPAPRFPLPPPLAWDARGGSLKTFRALLTLAAGADGPPRQ
SRSEPRWHVSARQPRPEESAAVHGGVFWSRGLEEQVPPGFSEAQAAAWLEAARGARMVALERGGCGRSSNRLARFADGTRACVRYGINPE
QIQGEALSYYLARLLGLQRHVPPLALARVEARGAQWAQVQEELRAAHWTEGSVVSLTRWLPNLTDVVVPAPWRSEDGRLRPLRDAGGELA
NLSQAELVDLVQWTDLILFDYLTANFDRLVSNLFSLQWDPRVMQRATSNLHRGPGGALVFLDNEAGLVHGYRVAGMWDKYNEPLLQSVCV
FRERTARRVLELHRGQDAAARLLRLYRRHEPRFPELAALADPHAQLLQRRLDFLAKHILHCKAKYGRRSGT

Leader sequence underlined.

MAGED4B truncations

MAGED4B sv1 (SEQ ID NO: 35)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTRLSLLLVILYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTS
STIRTRNAARAGASFFSWIQHR

MAGED4B sv2 (SEQ ID NO: 36)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDL
RKLITDDFVKQKYLEYKKIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHARAQMRAQ
MNIGDEALIGRWSWDDIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTR
NAARAGASFFSWIQHR

MAGED4B sv3 (SEQ ID NO: 37)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERA
TYTLEKKFGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTRNAAR
AGASFFSWIQHR

Alternative fusions – helper motifs:

PVXCP (SEQ ID NO: 38)

MSAPASTTQPIGSTTSTTTKTAGATPATASGLFTIPDGDFFSTARAIVASNAVATNEDLSKIEAIWKDMKVPTDTMAQAAWDLVRHCADVGS
SAQTEMIDTGPYSNGISRARLAAAIKEVCTLRQFCMKYAPVVWNWMLTNNSPPANWQAQGFKPEHKFAAFDFFNGVTNPAAIMPKEGLIR
PPSEAEMNAAQTAAFVKITKARAQSNDFASLDAAVTRGRITGTTTAEAVVTLPPP

MIP3a (SEQ ID NO: 39)

MCCTKSLLLAALMSVLLLHLCGESEAASNFDCCLGYTDRILHPKFIVGFTRQLANEGCDINAIIFHTKKKLSVCANPKQTWVKYIVRLLSKKVKN
M

MITD (SEQ ID NO: 40)

IVGIVAGLALFGAVITGAVVAAVMWRRKSSDRKGGSYSQAASSDSAQGSDVSLTA

MAGED4B Human isoform 2 (SEQ ID NO: 41)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERA
TYTLEKKFGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDLRKLITDDFVK
QKYLEYKKIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHARAQMRAQMNIGDEALI
GRWSWDDIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPSTSSTIRTRNAARAGASF
FSWIQ

MAGED4B Human isoform 3 (SEQ ID NO: 42)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKVRSPCPLPPPHPLAP
VLSFSSLSCSSPPSPLPLLPLFSSFPSFSPHLPSPPLLSSQLVHVSPTQVC

MAGED4B Human isoform 4 (SEQ ID NO: 43)

MAEGSFSVQSESYSVEDMDEGSDEVGEEEMVEGNDYEEFGAFGGYGTLTSFDIHILRAFGSLGPGLRILSNEPWELENPVLAQTLVEALQLDP
ETLANETAARAANVARAAASNRAARAAAAAARTAFSQVVASHRVATPQVSGEDTQPTTYAAEAQGPTPEPPLASPQTSQMLVTSKMAAPE
APATSAQSQTGSPAQEAATEGPSSACAFSQAPCAREVDANRPSTAFLGQNDVFDFTQPAGVSGMAFPRPKRPAPAQEAATEGPSAASGVP
QTGPGREVAATRPKTTKSGKALAKTRWVEPQNVVAAAAAKAKMATSIPEPEGAAAATAQHSAEPWARMGGKRTKKSKHLDDEYESSEEER
ETPAVPPTWRASQPSLTVRAQLAPRPPMAPRSQIPSRHVLCLPPRNVTLLQERANKLVKYLMIKDYKKIPIKRADMLKDVIREYDEHFPEIIERA
TYTLEKKFGIHLKEIDKEEHLYILVCTRDSSARLLGKTKDTPRLSLLLVILGVIFMNGNRASEAVLWEALRKMGLRPGVRHPFLGDLRKLITDDFVK
QKNPELREETPLSMAPSWYLEYKKIPNSNPPEYEFLWGLRARHETSKMRVLRFIAQNQNRDPREWKAHFLEAVDDAFKTMDVDMAEEHAR
AQMRAQMNIGDEALIGRWSWDDIQVELLTWDEDGDFGDAWARIPFAFWARYHQYILNSNRANRRATWRAGVSSGTNGGASTSVLDGPS
TSSTIRTRNAARAGASFFSWIQHR

