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Prospective cancer vaccines for prevention and treatment of cancer

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ABSTRACT

To study tumour immunity and vaccines, knowledge of tumour antigens and genes that encode them is essential. Cancer vaccines and cancer immunotherapy have been developed as a fourth type of treatment. There are two main categories of cancer vaccines: prophylactic (preventative) or therapeutic (curative). Prophylactic vaccines are given to healthy individuals whereas, therapeutic cancer vaccines are administered to cancer patients as a treatment, and the option of therapeutic vaccines is the personalized vaccines which are specific for tumour type and individual patient. The identification and characterization of tumour antigens that are highly immunogenic in human tumours are the key issue in tumour immunology. The candidate tumour antigen for cancer vaccine should have a potent effect in elicitation a specific humoral B cell and cellular T cell responses. To design cancer vaccines, it is necessary to identify a tumour antigen that can generate helper and cytotoxic T cells response and present tumour epitopes effectively. Several strategies have been developed to improve T cell-mediated immunotherapy and tumour vaccines. The current review highlights the tumour antigens as the main target for cancer vaccines; presentation of tumour antigens to CD4+ T cells, and strategies for cancer vaccines in terms of effectiveness of this treatment and the factors that affect the efficacy. Prophylactic, therapeutic, and personalized cancer vaccines have significant promise, but significant work remains to realize that potential. Therefore, technological advancements and a better understanding of tumour-host immune interactions are recommended to realize the mechanisms of resistance to the anti-tumour immune response.

اللقاحات السرطانية المستقبلية للوقاية وعلاج السرطان

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الكلمات المفتاحية:
اللقاحات السرطانية
اللقاحات السرطانية المتخصصة
اللقاحات السرطانية المعتمدة على الخلايا الغصنية
اللقاحات السرطانية الوقائية
اللقاحات السرطانية العلاجية
المناعة السرطانية

الملخص

لدراسة المناعة السرطانية واللقاحات ، لابد من الإلمام بالأنتيجينات (البروتينات) السرطانية ومعرفة الجينات المسئولة عنها . تم تطوير اللقاحات السرطانية والعلاج المناعي للسرطان ليكون النوع الرابع من العلاج . يوجد فئتين من اللقاحات ضد السرطان وهى : اللقاحات الوقائية واللقاحات العلاجية ، ويتم إعطاء اللقاح الوقائي للأشخاص الأصحاء ، فى حين يتم إعطاء اللقاح العلاجي لمرضى السرطان ، والخيار الأفضل للقاحات العلاجية هى اللقاح المتخصص والذى يكون متخصص لنوع السرطان ولكل فرد. ويعتبر تمييز و تحديد الأنتيجينات السرطانية الأساس المهم فى المناعة السرطانية . و الأنتيجين السرطاني المختار لصناعة اللقاح يجب ان يكون فعال فى تحفيز استجابة الخلايا التائية والبائية . ولتصميم اللقاحات السرطانية ، يتم تحديد واختيار الأنتيجين الذي يتم عرضه للجهاز المناعي بفعالية و ينتج عنه نوعي الإستجابات المناعية المساعدة والسمية . هناك العديد من الإستراتيجيات التى تم تطويرها لتحسين العلاج المناعي واللقاح السرطاني المفعل عن طريق الخلايا التائية . تهدف الدراسة المرجعية الحالية الى تسليط الضوء على الإنتيجينات السرطانية كأساس للقاحات ، وأيضا الى آليات عرض الإنتيجينات السرطانية الى الخلايا التائية المساعدة ، وتهدف المقالة ايضا الى الإشارة الى

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استراتيجيات تصنيع اللقاحات السرطانية من ناحية أنواعها وفعاليتها والعوامل المؤثرة على الفعالية .. خلصت هذه المراجعة البحثية الى ان اللقاحات الوقائية والعلاجية والمتخصصة ذات تنبؤ مستقبلى للوقاية والعلاج و التقليل من نسبة حدوث السرطان ، ولكن يبقى الكثير من العمل لزيادة نسبة نجاح هذه اللقاحات ، ومن ضمن الأعمال التى يجب التركيز عليها هى التطورات التكنولوجية فى هذا المجال والفهم الجيد للتداخلات بين الجهاز المناعي والسرطان حتى تتضح معرفة آلية مقاومة السرطان للإستجابة المناعية.

Introduction

The immune system plays a fundamental role in immune responses against a wide range of diseases, including cancer. One important set of molecules in the immune system is the Class I and Class II major histocompatibility complex (MHC) antigens, which deliver peptides from different cellular compartments to the cell surface for presentation to T cells. The presented antigen can be either self or non-self-antigen. Exogenous antigens bind to MHC class II molecules and are presented to $CD4^+$ T lymphocytes, whereas endogenous antigens are loaded into MHC class I molecules for presentation to $CD8^+$ T cells However, it has been demonstrated that MHC class II molecules can also be loaded with endogenous peptides, such as tumour antigens **[1-2].** Thus, tumour epitopes can be recognized by $CD4^+$ T cells in the context of MHC class II molecules.

