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Chapter

Cancer Vaccines

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Abstract

Recent advances in immuno-oncology have allowed for the design of more specific and efficient cancer vaccine approaches. There has been an improvement in molecular biology techniques, as well as a greater understanding of the mechanisms involved in the activation and regulation of T cells and the interplay between the components of the immune system and the escape mechanisms used by cancer cells and the tumour microenvironment. As a result, many interesting developments in therapeutic cancer vaccines are ongoing, with influence on survival still to be proven. The spectrum of tumour antigens that are recognised by T cells is still largely unchartered and, most importantly, dynamically evolving over time, driven by clonal evolution and treatment-driven selection. Vaccine approaches currently in development and tested in clinical studies are based on tumour antigens specifically identified for each tumour type, on tumour cells or dendritic cells, the latter having the potential to be modified to incorporate immunostimulatory genes. However, interplay between the immune system and the tumour and the inhibitory mechanisms developed by tumour cells to subvert immune responses are crucial issues that will need to be targeted in order for efficient therapeutic vaccines to emerge.

Keywords: vaccine, T cells, tumour antigens, immune system

1. Introduction

Cancer constitutes one of the biggest burdens in the Western society with lung, breast, prostate and colorectal cancer being the most prevalent. Despite declining rates in the Western societies [1], there have been an estimated 9.6 million deaths by cancer worldwide in 2018 [2, 3], and it is expected that this number will further increase over time.

The molecular nature of human cancers is complex and varies among tumours and individuals. For that reason, the approach towards a more personalised cancer treatment has gained intense interest. Treatment approaches are being increasingly changing from histology- to molecular-based therapies, including targeting the interplay between cancer and the immune system.

In the last few decades, major advances have been made in recognising that an effective immune system—or the lack thereof—plays an important role in cancer development, growth and metastasis. For example, the presence of tumour-infiltrating lymphocytes (TILs) has been identified as a positive prognostic factor in multiple cancer types [4]. Utilising the body's defences and reactivating the antitumour immune response, initially regarded as a simple paradigm, have created a scientific and therapeutic revolution [5]. After decades of attempts, immunotherapy

has achieved a major breakthrough with the sensational successes of immunomodulation with checkpoint blockade (i.e. PD-1/PD-L1 and CTLA-4 inhibitors, among others). In addition to that, the recent development of immune effector cell therapy in the form of chimeric antigen receptor T cells for haematological malignancies has opened exciting horizons for solid tumours as well [6, 7]. Up to now, therapeutic vaccination against cancer, despite few examples like Bacillus Calmette-Guérin (BCG) treatment for superficial bladder cancer [8], the oncolytic virus-based talimogene laherparepvec (T-VEC) [9] and the Sipuleucel vaccine in prostate cancer [10], has not achieved similar results.

Nevertheless, new vaccine development techniques as well as immunotherapy combination strategies shape the pipeline of current trial development and represent a promise and challenge for the future.

1.1 The T-cell response to cancer

The immune system and in particular dendritic cells (DCs) and macrophages are capable, to a variable extent, to recognise damage-associated molecular patterns (DAMPs), thus eliciting an innate immune response. The major DAMP driving innate host antitumour immune responses is tumour-derived DNA, which is detected via the stimulator of interferon gene (STING) pathway and results in type I IFN production [11].

The adaptive immune response begins when cancer antigens are presented to T and B cells by DCs. Although B-cell responses are probably playing a role in antitumour immunity, not much is yet known about them [12]. The following text will therefore mostly refer to antitumour T-cell responses. Tumour antigens are transported via lymphatic vessels to the lymph nodes, where they are captured by lymph node-resident DCs. Alternatively, tissue-resident DCs capture antigens at the tumour site and migrate to induce T-cell responses in the lymph node [13]. DCs present protein antigens in the context of major histocompatibility complex (MHC) class I and II molecules, allowing the stimulation of rare antigen-specific $CD8^+$ or $CD4^+$ T lymphocytes, respectively. Upon antigen encounter, CD8 T cells differentiate into cytotoxic T lymphocytes (CTLs) that have tumour-killing capacities, whereas CD4 T cells will provide CD8 T-cell help [13]. CD4 T cells can also be induced to become $FoxP3^+$ regulatory T cells (Tregs), which are then able to inhibit antitumoural immune responses [14].