The invention defines:

A. 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.

B. The cancer vaccine according to paragraph A, wherein the MAGED4B protein comprises or consists of the sequence of SEQ ID NO: 3, or a variant thereof.

C. The cancer vaccine according to paragraph A or paragraph B, wherein the FJX1 protein comprises or consists of the sequence of SEQ ID NO: 4, or a variant thereof.

D. The cancer vaccine according to any one of the preceding paragraphs, wherein the immunogenic fragment of tetanus toxin comprises or consists of the p30 MHC II epitope of tetanus toxin.

E. The cancer vaccine according to any one of the preceding paragraphs, wherein the immunogenic fragment of tetanus toxin comprises or consists of DOM.

F. The cancer vaccine according to any one of the preceding paragraphs, wherein the MAGED4B and FJX1 antigens are encoded as a single fusion protein.

G. The cancer vaccine according to any one of the preceding paragraphs, wherein the immunogenic fragment of tetanus toxin, MAGED4B and FJX1 are encoded as single fusion protein.

H. The cancer vaccine according to any one of the preceding paragraphs, wherein linker residues are provided between one or more, or all, of the antigens of the immunogenic fragment of tetanus toxin, MAGED4B and FJX1.

I. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid further encodes a signal peptide for enhancing the efficacy of secretion.

J. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid further encodes one or more promoters.

K. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid further encodes a polyA transcription termination sequence.

L. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid comprises sequences encoding SEQ ID NOs: 2-4, or variants thereof.

M. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid comprises sequences encoding SEQ ID NOs: 1-6.

N. The cancer vaccine according to any one of the preceding paragraphs, wherein the nucleic acid comprises or consists of the sequence of SEQ ID NOs: 12, 13 or 14.

O. A composition comprising the cancer vaccine according to any one of the preceding paragraphs.

P. A cancer vaccine according to any one of paragraphs A-M, or a composition according to paragraph O, for use as a medicament.

Q. A cancer vaccine according to any one of paragraphs A-M, or a composition according to paragraph 1O, for use for treating or preventing cancer in a subject.

R. A method of treating or preventing cancer in a subject, the method comprising the administration of the cancer vaccine according to any one of paragraphs A-M, or a composition according to paragraph O, to the subject.

S. The cancer vaccine or composition for use according to paragraph Q, or the method of treatment or prevention according to paragraph R, wherein the cancer to be treated or prevented is oral and/or oropharyngeal cancer.

T. The cancer vaccine or composition for use according to any one of paragraphs P, Q or S, or the method of treatment or prevention according to paragraph R or S, wherein the cancer vaccine or composition is used in combination with the administration of a checkpoint inhibitor to the subject.

U. The cancer vaccine or composition for use according to paragraph T, or the method according to paragraph T, wherein the checkpoint inhibitor comprises an anti-PD1 or anti-CTLA4 binding molecule, or nucleic acid encoding an anti-PD1 or anti-CTLA4 binding molecule.

V. A kit for the treatment or prevention of cancer, the kit comprising:

-a cancer vaccine according to any one of paragraphs A-M; and

-a checkpoint inhibitor agent, such as an anti-PD1 or anti-CTLA4 binding molecule.

W. A kit for the treatment or prevention of cancer, the kit comprising:

-a first cancer vaccine according to any one of paragraphs A-M, wherein the nucleic acid encodes MAGED4B;

-a second cancer vaccine according to any one of paragraphs A-M, wherein the nucleic acid encodes FJX1; and optionally

-a checkpoint inhibitor agent, such as an anti-PD1 binding molecule.