Two groups of tumour antigens have been recognised and characterised: tumour specific antigens (TSAs) or unique antigens, and tumour associated antigens (TAAs) **[3-4].** (TSAs are unique to malignant cells and cannot be expressed on normal cells, whereas TAAs may be expressed during a specific stage of development or may be expressed at low levels on normal cells and at high levels in tumours. To study tumour immunity, knowledge of tumour antigens and genes that encode them is essential. This will inform strategies aimed to control the expression of these genes and to study the mechanisms of antigen processing for presentation to T cells **[3,5].** The aim of such research is to study the uptake of tumour antigens by antigen presenting cells (APCs) and to direct the antigens to the appropriate antigen processing compartments for effective presentation to T lymphocytes, where cytokines, enzymes and molecules are enrolled in the mechanisms of antigen processing and presentation **[2].** This could be used to facilitate antigen processing towards effective tumour immunotherapy, or the development of cancer vaccines.

Previous project estimates that one person in three in the United States will develop cancer and that one in five will die from it' **[4].** Recently, it has been estimated that new cases of cancer worldwide will reaches around 18 million cases, and more than half of this cases will be deaths **[6].** Therefore, further studies in cancer treatments are essential as the three well-known treatments of cancer, i.e., surgery, chemotherapy, and radiotherapy cad be effective in earlier stage but in most cases ineffective in the advanced or recurrent stages **[7].** Cancer immunotherapies have been developed as a fourth type of treatment. Cancer vaccines and cancer immunotherapy have been focussed over the last half century. That gives new advent in cancer treatment **[8-10].** This kind of cancer therapy has demonstrated a good success for some patients and some cancer types. However, some patients and several cancer types do not respond to the immunotherapies. Recent advances in molecular biology and immunology have led to the identification of tumour antigens that enhance antigen-specific T-cell responses and anti-tumour immunity to achieve best clinical outcomes [**8,10].** There are two main categories of cancer vaccines: prophylactic (preventative) or therapeutic (curative), and the option of therapeutic vaccines is the personalized vaccines which are specific for tumour type and individual patient **[8,11-14].** 'Unlike prophylactic vaccines that are generally administered to healthy individuals, therapeutic cancer vaccines are administered to cancer patients and are designed to eradicate cancer cells through strengthening the patient' s own immune response' **[15].** While the personalized cancer vaccines are designed and produced to meet cancer patients' neoantigens, which arise from mutated proteins in cancer cells. These mutated proteins are considered cancer-specific antigens which are differ from patient to patient and could be highly immunogenic **[16].** The current review highlights the tumour antigens as the main target for cancer vaccines; presentation of tumour antigens to CD4+ T cells, and strategies for cancer vaccines in terms of effectiveness of vaccines and the factors that affect the efficacy.

1. Tumour antigens and tumorigenicity

Tumour antigens are proteins that express abnormally inside the cell or on the cell surface. They can degrade into peptides where they bind into MHC molecules and could be presented to induce T lymphocyte-mediated immune responses **[4].** Several techniques for identification of tumour antigens have been reviewed by **[3].** A good tumour antigen will bind to MHC molecules with a high affinity and induce a T cell response with a repertoire that is specific for tumour epitopes These epitopes can be used for the development of immunotherapy and/or vaccines. The identification and characterization of tumour antigens that are highly immunogenic in human tumours are the key issue in tumour immunology **[17].**

Most tumour antigens have been identified as being recognized by cytotoxic T lymphocytes (CTLs); CD8+ lymphocytes in the context of MHC class I molecules. An example is the melanoma-associated antigen (MAGE), the first human leukocyte antigen (HLA) class I-restricted tumour antigen described **[17].** However, some tumour antigens have been shown to be recognized by CD4+ T helper lymphocytes. One such antigen is tyrosinase that expresses in melanoma cells. It is HLA-DR4 restricted as it binds to this allele with high affinity and induces T cell response. Tyrosinase was the first tumour antigen identified as being recognized by CD4+ T cells **[18].** However, several studies have been carried out to identify tumour antigens presented to CD4+ helper T cells. The majority of these have examined melanoma **[17,19].** To date, only a limited number of human MHC class II-restricted tumour antigens have been identified and more research is needed on different types of cancer **[19].**

There are two classes of MHC molecule known as MHC class I and MHC class II that deliver peptides (both self and non-self) from different cellular compartments to the cell surface. Peptides from the cytosol or endogenous antigens (antigens that are generated within a cell, such as viral proteins in a virus-infected cell) are bound to MHC class I molecules and are recognized by $CD8^+$ T cells. Peptides generated in intracellular vesicles or exogenous antigens are bound to MHC class II molecules and recognized by $CD4^+$ T cells **[20].** The mechanisms of antigen processing and presentation are starting by the degradation of the antigen (protein) into peptides inside APCs (macrophages, dendritic cells, and B cells). The peptide fragments bind to MHC molecules and are carried to the cell surface for recognition by T cell receptors (TCRs) on T cells, leading to the elimination of pathogens. Three main processes take place in the mechanisms of antigen processing and presentation are: **Antigen uptake**, where professional APCs take up antigen via phagocytosis, pinocytosis, or receptor-mediated endocytosis; **Antigen processing**, which refers to the generating of epitopes by the degradation of the native antigen into peptides through enzymatic degradation in APCs. The resulting peptides bind to MHC molecules which are transported to the APC surface for recognition by T cells via TCRs, and **Antigen presentation**: where there are two main pathways of presentation of the antigen: MHC class I and class II pathways. Within MHC class I pathway (the cytosolic or endogenous pathway), the antigenic