A tumour mass is not composed solely of tumour cells, but contains immune cells, stromal cells and vessels, a concept known as the tumour microenvironment. Tumours are organised in various reciprocal, local and systemic relations with myeloid and lymphoid immune cell populations, both being key factors in regulating immune responses to cancer. During progression, tumours are able to modulate the immune response and highjack it to their advantage, in order to invade and grow. Macrophages can be polarised to a pro-tumoural and anti-inflammatory (called M2) phenotype at the tumour's advantage. In addition, myeloid-derived suppressor cells (MDCSs) accumulate in the tumour microenvironment and are able to suppress antitumour T-cell responses [15, 16].

1.2 The three phases of tumour immunoediting: elimination, equilibrium and escape

The principles of cancer immunoediting have set the basis for understanding the dual host-protective and immuno-sculpting effects of immunity on cancer [17]. During cancer immunoediting, the host immune system influences tumour fate in three phases through activation of innate and adaptive immune mechanisms: elimination, equilibrium and escape.

1.2.1 Elimination

The elimination phase occurs when cancer cells are eradicated by a competent immune system. This is evidenced by immunodeficient mice that have an increased propensity to develop carcinogen-induced and spontaneous cancers than wild-type mice [18]. In addition, tumours that come from immunodeficient mice are more immunogenic than those from immunocompetent mice, as they have not been edited by the immune response. Patients suffering from AIDS [19] or being under immunosuppression are similarly more prone to develop cancer [20, 21]. The role of CD8 T cells has been more extensively studied; however interplay with CD4 T-cell responses is also required in order to have an integrated and efficient response [22, 23].

1.2.2 Equilibrium

The sporadic tumour cells that survive immune destruction will enter into the equilibrium phase where editing arises. Immune pressure is mostly mediated by CD4 and CD8 T cells [24]. Upon tumour editing, more mutations will be acquired, which will favour entry into the escape phase of immunoediting. Importantly, the process of incomplete elimination promotes the generation of tumour cell variants with decreased immunogenicity [23]. The identification of hidden cancer cells in an equilibrium state remains a challenge; however, advances in technology and biomarkers may allow for circulating tumour cells and niches to be investigated further.

1.2.3 Escape

The escape phase represents the final phase of the process, where immunologically sculpted tumours begin to grow progressively, becoming clinically apparent. Tumour escape can result from many different mechanisms including reduced immune recognition, through loss of MHC class I, co-stimulatory molecules or tumour antigens. In addition, the tumour induces many molecules and cells to induce an immunosuppressive tumour microenvironment. Cytokines such as VEGF and TGF-β, immunoregulatory molecules such as indoleamine 2,3-dioxygenase (IDO), programmed death-ligand 1 (PD-L1) and ligands for Tim3 and lymphocyte-activation gene 3 (LAG-3), among others, are induced to supress the incoming CD4 and CD8 T cells. In addition, many cellular components of the tumour microenvironment, such as macrophages and neutrophils, are being redirected in an anti-inflammatory pro-tumoural state [25–27].

1.3 The principles and means of immunotherapy

Although cancer cells have the unique ability to escape from the immune response, the knowledge that immune cells are able to recognise tumours allows development of therapies that utilise the immune system [28]. Cancer immunotherapies focus on exploiting both the innate and adaptive arms of the immune system. They can be classified into vaccines, monoclonal antibodies (including immune checkpoint inhibitors), recombinant cytokines, small molecules and adoptive T-cell transfer, including chimeric antigen receptor (CAR), TCR and TIL therapy [29–32].

1.3.1 Vaccines

The aim of cancer vaccination is to prime cellular immune response against tumour-specific antigens. Despite its limitations mainly owing to heterogeneous tumour antigen composition and expression and their susceptibility to various

mechanisms of immune suppression, it is being intensively developed. Currently revisited with strategies aiming at combinations with other immunotherapies, cancer vaccines will be addressed in detail in this chapter.