The present invention has been extensively demonstrated and material and methods for the data obtained and presented in the Figures included herein are presented below, with specific information, on matters such as treatments strategies, are included in the Figures and legends. The methods and data presented here are to support the present invention and are exemplary, depicting some alternative vaccine constructs and the like.

Examples

Example 1

Evaluation of immunogenicity of DNA vaccine targeting MAGED4B/ FJX antigens in HPV-ve HNSCC

Target antigens expression and pre-existing T cell responses in patients with HPV independent HNSCC

While there is intriguing potential for the development of patient-specific vaccines based on an individual's tumour mutanome, the costliness and technical difficulty of such an approach means that, even if successful, it is unlikely to benefit most patients. Identifying common tumour antigens that are shared between patients, to the production of generic cancer vaccines that would provide a cheap and widely available treatment for OSCC. Among the different types of TAA, cancer/testis (CT) antigens are highly promising therapeutic targets; cellular and humoral immune responses to CT antigens are frequently observed in cancer patients, and there is an association between CT antigen expression and cytolytic activity of tumour immune infiltrates. The immunogenicity and cancer-specificity of CT antigens have made them prioritised targets for cancer immunotherapy, and their therapeutic function has been tested in a variety of clinical settings. CT antigen vaccines are generally well tolerated, and there are presently a large number of ongoing cancer vaccination trials assessing their therapeutic efficacy. We selected two cancer testis antigens MAGED4B and FJX1 that are identified as frequently expressed in OSCC. We then extended these data to analyses using the CGA (Cancer Genome Atlas) data set and confirmed the expression. Overall the two antigens are

expressed at 96% of OSCC cases at the RNA level. Furthermore we have 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 (Figure 2). Expression data in healthy tissues at protein levels 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 MAGED4B tetramer positive CD8+ T cells were observed in 4/6 HLA-A2 patients (0.04-0.1% of total CD8+ T cells) (Figure 3) with 2/2 expanded TILs also having the tetramer positive at a similar frequency (Figure 4 A and Figure 5). 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 reactivities beyond HLA-A2 restriction (Figure 4B). For FJX1 so far CD8 T cell reactivity has been only evaluated in expanded TIL samples using OPP with demonstration of CD8 reactivities in HLA-A1 patients coexisting with MAGED4B CD8 T cells (Figure 4B). The patients' data indicate a significant immunogenicity of both antigens more so pronounced for MAGED4B.

Example 2

Preclinical data on DNA vaccines efficacy/ immunogenicity

Vaccine design

In HPV+ cancers DNA vaccine encoding immunogenic viral antigens have advanced significantly with the recent results of a randomised phase 2b clinical trial in cervical neoplasia demonstrating histopathological regression of the disease. Our approach for DNA vaccination has been to deliver tumour-specific peptides or antigens in the context of immunogenic sequence of tetanus toxin domain (Dom). The vaccine design is aimed to provide linked CD4 T cell help for optimal induction of CD8+ T cells in patients with cancer, a potent strategy that breaks tolerance. Phase II clinical data suggest that DNA vaccination is able to overcome peripheral tolerance in tumour tissue with CD8 T cells response to tumour antigen (carcino-embryonic antigen; CEA) detected post-vaccine and with indication of clinical benefits. We therefore applied Dom based design to generate DNA vaccines encoding full length MAGED4B and FJX1 antigens (p.Dom-MAGED4BFL and p.Dom-FJX1FL). Here we opted for full-length antigen design to achieve a wider populational coverage and not focused on targeting individual HLA alleles (HLA-A2).

Mouse models

For immunogenicity HLA-A2 transgenic mice HHD were used without tumour challenge. B16/F10 was transfected with human HLA-A2, MAGED4B and FJX1 constructs to mimic the expression of these antigens in HNSCCs is used in the humanised mouse model (B6.Cg-Tg (HLA-A/H2-D)2Enge/J) transgenic for the HLA-A2. The tumour was given subcutaneously.