peptides are generated inside the cell and bind to MHC molecules **[1-2,20].**

2. The role of $CD4^+$ T lymphocytes on tumour immunity

B and T lymphocytes are the immune system's antigen-specific cells requiring antigen to stimulate the proliferation and differentiation of both cell types. Specific responses to pathogens or foreign substances demand recognition of the antigen, and the peptide antigen induces a signal transduction by way of receptors on the surface of these cells leading to the initiation of acquired immune responses **[2].** In addition, T lymphocytes comprise the main regulatory cells of the immune system. Their fundamental function is largely mediated by secretion of small proteins (known as cytokines) because of antigen stimulation. These cytokines act by binding to high-affinity receptors expressed on the target cells and by inducing biochemical signals within these cells **[21].** As a result, the cytokines activate phagocytic cells to internalize pathogenic organisms and eliminate them. Moreover, the induction of T cells stimulates B cells to produce antibodies specific to the antigen. T lymphocytes have an essential role in suppressing the growth of tumours and the lysis of tumour cells whereas, $CD8^+$ T cells can kill tumour cells directly. However, there is growing knowledge that indicates $CD4^+$ T cells play a central role in initial anti-tumour responses and that is long-lasting effect **[19,22].** The role of $CD4^+$ T helper cells in anti-tumour responses has been attributed to providing regulatory signals required for the activation of MHC class I-restricted $CD8^+$ cytotoxic T cells which serve as the effector of cell-mediating tumour killing. Thus, $CD4^+$ T cells have been considered as essential elements for priming tumour-specific $CD8^+$ T cells. In addition, $CD4^+$ T cells are critical components for generating long-term $CD8^+$ T memory cells and production of antibodies **[22].** Therefore, the induction of optimal anti-tumour immunity involves the activation of both $CD4^+$ and $CD8^+$ T cells specific for tumour-associated antigens **[23].** Taken together, $CD4^+$ T cells are one of the key elements of anti-tumour immunity and their activation is the main basis on which effective vaccines can be developed. **Table 1** illustrates some of human tumour antigens that are presented to CD4+ T cells **[19].**

Table 1. Human MHC class II-restricted tumour antigens presented to $CD4^+$ T cells [19]

Class	Tumour antigen	Restricting allele
Tissue specific:	Tyrosinase	HLA-DR
	TRP-1	HLA-DR
	MART-1	HLA-DR
	gp100	HLA-DR
	MAGE-3	HLA-DR/DP
	CEA	HLA-DR
Shared:	NY-ESO1	HLA-DR/DP
	CAMEL	HLA-DR
	COA-1	HLA-DR
	LAGE-1	HLA-DR
	HER-2/neu	HLA-DR
	p53	HLA-DR
	MUC-1	HLA-DR
Common:	Telomerase	HLA-DR
Viral:	HPV16/E2	HLA-DR
	EBV/EBNA1	HLA-DR

3. The role of MHC class II on tumour immunity

Presentation of MHC class II-restricted tumour antigens to $CD4^+$ T cells has been trialled in tumour immunology. A functional role for MHC class II in the presentation of tumour antigen and induction of anti-tumour immunity has been demonstrated in several animal and some human tumours **[24].** The enhancement of MHC class II expression on tumour cells has been used to produce effective cell-based cancer vaccines which result in long-lasting anti-tumour immunity **[25].**

Most tumour antigens that have been shown to be presented by MHC class II molecules are derived from cytosolic (e.g., MAGE-A3) or cell surface proteins of tumour cells **[26].** However, one study has demonstrated that some tumour antigens loaded by MHC class II are derived from the nucleus and cell organelles such as, mitochondria and endoplasmic reticulum **[27].** For tumour cells that express MHC class II molecules, processing and presentation of tumour antigens expressed on these cells by MHC class II pathway can take place effectively **[26].** Tumour cells that do not express MHC class II molecules can be transfected with MHC class II molecules permitting them to act as antigen presenting cells for effective presentation of their antigens **[28].** Nevertheless, the question of how tumour antigens gain access to MHC class II pathway remains. One possible mechanism is autophagy, where the cells can transfer intracellular antigens into the class II pathway **[26].** The identification of MHC class II-restricted tumour antigens is important because such antigens would be able to generate $CD4^+$ and $CD8^+$ T cell responses. This is a novel approach in tumour immunology