1.3.2 Monoclonal antibodies

Antibodies target (a) factors that regulate signal pathways used by cancer cells in division and angiogenesis (such as the VEGF inhibitor bevacizumab) [33]; (b) tumour-associated antigens, activating antibody-dependent cellular cytotoxicity (such as the Her2-directed antibody trastuzumab) [34]; (c) complement-dependent cytotoxicity (such as the anti-20 and anti-EGFR antibodies rituximab and cetuximab); and (d) immune blockade with checkpoint inhibitors such as anti-CTLA4 antibodies (ipilimumab and tremelimumab), anti-PD1 antibodies (nivolumab and pembrolizumab) or anti-PD-L1 antibodies (atezolizumab, durvalumab, and avelumab) [31].

1.3.3 Recombinant cytokines

Immunostimulatory recombinant cytokines promote lymphocyte activation via control of transcriptional and metabolic programmes [35]. An example is recombinant IL-2 (aldesleukin, Proleukin®) that has been used to treat renal cancer and melanoma [36]. Another recombinant cytokine approved by the US Food and Drug Administration (FDA) for the adjuvant treatment in resected melanoma patients is pegylated interferon α-2β (Sylatron®), a member of the IFN cytokine family [37]. Concurrent administration of immunostimulatory cytokines such as IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) may also enhance the efficacy of antibody therapy [38]. Limitations include their antigenicity, poor pharmacokinetics and high toxicity [29].

1.3.4 Small molecules

The use of small molecules in cancer immunotherapy has been increasing, given their ability to target both intracellular and surface targets. Plerixafor is a small molecule that inhibits the binding interaction of stromal cell-derived factor 1 (SDF-1) to the chemokine receptor CXCR4, used as a haematopoietic stem cell mobiliser [39]. This small molecule aims to prevent the development of cancer metastasis in cancer patients, principally in pancreatic ductal adenocarcinoma patients [29]. Another known small molecule called imiquimod, used for the treatment of basal cell carcinoma, is an agonist for toll-like receptor (TLR)-7. Imiquimod-mediated TLR7 activation induces production of proinflammatory cytokines, inhibits Tregs and induces activation of natural killer (NK) cells to eliminate cancer cells [29]. IDO inhibitors are being tested as well in multiple malignancies, but results as monotherapy have been disappointing [40]. Combinations with other immunotherapeutic agents or with chemotherapy/radiation are being currently investigated. A much anticipated combination, however, of the small molecule IDO1 inhibitor with pembrolizumab failed to provide significant benefit in a phase 3 trial in unresectable or metastatic melanoma [41]. Finally, ongoing research evaluates the adenosine signalling with adenosine receptor inhibitors [42].

1.3.5 Adoptive T-cell therapy

The use of cancer patient's own immune effector cells is a novel cancer immunotherapy, also called adoptive cell therapy. Starting with TILs, it has moved to the generation of artificial T cells that are genetically altered to express an antitumour

antibody (CARs) or a selected TCR [30]. These cells are multiplied and subsequently transferred back to the patient, who usually receives conditioning chemotherapy. TIL therapy has shown some clinical evidence of efficacy in the treatment of melanoma [43] and cervical cancer [44], with the LN-145 TIL therapy recently obtaining breakthrough therapy designation by FDA, while its potential is being further investigated. Generation of tumour-specific T cells through expression of a TCR that has shown antitumour properties is ongoing for several malignancies [44]. CAR T cells, which are engineered to express part of a tumour-specific antibody, linked to intracellular T-cell signalling domains are gaining major interest [45]. Two anti-CD19 CAR T-cell therapies have so far received FDA approval in haematological malignancies, notably tisagenlecleucel for diffuse large B-cell lymphoma (DLBCL) and acute lymphoblastic leukaemia and axicabtagene ciloleucel for primary or transformed DLBCL, mediastinal and high-grade B-cell lymphoma [46]. Research on CAR T-cell therapies in solid malignancies is currently ongoing.

2. Cancer vaccines

2.1 Introduction

Cancer vaccination seeks to generate, amplify or skew (or the combination thereof) antitumour immunity. In order to reach such an ambitious goal, many approaches are in development, including the administration of tumour antigens, often with antigen presenting cells or other immune modulators.

Current technological advances in genomics, data science and cancer immunotherapy enable the fast mapping of alterations within a genome, as well as the rational selection of vaccine targets and on-demand production of a therapy that has been customised to a patient's individual tumour. With the development of vaccination being promoted by emerging innovations in the digital era, vaccinating patients according to their individual tumour mutational profile may become the first truly personalised treatment for cancer.