Immunogenicity of each vaccine has been confirmed followed a single dose of the vaccines given with electroporation in non-tumour bearing mice (Figure 7). An overlapping peptide pool (OPP) covering the entire sequence of each antigen (MAGED4B 183 peptides; FJX1 107 peptides) was used to measure the antigen specific T cell responses.

In tumour challenge experiments mice were vaccinated when the tumours were palpable at day 3 (size). The DNA vaccines were given as a mixture twice 3 weeks apart with or without anti-PD1 (In

vivo Mab antimouse PD1 (RMP1-14, BEO146 from BioXcell) with anti-PD1 serving as a comparator. The experiments were paralleled by immunogenicity measurements using OPP as above. The DNA vaccine was able to reduce/supress the tumour growth at a similar level as anti-PD1 against the control DNA vaccine (p.Dom backbone) with a remarkable synergistic affect when combined together (Figure 9B). The data were paralleled by demonstrating of induction of MAGED4B –specific T cells and an increase in MAGED4B specific T cells upon combination with anti-PD1 (Figure 9C). Collectively the preclinical data demonstrates the DNA vaccines targeting MAGED4-B/FJX1 have a significant potential to suppress the growth of tumour expressing these antigens and this can be further enhanced by combination with anti-PD1.

Example 3

Alternative gene fusion partners – helper motifs - MITD, PVXCP, MIP3α

MHC Class I trafficking signal (MITD) attached to the C-terminus of target antigen has been shown to promote presentation of both MHCI and MHCII epitopes leading to polyepitope expansion of CD4 and CD8 T cells (Kreiter S, *et al.* J Immunol. 2008;180(1):309-18).

PVXCP (potato virus X coat protein) is a helper sequence which has been shown to enhance induction of T cell responses to fused cancer antigen through the mechanism of linked T cell help similarly to DOM helper sequence from tetanus toxin (Savelyeva N *et al* Nature biotechnology. 2001;19(8):760-4, and Stegantseva MV *et al*, Cancer immunology, immunotherapy : CII. 2020).

Antigens fused to chemokine MIP3α have been shown to direct to immature DCs via chemokine receptor CCR6 (Biragyn A *et al.* J Immunol. 2001;167(11):6644-53). Following the receptor mediated uptake fused antigens are presented by both MHC class I and II, activating significant responses CD4+ and CD8+ T cell responses (Biragyn A *et al*. Blood. 2004;104(7):1961-9, Biragyn A, *et al*. J Immunol. 2007;179(2):1381-8.)

These fusions are depicted in Figure 13 and the figure legend provides further information.

Assembly of the fusion constructs with helper motifs

MITD (165bp) encodes the HLA-A2 trafficking signals. PVXCP (732bp) encodes the potato virus X coat protein. MIP3α (252bp) encodes macrophage inflammatory protein 3 alpha. MITD PVXCP and MIP3α gene were codon-optimised and synthesised by GeneArt (Invitrogen). The leader sequence encoding mouse (mus) IgH signal peptide (MGWSCIIFFLVATATGVHS) was inserted at the N terminus of each construct to enhance secretion, with the exception of MIP3α fusion constructs, which has its own signal peptide. Fusion partners and the gene of interest (MAGED4B or FJX1) were linked with a seven amino acid linker (AAAGPGP). With the exception of MITD, all other fusion partners were fused upstream of the target cancer antigens (Figure 13). MITD sequence was added downstream of the antigenic sequence. The genes for MAGED4B, FJX1 or their fusions of interest were inserted into pcDNA3 vector at *Not*I, *Xho*I and *Xba*I restriction enzyme sites to generate the DNA vaccine constructs.

Evaluation of immunogenicity of fusion constructs containing DOM and different fusion helper motif partners:

Generic vaccination protocol for evaluating immunogenicity of DNA vaccines alone or in combination

with electroporation was prime/ boost (Figures 17; 21-26) (specific vaccine constructs are indicated in each figure legend):

Three groups of 5-6 non-tumour bearing HHD (transgenic for the human HLA-A2 allele) mice were vaccinated with 50 μg of p.Dom-MAGED4B, p.Dom-FJX1 or p.Dom individually on day 1 following by a booster injection of the same DNA vaccine with electroporation on d 22. Their immunogenicity was evaluated by IFN-γ ELISPOT. Lymphocytes isolated from mouse spleens were plated to ELISPOT plates on day 35 (Figure 8). In experiments in Figure 21-26 varying doses of dbDNA vaccines indicated in the figure legends.