An example of tumour antigen that induce cellular and humoral immune responses is HER2/neu I (an oncogenic cell-surface protein). It has been demonstrated that HER2/neu expresses on human breast cancer cells and many types of epithelial cancers. Interestingly, this antigen is defined by antibodies as well as T cells, since HER2/neu peptides have induced $CD4^+$ helper T cell responses of breast cancer patients. It has also been found that HER2/neu antigen is homologous with epidermal growth factor receptor. Anti-HER2/neu antibodies are also believed to function as the natural ligands of epidermal growth factor receptor. In consequence HER2/neu antigen is a good immunogen and current research is primarily focused on the conduct of clinical trials **[29-33].**

It has been shown that the failure of T-cell-based vaccines in patients with tumours is attributable to an abnormality in the mechanism of antigen processing and presentation by MHC class I and class II molecules **[34].** Such defects are the result of altered expression of cytokines, costimulatory molecules, and chaperones. In contrast, effective processing and presentation of tumour antigen is necessary for successful T cell-based immunotherapy. A study demonstrated the role of endosomal compartments in presentation of endogenous MHC class II-restricted tumour peptides and suggested several methods for determining vaccine efficacy **[35].** A better understanding of the mechanisms of processing and presentation of tumour antigens is therefore essential for the development of successful cancer vaccines.

Functional experiments have demonstrated the role of MHC class II cytoplasmic domain on tumour immunogenicity. That indicated the role of MHC protein in the presentation of tumour antigens **[1].** Importantly, the immunogenicity of a tumour cell-based vaccine was found to be inhibited by truncation of the cytoplasmic domain of MHC class II molecules resulted in a decrease in vaccine immunogenicity **[36].** That is indicating the importance of the MHC class II molecules in tumour immunity.

4. The role of invariant chain on tumour immunity

CD74, also known as the invariant chain (Ii), is a non-polymorphic glycoprotein that has diverse immunological functions. One of the main functions of the MHC class II-invariant chain, in the MHC class II- antigen presentation pathway, is to block the peptide-binding site of MHC-II molecules in the endoplasmic reticulum, thereby preventing the binding of endogenous peptides to MHC class II molecules **[37-38].** A previous in vivo study showed that expression of Ii on tumour cells prevents MHC class II molecules from being loaded with endogenous tumour peptides **[1].** Subsequent studies have also demonstrated that some epitopes can be presented effectively in the absence of Ii. In other words, these epitopes have no need of Ii for their presentation, since the immunogenicity of tumour cell-based vaccines **[28, 39]** and dendritic cell-based vaccines **[40]** was found to be enhanced by the inhibition of the invariant chain. Similarly, it was found that down-regulation of Ii increases the immunogenicity against leukaemia **[41].** More recent study observed accumulation and interaction between CD74 and CD44 in cytoplasmic compartments, suggesting they associate with each other to facilitate tumour growth and metastasis **[42].** Another study showed that the knockdown of CD74 reduced the proliferation of breast cell lines and increased the level of apoptosis significantly **[43].**

Genetic modification of tumour cell-based vaccines was reviewed by **[25].** They critically showed that a high expression of MHC class II molecules increases the effectivity of this vaccine. In contrast, the expression of Ii inhibits the vaccine's efficacy by preventing

endogenous antigens from binding MHC-II molecules in the endoplasmic reticulum for presentation to $CD4^+$ T cells. Thus, the transfection of tumour cells with MHC class II molecules and the inhibition of Ii expression are two strategies for developing effective cell-cancer vaccines.

Earlier studies in tumour immunity were performed on mice tumours. However, recent research has been conducted on human tumours to assess the role that Ii plays in immunity to cancer. This research would seem to indicate that the inhibition of Ii leads to an effective cell-based cancer vaccine. This is supported by other research which has shown that Ii is expressed by multiple tumours, for instance in myeloma cells **[44],** while many normal cells do not express Ii **[25].** The rapidly internalized anti CD74 monoclonal antibody has therefore been used as a target immunotherapy for myeloma and other $CD74^+$ tumours.

5. Involvement of cancer testis antigens in tumour immunity

Tumour cells are characterised by expression of tumour-specific and tumour-associated antigens. That characteristic makes them different from normal cells. However, some of those tumour antigens are found in both tumour and normal cells. An ideal TSA should meet the following criteria **[45]:** it should express only in tumour cells and not in normal one; it should be recognized by the adaptive immune cells (i.e., CD4 and CD8 T cells); and B cell response should be generated after T cell response by producing antibodies against those antigens. However, TSAs are very rare and usually arise in cancer cells by different reasons, mostly are genetic alterations. Several tumour antigens have been identified so far which express in different types of tumours. The attractive group of the identified tumour antigens is cancer testis (CT) antigens that appear to be the focus for immunological studies and become the promising candidate of tumour vaccine. Moreover, it has been mentioned that some of CT antigens might have a role in the pathogenesis and progressive of cancer disease **[46].**

A multiple of cancer antigens have been defined thus far since 1990s **[47],** of which Cancer testis (CT) antigens have a particular attention. Their attractive is relied on their limited expression to cancer tissues, as they are restricted to normal tissues mainly testis **[48].** Therefore, CT antigens' name is attributed to their restricted presence in germ cells of the testis as a normal expression and their expression in several types of tumours. The nomenclature of CT antigens was based on that characteristic that makes them the most attractive target for cancer immunotherapy. They are a multigene family, and they have a shared location, frequently, on X chromosome **[49].** MAGE-1 (Melanoma antigen-1) was the first tumour antigen that was cloned in 1991 **[47].** Subsequent cloning of some T cell epitopes along CT antigens has been applied to identify new antigens **[50-51].**