It is important to distinguish vaccines that are designed to prevent cancer from the ones that are designed to treat cancer. The mode of action of the HPV vaccine for the prevention of cervical and other HPV-associated cancers [9] and of hepatitis B virus (HBV) vaccine for the prevention of HBV infection that carries a risk of development of hepatocellular carcinoma [47] is the prevention of infection itself. Their action is based on the generation of antiviral antibodies and has led to a net reduction in the incidence of these cancers in vaccinated individuals [48]. The development of therapeutic cancer vaccines has been more challenging. The nature of the antigen, which, in the case of therapeutic cancer vaccines, is derived from self-antigens against which the host has been tolerised and the presence of a hostile tumour microenvironment are key limiting factors.

2.2 Therapeutic vaccines

2.2.1 FDA-approved vaccines

Three therapeutic cancer vaccines have been approved by the FDA. The Bacillus Calmette-Guérin (BCG, TheraCys®, TICE®) vaccine, based on a live attenuated strain of *Mycobacterium bovis*, was the first approved cancer vaccine for use in non-muscle invasive bladder carcinoma following transurethral resection. It showed a prolongation in disease-free survival (DFS) of 30 months in patients with bladder carcinoma in situ (CIS) and of 22.5 months in patients with Ta/T1 urothelial

carcinoma compared to 4.9 months in bladder CIS and 10.5 months in Ta/T1 patients treated with topical doxorubicin [49].

Sipuleucel-T (Provenge) is an autologous DC vaccine for patients with minimally symptomatic or asymptomatic metastatic castrate-resistant prostate cancer (mCRPC). Patient's DCs are being injected with a recombinant fusion protein, PA2024, which consists of a tumour antigen, the prostate acid phosphatase (PAP) and GM-CSF, before reinfusion. The phase 3 IMPACT study, a double-blind, placebo-controlled, phase 3 trial of 512 mCRPC patients randomised to receive either three infusions of Sipuleucel-T or placebo 2 weeks apart, demonstrated statistically significant improvement of 4.1 months in median overall survival (OS) (25.8 months in the Sipuleucel-T group compared to 21.7 months in the placebo group) [10]. However, this study elicited significant criticism in regard with the observed—albeit modest—OS benefit without correlation with a progression-free survival (PFS) benefit or a T-cell response, the lack of association between survival benefit and T-cell proliferation responses, the fact that T-cell proliferative responses to the chimeric antigen (PA2024) did not cross-react to the physiological human PAP and hence the absence of alternative mechanisms to explain the survival benefit [50].

The third approved vaccine, called talimogene laherparepvec (T-VEC or Imlygic), is an oncolytic herpes virus 1-based vaccine for advanced melanoma. In this vaccine, two viral genes governing neurovirulence and blockade of antigen presentation are deleted, and the virus is modified to produce GM-CSF to enhance immunogenicity [8]. T-VEC was approved based on data published on the phase 3 OPTiM trial. The vaccine virus, injected intralesionally, infects both the cancer and normal cells but can only replicate within cancer cells. The OPTiM trial showed more durable response rate ($\geq$6 months) with T-VEC than GM-CSF alone, as well as higher overall response rate and a longer median OS (23.3 months compared to 18.9 months with GM-CSF alone) in patients with stage IIIB, IIIC or IV M1a melanoma [51].

2.2.2 Mode of action of therapeutic vaccines

The mode of action of most therapeutic vaccines involves development of cell-mediated immunity directed against tumour antigens; such antigens ought to ideally not be expressed in normal cells or have restricted normal expression, be of high expression on cancer cells, be highly immunogenic and be necessary for cancer cell survival [32]. Tumour antigens can be delivered as peptides, proteins, DNA or viral vectors or tumour cells themselves. They are usually administered with an adjuvant (see Section 2.5), in order to potentiate the immune response. Tumour antigens can also be generated via antigen spreading, which is the exposure of novel antigens after an initial antitumour response [52].

2.3 Tumour antigens

2.3.1 Tumour-associated antigens (TAAs)

TAAs are self-antigens commonly expressed in a specific tumour type among different patients. They are derived from non-mutated proteins that are overexpressed in tumour cells as compared to normal cells. The first TAAs were discovered after the cloning of gene-encoding proteins that generated epitopes recognised by tumour reactive TILs [53]. The first gene discovered that was reported to encode a tumour antigen recognised by T cells was MAGE-1 [53]. Since the discovery of MAGE-1, a large number of TAAs have been described, and they are classified into shared TAAs and unique TAAs [54], the latter being present only in individual patients.