In experiments in Figures 11, 12, 14 vaccinations were given at day 1 and 8 and spleens for ELISPOT were taken on day 22 (specific vaccine constructs are indicated in each figure legend).

In Figures 15, 19 and 20 the response was evaluated after priming only following generic (specific vaccine constructs are indicated in each figure legend) protocol: non-tumour bearing C57B/6 mice were vaccinated 50 μg pDOM plasmid vaccine (as a negative control, 3 mice), 25 μg DB-DOM-FJX1 CO (5 mice), and 25 μg pDom-FJX1 plasmid (5 mice). The vaccines were administered i.m. with EP on day 1.). In experiments in Figure 15 mice received 50 μg of DNA vaccine. Lymphocytes isolated from mouse spleens were plated to ELISPOT plates on day 14.

Generic ELISpot protocol as used herein:
IFNγ ELISpot was performed according to manufacturer's protocol; BD Biosciences). Briefly, lymphocytes were isolated from the spleens of vaccinated mice using Lymphoprep™ and plated to ELISpot plates at 2.5 x 10^5 cells per well. Overlapping peptide pools (OPP) for each target antigen MAGED4B or FJX1 were added to a final concentration of 1μM and incubated for 40 hours at 37°C at 5% in RPMI supplemented with 10% FCS, 20 mM L-Glutamine, 10 U/ ml penicillin/streptomycin. The overlapping peptide pools for the entire sequence of each antigen consisted 15 mer peptides with 11aa overlap; 183 peptides were pooled for MAGED4B and 107 peptides for FJX1 (JPT, Germany). FJX1 OPP served as a negative control for MAGED4B targeting vaccines and *vice versa*. Spots forming units (SFUs) corresponding to individual responding T cells were imaged and enumerated with AID ELISpot plate reader system ELR04 and software (AID Autoimmun Diagnostika GmbH, Strassburg, Germany). The graphs were generated using PRISM graphpad package.

Example 4

Identification of MAGED4B antigen on CAF

Immunohistochemical analysis of HNSCC cases demonstrating strong MageD4B expression in cancer associated fibroblasts as well as cancer cells. Anti-MAGED4B monoclonal antibody (Santa Cruz, G12; sc-393059) was used on HNSCC tissues at 1:50 dilution following antigen retrieval using high pH (Tris-EDTA (pH (9)) buffer. Deparaffinization, rehydration, antigen retrieval, and IHC staining were performed using a Dako PT Link Autostainer (using EnVision FLEX Target Retrieval Solution, High pH (Agilent Dako) and DAKO Auto-stainer Link48 in Cellular Pathology Department of University Hospital of Southampton NHS Trust. DAKO Envision FLEX Mouse linker was applied to sections for 15 minutes; DAKO Envision FLEX HRP (20 minutes) and DAKO Substrate Working Solution (10 minutes) and then counterstained with DAKO Envision FLEX Haematoxylin for 5 minutes. Images were captured using ZEISS Axio scanner in WISH Lab.

Six individual HNSCC cases are presented (Figure 27)

Analysis of 10 cases of HNSCC confirmed tumour cell expression of MAGED4B in 9/10 cases. Level of tumour cell expression was assessed using the *H Score* (the product of staining intensity (scored 0-3) and percentage of positive cells (scored 0-100); giving a maximum possible score of 300 ie 100% of tumour cells showing strong staining. Notably, (and unexpectedly), high expression of MAGED4 was also observed in CAF (7/10 cases) indicated as CAF+ in the table below. CAF staining was assessed as positive or negative. We confirmed CAF MageD4B expression by analysing scRNASeq HNSCC transcriptomic data (which also confirmed MAGED4B expression by HNSCC cells) (Figure 28A). Single-cell RNASeq (scRNASeq) has emerged as a powerful method for quantifying the transcriptome of individual cells, and appropriate protocols are outlined in Andrews, T and Hemberg, M, Molecular Aspects of Medicine Volume 59, February 2018, Pages 114-122.