So far, at least 70 families of CT antigens, accounting for about 140 members **[52]** have been identified. Each of those antigens has a role in the normal cells and their functions in tumour are still under investigation. The abnormal expression of CT antigens in cancer cells has been found in multiple cancer types at the level of proteins, and at the level of genes, and the CT gene database was established to include all the information regarding those genes **[49,52].**

CT antigens have been shown to express in many types of cancer. The wide expression of this group of tumour antigens indicates the high importance of this family and the necessary of addressing its role in normal and tumour cells. Moreover, its role as an immune target for tumours should be focussing on. However, some studies have demonstrated the expression of some CT antigens in normal and malignant tissues **[53-55].**

The restricted expression of CT antigens in cancer cells has led the researchers to target them as tumour vaccines, since the development of antigen-specific cancer vaccine depends mostly on the antigens which have a restriction expression in tumours and have no expression on normal organs. Furthermore, the candidate tumour antigen for cancer vaccine should have a potent effect in elicitation a specific humoral B cell and cellular T cell responses **[48].** With this regard, several studies have focussed on identification of T cell epitopes from CT antigens aimed at generating potent cellular tumour immunity. The successful immune response against tumours demands recognition of epitopes in the tumour cells to create effective T-cell responses targeted to those tumours associated antigens (TAAs). Potent immune responses against the previous studied TAAs, such as HER2, CEA, MUC1 and p53 may be limited due to immune tolerance because they are self-antigens. However, CT antigens may be considered as non-self-antigens that could induce strong anti-cancer immunity **[56].** As a result, their role in tumour immunology has been highlighted in the recent studies. Among CT antigens, NY-ESO-1 is the focus as it is considered the most immunogenic CT antigens known to date. Therefore, more attention has been paid on it due to its cellular and humeral immunogenicity. In addition, it's widespread among many tumours that make it a potential candidate for cancer vaccine against many cancers **[57].**

It was found that both CD4 and CD8 T cells play a critical role in generating strong immune responses against tumours. However, CD4 T cells have a major role in the anti-tumour immunity **[19,22].** Therefore, identification of both CD4 and CD8 T cell epitopes from tumour antigen is leading to the development of effective cancer vaccines.

Due to the immunogenicity demonstrated against many of CT antigens, this group of tumour antigens has been used for vaccine and/or immunotherapy trials. **Table 2.** presents a list of some CT antigens of known spontaneous immunity in cancer patients **[48-49].**

Table 2. List of some CT antigens of detected spontaneous immunity [48-49].

Immune response	Antigens
Cellular and Humoral	MAGE-A (CT1); SSX-1 (CT5); SSX-2 (CT5); SSX-4 (CT5); NY-ESO-1 (CT6)
Cellular	BAGE (CT2); MAGE-B (CT3); GAGE-A (CT4); NA88 (CT18)
Humoral	LAGE-1; MAGE-C (CT7); SCP-1 (CT8); TPTE (CT44); cTAGE-1 (CT21); SPA17 (CT22); OY-TES-1 (CT23); CAGE (CT26) HOM-TES-85 (CT28); HCA661 (CT30); FATE (CT43); NY-SAR-35 (CT37); CT10

6. Strategies for cancer vaccines

6.1 History of cancer vaccines

William Coley was the first who did an attempt for improving a patient with cancer in 1891 by intratumoral injections of what was known by Coley's toxin **[58].** This idea was the starting of stimulation of the immune system to fight cancer cells. The mechanisms of stimulation the immune system by therapeutic vaccination based on attack the cancer cells without affecting the normal ones. As a result, therapeutic cancer vaccines can be used to inhibit the growth of cancer cells at advanced stages, and that could be beneficial also in case the conventional therapies (surgery, radiation, and chemotherapy) are not effective **[15].** Cancer immunotherapy and vaccinations is relied on understanding the mechanisms of immune response and immune resistance.

'To design the vaccines of the future we need to fully exploit microbial genomes and understand the basic mechanisms of the immune system' **[59].** To design cancer vaccines, it is necessary to identify a tumour antigen that can generate helper and cytotoxic T cells response and present tumour epitopes effectively. In the context of strategies for breast cancer vaccines and immunotherapy, it has been shown that breast cancer cells express multiple tumour-associated antigens (TAAs), such as MUC-1, which is expressed in 80% of breast cancers and erbB-2 which is overexpressed in about 30% of breast carcinoma. It has been shown that the monoclonal antibody DF3 can cause phagocytosis and lysis of Mucin-1-expessing breast cancer cells **[60].** Another antigen that expresses on human breast cancer cells is HER2/neu which is defined by antibodies as well as T cells. HER2/neu also shows overexpression