Shared TAAs can be classified in three main groups, cancer/testis antigens, overexpressed antigens and differentiation antigens [54].

Cancer testis antigens (*CT*) are a large family of TAAs expressed in human tumours of different histological origins but not in normal adult tissues, with the exception of immune-privileged cells such as testis and placenta [55]. These antigens result from the reactivation of genes that are normally silent in adult tissues but that are transcriptionally activated in tumours. This quasi-exclusive tumour-restricted expression pattern (sparing normal germ cells that do not express HLA class I molecules) as well as their high prevalence make them ideal vaccine candidates [55]. They have been identified and tested in many human clinical trials [56]; however, there is usually very little knowledge about their specific function, especially with regard to tumour transformation. CT antigens include, among others, the MAGE-A, MAGE-B, MAGE-C, NY-ESO and SSX-2 families.

Overexpressed antigens are expressed at a higher level in tumour cells than in normal tissues. Expression of these antigens at variable levels in normal cells conveys the risk of autoimmune attack upon vaccination, but a large number of clinical trials have used these antigens with up to now few side effects [57]. Some examples of this group of antigens are tumour suppressor proteins such as p53 and the antiapoptotic proteins hTERT and Mucin 1 (MUC-1).

Tissue-specific (cell lineage) differentiation antigens are shared between the tumour and the normal tissue of origin, albeit with variable specificity. They include carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), HER2/neu and melanoma lineage antigens such as gp-100, Melan-A/Mart-1 and tyrosinase, expressed in melanoma [58]. As for overexpressed antigens, they are endowed with a risk for autoimmune reactions.

The advantage of TAAs is that they are frequently expressed by the majority of patients and can therefore be used to treat many patients. The disadvantage of TAAs is the fact that some of them retain a level of expression in normal tissues, entailing the potential risk of autoimmune damage upon efficient vaccination. In addition, as TAAs derive from self-antigens, specific T cells have undergone negative selection, leaving only T cells with low avidity of antigen recognition, which are not able to generate strong immune responses.

2.3.2 Tumour-specific antigens (TSAs)

TSAs are antigens resulting from point mutations. They represent neoantigens mostly expressed by individual tumours. TSAs are tumour-specific, and it is usually viewed that their immunogenicity is not restricted by central tolerance, which is true when the mutated epitope is different enough from the wild-type one. Additionally, induced T-cell responses are not expected to result in autoimmune toxicity [59]. Moreover, neoantigens may be more resistant to immune selection, as they are critical for the oncogenic process and, therefore, essential for keeping the neoplastic state. In contrast, the fact that TSAs are patient-specific prevents broad vaccination and requires identification in a patient-specific manner. However, recent availability of sequencing technologies and epitope prediction algorithms allows for a rapid identification of potential neoantigens. Methods of in silico prediction of neo-epitope candidates with a high potential for neoantigen generation potentially present in multiple patients. These neoantigens hold the potential for development of "off the shelf" T-cell therapies, aiming to complement individualised, patient- and tumour-specific precision medicine approaches [32]. Nevertheless, it should be kept in mind that only a small percentage of mutations are being presented on MHC molecules at the tumour cell surface, making verification of the presence of a neo-epitope at the tumour cell surface a prerequisite [60, 61].

2.4 Types of vaccines

2.4.1 Peptide vaccines

Peptide vaccines consist in the delivery of MHC class I- or class II-restricted peptide epitopes derived from tumour antigens with the intent of activating $CD8^+$ and $CD4^+$ T cells. As peptides are not immunogenic per se, they need to be injected with an adjuvant [62]. GM-CSF, Montanide and TLR agonists, among others, have shown clinical benefit in small- and larger-scale clinical trials [63–65]. Peptide vaccines have the limitation of being applicable only to patients that have the HLA allele the peptide is restricted to. In addition, most vaccines are made of MHC class I-restricted peptides, therefore not eliciting CD4 T-cell help [66, 67]. In order to overcome this issue, the addition of non-tumour-specific peptides has been used, but limited data is available on the improvement provided by such heterologous helper peptides [68]. Overall, the numerous clinical trials performed in different tumour types have not provided satisfactory results yet [69].