Intensity sco	0-None	1- Low	2 - Moderate	3 - Strong	Automatic	
	Proprtion Score	Proprtion Score	Proprtion Score	Proprtion Score	H Score	
17HS10920P	30	10	10	50	180	
16HS19001B	85	5	5	5	30	CAF+
17HS15081C	90	5	5	0	15	CAF+
16HS33330H	85	5	5	5	30	CAF+
19HS16208P	95	5	0	0	5	CAF+
19HS1407D	80	5	5	10	45	CAF+
18HS5113K	85	20	5	0	30	CAF+
19HS233L	95	5	0	0	5	
19HS7587F	100	0	0	0	0	
19HS12674C	85	5	10	0	25	CAF+

The table shows the intensity of staining of tumour cells. CAF+ indicates strong staining for MAGED4B in CAF.

Example 5

Identification of MAGED4B expression in tumour and normal tissues

These studies were designed to evaluate MAGED4B and FJX1 expression in both tumour and normal tissue samples. Tumour biopsy samples from OSCC patients and normal tissue microarray samples were obtained and subjected to immunohistochemistry analysis. Strong expression of both antigens was evident in all samples from patients with OSCC. 5/5 samples using HPV- HNSCC and 5/5 in oral dysplastic tissues for each antigen (Southampton, UK cohort). 28 samples were evaluated for antigen expression with 28/28 samples being positive for MAGED4B and 27/28 samples positive for FJX1 (Malaysian cohort).

In addition, 5 samples from patients with lung cancer (3 LUAD, 2 LUSC) exhibited strong expression of MAGED4B.

The suitability of targeting either of these two antigens was suggested in the analysis which demonstrated no/negligible expression in healthy tissue and confirmed by low staining in the TMA

samples (data not shown). Thus, the vaccine is not expected to induce an immune response to normal tissues.

This work established the expression of both antigens in multiple HPV-ve HNSCC samples from patients in Malaysia and the UK and confirmed the favourable tissue expression patterns in tissue microarray panels of major organs including non-dysplastic oral tissue.

For Southampton OSCC patient samples: samples were stained using an automated DAKO autostainer following the manufacturer's instructions.

Table 1: Summary of approach (UK):

Primary Antibody	
MAGED4B – NBP1-89594 (Novus Biological)	FJX1 - NBP2-32442 (Novus Biological)
Dilution Factor	
1:200	1:100
Target Retrieval	
Heat-Induced	Heat-Induced
Linker Type	
Rabbit IgG	Rabbit IgG

For Malaysian cohort of OSCC patients, FFPE blocks were identified and sectioned at 4µm on positively-charged glass slides. Briefly, wax from the sections were melted for 10 minutes at 65°C for 10 minutes, followed by deparaffinisation by 2 washes of xylene substitute at 5 minutes each. The sections were then rehydrated in graded ethanol (100% ethanol X 2, 95% ethanol X 2, 70% ethanol X 1) and washed in distilled water for a minimum of 30 seconds. It was followed by heat-induced antigen retrieval (citrate buffer pH 6 for MAGED4B; Tris-EDTA buffer pH 9 for FJX1) for 20 minutes at 99°C. Non-specific binding was blocked by incubating sections in Dual Endogenous Enzyme Blocking Reagent from the Dako cytomation Envision+ Dual Link System HRP (DAB+) kit for 10 minutes at room temperature. Sections were then incubated with anti-MAGED4B (1:100 dilution) and anti-FJX1 antibodies (1:200 dilution) for 16 hours in 4°C. After incubation, sections were washed and incubated with peroxidase-labelled polymer (conjugated to goat anti-mouse and goat anti-rabbit) for 30 minutes at room temperature. Positive binding to respective antibodies was developed under microscope with DAB+ chromogen substrate and mounted with coverslips after counterstained by haematoxylin and dehydrated in graded ethanol.

Table 2: Summary of approach, alternative antibodies (Malaysia):

Primary Antibody	
MAGED4B –HPA003554	FJX1 - HPA059220
Dilution Factor	
1:100	1:200
Target Retrieval	
Heat-Induced	Heat-Induced
Linker Type	
Rabbit IgG	Rabbit IgG

This work confirmed:

Strong expression of both antigens in HNSCC/OSCC patient samples from the UK and from Malaysia.