in other types of cancers **[61-64].** In contrast, however, it shows low expression in normal cells **[65].** It has been determined that HER2/neu is a CTL-specific human breast cancer antigen by using dendritic cells loaded with epitope from the HER2/neu protein [66]. In addition, HER2/neu peptides have been shown to induce antigen-specific T cell and antibody responses in breast cancer patients **[67].** More recently, DCs were used as APCs to study the antigen uptake of HER2 antigen by DCs and use these cells as a model of breast cancer vaccine **[68].** One of the aims of this work was to look at the internalization of the breast tumour antigen (HER2) by DCs and use these cells as a model of breast cancer vaccine to be tested in HLA-DR transgenic mice. The preliminary result of that study showed that HER2 has been internalized by DCs, and that would be a type of DC-based cancer vaccine design (work in progress). Other TAAs that express on breast cancer cells are MAGE-1, BAGE, mammaglobin and MENA. All these antigens have been identified as targets for effective presentation to CTLs **[69-70].**

Since there are a huge number of TAAs in breast cancer patients, it could be possible to induce immunity against these TAAs **[70].** One successful result has concluded that a group of undefined breast cancer-associated antigens was highly immunogenic in transfected antigen-presentation cells, since immunization of breast tumour-bearing mice with transfected fibroblasts resulted in prolonging the survival of these mice **[69].** Collectively, these characteristics make such tumour cells a target for research in cancer vaccines, and more studies need to be carried out using advanced immunological techniques to develop new successful tumour vaccines and/or immunotherapies. This kind of research may provide an insight into the presentation of tumour antigens to helper (CD4+) T cells for effective immune responses.

6.2 Types of cancer vaccines

Several design strategies have been developed to improve T cell-mediated immunotherapy and tumour vaccines. These take into consideration all the immunological factors that enhance immune responses against cancers. The more practical of the strategies include:

Peptide / protein cancer vaccines: Peptides are a short subunit of proteins resulting from degradation of the proteins inside the antigen presenting cells and are presented on the cell surface via MHC molecules for T-cell recognition in this type of vaccines, an immunodominant peptide derived from a tumour antigen are used as the candidate vaccine **[9].**

Several studies have focused on NY-ESO-1 (a melanoma antigen), which belongs to CT antigens. The cellular and humoral immune responses produced against NY-ESO-1 have led the researchers to use it in vaccine trials and or/immunotherapy in different types of cancer. All these trials aimed to generate long lived cellular immunity. Some of these studies used full length NY-ESO-1 (recombinant protein) applying different adjuvant **[71-75].** Others used synthetic peptides mostly ESO-1157-170 epitope, which is considered the highly immunogenic T cell epitope **[76-78].** Based on the critical role of brother of the regulator of imprinted sites (BORIS) which is a new CT antigen in cancerogenesis and its expression pattern in cancer cells, protein-based mouse BORIS vaccines using a non-DNA-binding version of the BORIS molecule were generated by [**79].** They have generated the vaccines by using BORIS molecule without DNA-binding zinc fingers domain to avoid the risk of BORIS protein in the progression of cancer. This type of vaccine produced Ag-specific CD4+ T cell and some cytokines, production of antibodies and anticancer CD8+-cytotoxic T cells responses in immunized animals. The CD8+-cytotoxic responses were determined among different cancers in mice, e.g., mammary adenocarcinoma, glioma, leukaemia, and mastocytoma.

Tumour cell vaccines

That could be of two types: the first one is autologous tumour cell vaccines by using of patient-derived tumour cells (patient specific) and it is one of cancer vaccines that was first used the end of the seventies of the last century **[15].** The tumour cells are irradiated and stimulated with an immune adjuvant, such as BCG, then they will be injected to the patient who the tumour cells were isolated from. This type of vaccine has been tested in several cancers. The other type of tumour cell vaccines is allogenic tumour cell vaccines, (non-patient-specific) which contain human tumour cell lines to overcome the limitation with autologous tumour cells **[7,15,33].**

Tumour-based cancer vaccines can be also produced by transfecting of tumour cells with MHC class II and costimulatory molecules **[28,80].** So, the cells act as antigen presenting cells.

Dendritic cells (DCs) cancer vaccines: this utilizes dendritic cell-based vaccines, used to present tumour antigens **[81].** Dendritic cells (DCs) are considered a regulator cell of the immune system against a variety of diseases and are also critical for generating a specific anti-tumour immunity **[82].** Since their discovery in 1973, DCs have been identified as a key of antigen presenting, which are effective in processing and presenting of an antigen to the T cells with great potential in vaccine development **[9].** DCs have a unique capacity to activate and regulate adaptive immune response **[7,15,32-33].** Peptide epitopes are generated inside the DCs because of processing of an antigen which is bound to MHC class I or class II molecules for presentation to CD8+ or CD4+ T cells, respectively, on the surface of APC.