Using multiple peptides derived from different TAAs targeting several antigens at once could overcome such tumour escape mechanisms. This multipeptide approach has demonstrated in in vitro and in vivo studies that multiple peptides do not compete for MHC presentation, inducing a multi-specific T-cell response [70–72]. The use of synthetic peptides with improved DC-targeting mechanisms, such as integrating pattern recognition receptors or TLRs [73], the conjugation of synthetic peptides to a DC-targeting antibody [74] or the encapsulation of long peptides in structures such as nanoparticles, liposomes or nano-hydrogel systems to enhance T-cell priming by DCs [75, 76] are some of the strategies under investigation towards a more efficient processing and presentation pathway that would lead to greater T-cell activation.

In two trials (a phase 1 and a randomised phase 2) combining single-dose pre-vaccine cyclophosphamide with IMA901, a renal cell carcinoma (RCC) peptide vaccine containing 10 antigens (9 HLA class I-binding and 1 HLA class II-binding) adjuvanted with GM-CSF in $HLA\text{-}A02^+$ subjects showed that there was an improvement in survival with amplification of antigenic response and a reduction in suppressive circulatory T cells and MDSCs, with a disease control rate (DCR) at 6 months of 31% (95% CI: 3–35%) [77]. Mouse models also support combinations of multipeptide vaccines and chemotherapy. However, the addition of IMA901 to first-line sunitinib (an anti-angiogenic tyrosine kinase inhibitor) failed to show improvement in metastatic renal cancer, owing to low-level immune responses [78], despite the fact that sunitinib has been shown to decrease the number of Tregs in mice and patients with RCC [79, 80], as well as MDSCs in patients with RCC [81].

Owing to their tumour specificity, much effort has been made in order to exploit neoantigens for vaccine development. Such neoantigen-directed vaccines have been developed for melanoma, using either synthetic RNAs containing up to 10 predicted neoantigens or long peptides targeting up to 20 neoantigens [82, 83]. In these trials, neo-epitopes were chosen to bind HLA class I [82] or HLA class I and II molecules [83] and showed activation of $CD4^+$ and $CD8^+$ T cells in response to vaccination.

Peptide vaccines can also be helpful in the prevention of the progression of a premalignant lesion to cancer. The MUC-1 peptide vaccine has been tested as a prevention of progression of colon adenoma to colorectal cancer [84]. MUC-1 was highly immunogenic in about half of the patients evaluated. Moreover, response to the vaccine correlated with prevaccination levels of circulating MDSCs, as nonresponders had a significantly higher percentage of MDSCs ($p < 0.05$); interestingly, no such association was observed for regulatory T cells.

NeuVax is a peptide vaccine that has been developed for early-stage node-positive low or intermediate HER2-expressing breast cancer after standard of care treatment [85]. The vaccine is composed of a peptide isolated from HER2/neu proto-oncogene combined with GM-CSF. Final results of a phase 1/2 clinical trial showed a non-significant improvement in 5-year DFS of 89.7% in the vaccine group versus 80.2% in the control group ($p = 0.08$); the improvement in DFS was even greater in the sub-group of optimally dosed patients (94.6%; $p = 0.05$ versus the control group) [86]. The vaccine is now being tested in an ongoing phase 3 trial (NCT01479244).