Lung cancer samples also show strong expression of MAGED4B (FJX1 not analysed), confirming the overexpression data generated using the TCGA database (detailed in TGL-100_001-R TCGA)

These data correspond broadly with the available RNAseq data showing a favourable tissue expression pattern; strong expression in tumours and low/no expression in healthy tissue.

Example 6

Preclinical work in B16 mouse model

To demonstrate the impact of therapeutic vaccination with the vaccine described here on tumour progression, the B16 model expressing the antigens was employed to challenge HLA-A2 transgenic AAD mice subcutaneously. Tumours were allowed to establish for five days prior to vaccination at day 5 with the dual vaccine, tumour volume evaluation commencing once measurable at approximately day 10 post administration. Combination of the effect of vaccine treatment with anti-PD-1 was also evaluated.

Vaccine monotherapy delayed tumour growth compared with controls, with the effect further markedly enhanced upon combination with anti-PD-1. Evaluation of the tumour by immunohistochemistry revealed that in the tumours of mice vaccinated with the dual vaccine, increased T cell infiltrates were evident compared to pDOM control vaccinated mice. Flow cytometry further demonstrated that these infiltrates contained increased numbers of activated CD4+ and CD8+ T cells relative to the pDOM control. Increased expression of the T cell exhaustion marker PD-1 indicated that combining PD-1 inhibition with vaccination could be beneficial.

This work upholds our proposed mechanism of action that vaccination targeting novel antigens MAGED4B and/or FJX1 combined with PD-1 inhibition can effectively induce T cell mediated tumour attack.

Efficacy of two doses of the vaccine by intramuscular (i.m) injection was tested in the BAM model (B16 melanoma tumour genetically modified to express MAGED4B and HLA-A2) or the BAF model (B16 melanoma tumour genetically modified to express huFJX1 and HLA-A2) using the AAD mouse (HLA-A2+/Kb+). BAM cells have confirmed expression of MAGED4B and mouse FJX1 which has 95% homology to human FJX1. BAF cells have confirmed expression of huFJX1.

B16F10 melanoma cell line expressing the human HLA-A2 gene was kindly given by Professor Eric Tartour (Université Paris Descartes, Paris). This cell line was cultured in RPMI 1640 supplemented with 10% heat inactivated-foetal bovine serum, penicillin/streptomycin (100 U/ml) and 1 mg/ml G418. B16F10/HLA-A2 was validated to endogenously express mouse FJX1 and modified to express human MAGED4B (B16F10/HLA-A2/MAGED4B, “BAM”). In parallel, B16F10/HLA-A2 was modified to express human FJX1 (B16F10/HLA-A2/FJX1, “BAF”). The expressions of HLA-A2 in these cell lines were confirmed by flow cytometry using human HLA-A2–PE (clone BB7.2)-conjugated antibody.

MAGED4B and FJX1 expression levels were confirmed by western blotting using custom made anti-MAGED4B and anti-FJX1 antibodies respectively.

The immunogenicity and efficacy of DNA vaccine were tested on transgenic mice B6.Cg-Immp2lTg(HLA-A/H2-D)2Enge/J expressing chimeric HLA-A2.1/H2-Dd MHC Class I molecule (also known as the AAD mice) purchased from Jackson Laboratory, USA. AAD mice were bred in the animal laboratory for the use in subsequent experiments.

Mice were challenged with the BAM cell line (10^6 cells) at day 0 and then vaccinated with vaccine (50 µg of each vaccine) or 50 µg pDOM DNA vaccine control (intramuscular) in sterile saline on day 5 after palpable tumours were observed (measuring on average 25mm^2). The pDOM control expressed the tetanus toxin DOM helper sequence only. Booster injections were given at day 12. The tumour growth was monitored until mice were culled to assess the tumours for T cell infiltration by immunohistochemistry and flow cytometry. Tumour volumes were evaluated using the formula: volume = ½ (length X $width^2$).

Results for plasmid based vaccines are given on Figure 9 and 10.