The recent immunotherapeutic studies have focussed on using DCs as adjuvant for vaccination **[83-84],** as the immunogenicity against the antigen delivered by DCs have been reported in cancer patients **[83].** The new strategies of monocyte isolation from whole blood and from PBMCs will make the generation of monocyte-derived dendritic cells (Mo-DCs) more efficient, reliable, and easier than previous methods. These strategies will help to save resources and can be applied in research and clinical approaches. This technique can be applied for in vitro-generated DCs, and in efficient generation of immature and mature DCs, **[68,85].**

T cell-based vaccines

T cell-based vaccines **[59],** used to induce anti-tumour responses. T cell clone specific for NY-ESO-1, which was used as an immunotherapy for a patient with metastatic melanoma **[86].** This type of immunotherapy could be used as a personalized therapeutic vaccine for the cancer patient who the T cells were isolated from his blood. An efficient vaccine for NY-ESO-1 that can generate immune responses against tumour cells should be processed naturally **[87] or** can be delivered in the intracellular compartments of APC for efficient processing **[88].** The results achieved from the above trails demonstrated that NY-ESO-1 is a promising candidate for cancer vaccine and/or immunotherapy, but still a lot of improvements with the vaccine design are needed **[57, 89].**

Genetic vaccines

Genetic vaccines consist of DNA, RNA, or viral vector **[7].** By using this type of vaccines, it can stimulate the immune responses for several epitopes of the tumour antigen **[90].** Some other advantages of nucleic acid vaccines are low cost, stable, no need of adjuvants when an immunogenic viral vector is used. Both DNA and RNA vaccines have shown promise in stimulating the immunity against cancer antigens with some differences **[7, 15].** In this term, there is also gene-based cancer vaccines which is nucleoside-based cancer vaccines and comprise a good percent in preclinical and clinical development **[10].** Experimental attempts have developed a mutant variant of BORIS that lacks tumorigenic ability and have immunogenic epitopes that produce responses against tumour cells **[91].** They used a DNA-based vaccine and a protein-based vaccine. They determined that BORIS-DNA vaccine, but not recombinant protein vaccine, induced helper T-cell responses that significantly inhibited tumour growth and prolongs the survival of vaccinated mice. These studies demonstrate that DNA immunization is superior to recombinant protein-based vaccine. Other attempts used NY-ESO-DNA vaccine **[92]** and found that the NY-ESO-1 DNA vaccine was safely induced antigen specific effector CD4 and/or CD8 T cell responses in 93% of cancer patients.

6.3 The approval cancer vaccines

The clinical trials of therapeutic cancer vaccines that aim to treat cancer at late stages by stimulation the cancer patient's immune system, have led to the approval of some of these vaccines by the U.S. Food and Drug Administration **[15].** The first therapeutic cancer vaccines that has been approved was Sipuleucel-T (also, known as PROVENGE; Dendreon); **[93].** Currently, there are four preventive

cancer vaccine have been approved by FDA (**Table 3) [8,32].** In comparison, FDA has approved just two therapeutic cancer vaccines. The first one is Bacillus Calmette-Guerin (BCG) which treats early early-stage bladder cancer. It is given by instillation into the bladder. The second approved therapeutic vaccine is Sipuleucel-T(PROVENGE) which is used to treat the prostate cancer **[32].** In addition, there are several cancer vaccines currently under clinical trials **[9,32-33].** However, each cancer vaccine has advantages and disadvantages.

Table 3. List of FDA-approved prophylactic cancer vaccines with strategy [8,32].

Name of the vaccine	Strategy	Cancer type prevented
Cervarix	Viral antigens-based vaccine	Renal, cervical, head and neck, penile, vulvar, and vaginal cancers related to HPV
Gardasil-4	Viral antigens-based vaccine	Renal, cervical, head and neck, penile, vulvar, and vaginal cancers related to HPV
Gardasil-9	Viral antigens-based vaccine	Renal, cervical, head and neck, penile, vulvar, and vaginal cancers related to HPV
HBV vaccine (HEPLISAV-B)	Viral antigens-based vaccine	Hepatocellular carcinoma related to HBV

The most advantages of these preventive cancer vaccines are highly effective, safety and immunogenic, whereas the disadvantages that they are restricted to known pathogenic virus, i.e., they cannot treat a broad of viruses **[8].**

7. The factors that affect the response to cancer vaccines

The ideal immunological target for cancer vaccine development would meet the criteria of tumour specificity, immunogenicity, and vital dependency of the tumour on the functional activities of the antigenic target to avoid antigenic loss by mutation. Some of tumour antigens meets these criteria **[84,91].** However, the immunogenicity of these target antigens could differ from person to person. What are the reasons behind that and what are the factors that influence the immune responses? This section will highlight that factor that affect the responsiveness of cancer vaccines. Linked to the successful development of some cancer vaccines, they have given the patients new hope although not all of them work probably in cancer patients. One of most important reason is the weakness in the immune system itself which could be due to genetic causes or other diseases. It has been found that there are some gene mutations that prevent the body of the patients from responsiveness to the treatment including vaccines. Also, it has been investigated that some proteins cause an obstacle to successful pharmacotherapy of cancers as they prevent cellular uptake of many structurally and functionally diverse compounds, including most cancer therapeutics and in this way cause multidrug resistance **[94].**