CDX-110 is a peptide vaccine also known as the rindopepimut vaccine. It is a 14-mer peptide covering the EGFRvIII mutation (the commonest form of EGFR mutation in human glioblastoma multiforme (GBM), detected in 23–33% of tumours) [87], and it is linked to the adjuvant keyhole limpet hemocyanin (KLH) to stimulate a specific immune response against EGFRvIII expressing tumour cells. This vaccine has been evaluated in three phase 2 clinical trials (ACTIVATE, ACT II and ACT III trials) for newly diagnosed GBM and one phase 2 (ReACT trial) for recurrent GBM. The ACTIVATE trial demonstrated that patients with EGFRvIII-specific humoral responses had an improved median OS as compared to patients not displaying immune responses (47.7 months vs. 22.8 months OS) [88]. The ACT II and ACT III clinical trials demonstrated longer PFS and OS than with historically matched controls [88, 89]. Deceivingly, the phase 3 trial ACT IV for newly diagnosed GBM was terminated for futility at the second preplanned interim analysis (HR: 0.99 for rindopepimut versus control, 95% CI: 0.74–1.31) [90]. A lack of benefit was also observed in the intention-to-treat population. The study confirmed earlier-phase trial findings of rindopepimut-induced EGFRvIII-specific antibody responses in the majority of patients; however, the fact that loss of EGFRvIII was observed in patients receiving or not the vaccine suggests that this target is unstable and therefore not a suitable antigen for immunotherapy. The phase 2 ReACT trial, evaluating the combination of bevacizumab and rindopepimut for recurrent GBM, showed that the vaccine induced robust anti-EGFRvIII antibodies in the majority of patients. The primary endpoint of PFS at 6 months was improved for the rindopepimut arm, albeit non-significantly (28% vs. 16%, $p = 0.12$), with a similar outcome for OS and duration of response. Rapid anti-EGFRvIII antibody generation was shown to be associated with prolonged OS in the rindopepimut arm [91]. A major criticism for this study, which could explain the non-significant results, is that the EGFRvIII status was principally decided on diagnostic tumour specimens, despite the known fact that EGFRvIII expression is lost in half of tumours upon recurrence.

2.4.2 DC vaccines

In order to improve peptide presentation in vivo, cancer vaccines using DC have been developed. DC-based vaccines are safe and immunogenic, and they have the ability to promote clinically significant tumour regression in some patients [92–94]. Clinical trials performed with DC-based vaccines usually involve an individualised patient vaccination approach with single clinical trial arms, which makes it difficult to evoke firm conclusions about their efficacy. Several cells such as monocytes and $CD34^+$ progenitor cells, antigens including complex tumour lysates and synthetic MHC class I-restricted peptides have all been used in different trials [95]. Some promising and important clinical trials involving DC vaccines have been published. Sipuleucel-T (Provenge) is one of the three FDA-approved vaccines (see Section 2.2.1). In addition to the IMPACT trial that led to FDA approval, 42 men with localised prostate cancer received Sipuleucel-T in a phase 2 study prior to radical prostatectomy [96]. Increased incidence of T cells was observed in the post-operative prostate gland

histology compared to preoperative biopsies. Currently, clinical trials are investigating combination of Sipuleucel-T with other approved drugs, such as abiraterone acetate, enzalutamide, radium-223, ipilimumab and atezolizumab (NCT01487863, NCT01981122, NCT02463799, NCT01832870, NCT01804465, and NCT3024216).

A clinical trial that used DCs loaded with a MUC-1-derived peptide and heterologous pan DR epitope (PADRE) peptides (universal CD4 T-cell helper peptides) delivered subcutaneously in patients with RCC has shown encouraging objective clinical responses and immunologic responses [97]. A phase 1/2 clinical trial used autologous WT-1 (Wilms' tumour 1, a shared TAA) mRNA-loaded DCs in patients with acute myeloid leukaemia (AML) in remission after standard of care, with the aim of eradicating or controlling residual disease. This study showed clinical responses correlating with increased WT-1-specific $CD8^+$ T-cell frequencies, as well as elevated levels of post-vaccine-activated NK cells [98]. Another study used patient-derived AML cells fused with autologous DCs vaccination in post-chemotherapy remission AML patients, achieving a marked rise in circulating T cells recognising whole AML cells and leukaemia-specific antigens that persisted for more than 6 months, which was associated with prolonged survival [99].

DCVax is a DC vaccine that has been developed for GBM. Two phase 1/2 studies tested the vaccine, which collectively recruited 39 patients, 20 of whom had newly diagnosed GBM and the remaining had recurrent high-grade glioma [100, 101]. For the newly diagnosed patients, the median OS with the addition of DC vaccine to the standard of care chemoradiation was 36 months. Long-term survival was also reported for some patients; 33% of patients reached or exceeded a 4-year survival, 27% reached an OS of 6 years, and two patients achieved a 10-year survival. The first report of the DCVax 2:1 randomised phase 3 trial in newly diagnosed GBM unfortunately does not allow interpretation as it is endowed with methodological flaws [102].