Disease recurrence has been estimated as a significant problem in about 20% of breast cancer patients, as most of patients with metastasis and advanced stages of disease develop resistance to the targeted cancer therapies leading to a poor clinical outcome **[82].** However, vaccination of breast cancer patients with a tumour antigen; human epidermal growth factor receptor 2 (HER2) pulsed with DCs prior to surgery has eliminated HER2 expression in tumour **[82].** Additionally, the toxicity of some cancer vaccines is an important factor to determine for efficacy and safety of the vaccine. It has reported the toxicity end points of HER2 vaccines and summarized that treatment with HER2 vaccines, such as the DC vaccine and the DNA plasmid-based vaccines were well tolerated and discontinued due to treatment-related toxicity but will continue to be developed for the treatment of HER2+ breast cancer [31]. Moving forwards, promising results have shown that a therapeutic DC-cancer vaccine against HER2 using autologous DCs (10 and 20 million DC) had clinical benefit in 54% of patients and based on the safety data, a maximum increased dose to 40 million DCs has been approved **[30].** Additionally, previous study has demonstrated that the trastuzumab, the monoclonal antibody targeting HER2 protein, increases the uptake od HER2 and links innate and adaptive immune response (cross-presentation) by DCs **[67].** On the other hand, immunosuppressive tumour microenviroment is one of the major reasons for the limited success and development of effective therapeutic cancer vaccines. As soon as the immune response is suppressed, tumour escape and recurrence will occur **[15].** Furthermore, clinical studies have shown that less prior chemotherapy are generally more responsive to vaccines **[15]**. The immune responses after therapeutic vaccines need more time than the response to the chemotherapy **[15].** Nevertheless, clinical effects of cancer vaccines were often tested in patients with advanced stages of disease whom immune system was compromised by exposure to several other treatments (surgery, chemotherapy, radiotherapy), or by progression of the disease. Moreover, the immune status of individual patients may be affected by other factors such as age and past treatment history **[7].** Taken together, several reasons likely contribute to the inability of cancer vaccines to achieve potential role in all patients, such as type of tumour antigen, immune tolerance, and the development immunosuppressive tumour microenvironment **[8].**

For successful development of cancer vaccines, it is essential to develop accompanying delivery methods and adjuvants to adequately predict anti-tumour effect of the vaccines and to achieve the expected effects **[95-96].** The combination therapy with cancer vaccines has also the potential effect than individual therapy. Such combination therapies are immune checkpoint inhibitors, monoclonal antibodies, co-stimulatory molecules, and other immune modulators to boost anti-tumour immunity. The combination of different immunotherapies has indicated promising effects on enhancing anti-tumour immunity and will offer clinical benefit to patients with late stages metastasis **[8,16,97].** Last and not least, the psychological status of the patients and their wellbeing for recovery is very important factor for efficacious of any treatment including cancer vaccines, as the good psychological conditions enhance the immune system. Therefore, to impact the mechanisms of resistance to the anti-tumour immune response, novel combination strategies of therapies should be further investigated which should include technological advancements and a better understanding of tumour-host immune interactions are recommended **[33].**

Conclusion

Prophylactic, therapeutic, and personalized cancer vaccines have significant promise, but significant work remains to realize that potential**.** They have offered hope to cancer patients, but they are not effective for all the patients, although the evidence showed that cancer vaccines are able to generate antitumour responses in some patients. Recent clinical trial data using personalized cancer vaccines are highly encouraging but, however, several reasons likely contribute to their inability to achieve potential role in all patients, such as type of tumour antigen, immune tolerance, and the development immunosuppressive tumour microenvironment. In addition, combination therapy of other immunotherapies with cancer vaccines will be promising synergistic effects to produce a combined effect greater than each separate therapy. The factors that could affect the effectiveness of cancer vaccines are summarized as follow:

- The weakness in the immune system which could be due to genetic causes or other diseases.
- Gene mutations that prevent the body from responsiveness to the treatment.
- Some proteins prevent cellular uptake of many structurally and functionally diverse compounds, which cause an obstacle to successful pharmacotherapy of cancers.
- Most of patients with metastasis and advanced stages of disease develop resistance to the targeted cancer therapies leading to a poor clinical outcome and disease recurrence.
- The toxicity of some cancer vaccines is an important factor for efficacy and safety of the vaccine.
- Immunosuppressive tumour microenviroment is one of the major reasons for the limited success of effective therapeutic cancer vaccines.
- High exposure to the chemotherapy as less prior chemotherapy is generally more responsive to vaccines, and patients with advanced stages of disease whom immune system was compromised by exposure to several

other treatments or by progression of the disease are less responsive to vaccines.

- The status of the immune system of the patients which may be affected by other factors such as age and past treatment history.
- The combination therapy with cancer vaccines has also the potential effect than individual therapy. to boost anti-tumour immunity.
- The psychological status of the patients and their wellbeing for recovery is very important factor for efficacious of any treatment including cancer vaccines.
- Additionally, the procedure and cost of personalized cancer vaccines should be also highlighted.

Therefore, technological advancements and a better understanding of tumour-host immune interactions are recommended to realise the mechanisms of resistance to the anti-tumour immune response.

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