Some findings have suggested that the current DC vaccines can be optimised in order to get improved clinical outcomes. The discovery that the overexpression of CD40L in human DCs produces an increased stimulation of the T-cell response to tumour antigens such as gp100 and Melan-A is promising [103]. Additionally, DC function can be enhanced by stimulating antigen-specific Th1 and CTL responses through modulation of other co-stimulatory or co-inhibitory molecules, such as PD-1, CTLA4, CD28, OX40, etc. [104, 105]. On the contrary, suppressing the ubiquitin-editing enzyme A20 or the scavenger receptor SRA/CD204 in human DC helps in the development of IFN-α-producing Th1 cells and antigen-specific $CD8^+$ T cells [106, 107]. These developments suggest that there is promising data for the future in DC-based cancer vaccines.

2.4.3 Tumour cell vaccines

Tumours concentrate a high number of genetic modifications in somatic cells and therefore carry a large number of potential antigens. For that reason, vaccination with whole tumour cells has been an interesting strategy, with the limitation that they need to be patient-tailored. Autologous tumour cell vaccines have been evaluated in several cancer types such as lung cancer [108], melanoma [109, 110], RCC [111], prostate cancer [112] and colorectal cancer [113, 114]. In order to prepare the vaccine, a large amount of tumour tissue needs to be collected, which impedes its application in some tumour types or some individuals.

MVX-ONCO-1 is an autologous tumour cell vaccine containing irradiated tumour cells from a patient and a capsule implanted with a genetically modified allogeneic cell line that continuously releases the adjuvant GM-CSF [115]. Results of the first-in-human phase 1 trial testing of this vaccine reported an excellent safety profile, the main toxicity being a discomfort at the implantation site (20%) [116].

Over 50% of patients (8/15) experienced either partial response (PR) or stable disease (SD) including disappearance of lung metastases, with interesting activity in head and neck squamous cell carcinoma (HNSCC) and chordoma [117]. A phase 2 trial is ongoing in HNSCC (NCT02999646).

Canvaxin was the first allogeneic whole-cell vaccine to be developed and consisted of three melanoma cell lines in combination with BCG as adjuvant [118]. It showed promising results in a phase 2 clinical trial [119, 120], but failed in the randomised phase 3 trial [121]. Although the reasons for the lack of efficiency remain to be determined, it is possible that the induced immune response was not able to control the disease. To potentiate induction of immune response, tumour antigens utilised in vaccines should be linked with potent immunological adjuvants [122]. Such examples are tumour vaccines that have been modified genetically to express co-stimulatory molecules and/or cytokines. Such an example is the GVAX vaccine, an allogeneic whole-cell vaccine modified with the GM-CSF gene, which has been evaluated for recurrent prostate cancer [123, 124], breast cancer [125] and pancreatic cancer [126, 127], but impact on patient survival remains to be proven.

In order to improve the immunogenicity of allogeneic tumour cells, cell lines have been engineered to secrete antisense oligonucleotides to inhibit expression of immunosuppressive cytokines, such as TGF-β. The tumour vaccine Lucanix (Belagenpumatucel-L) has been designed using this strategy to target metastatic NSCLC and has shown significant improvement in OS in two phase 2 clinical trials [128, 129]. However, the phase 3 clinical trial in stage III/IV patients did not demonstrate prolongation of OS in the whole cohort of patients, a survival benefit being however observed in several subgroups of patients [130].

BiovaxID is a patient-specific therapeutic cancer vaccine composed of the patient clonal immunoglobulin molecule idiotype vaccine conjugated to the adjuvant KLH. In a phase 2 clinical trial, the administration of BiovaxID together with GM-CSF in patients diagnosed with follicular lymphoma in complete remission with minimal residual disease demonstrated induction of tumour-specific cellular and humoral immune responses, which translated into clinical benefit, with a median DFS of 8 years and an OS rate of 95% at 9 years [131]. A randomised, controlled phase 3 trial in patients achieving remission after chemotherapy showed a median DFS after randomisation of 44.2 months for the vaccine arm versus 30.6 months for control arm [132]. However, other phase 3 trials failed to demonstrate increase in survival for patients receiving the vaccine [133, 134].

The HyperAcute vaccines are made of tumour cell lines that have been genetically engineered to express the α(1,3)-galactosyltransferase enzyme in order to induce an hyperacute reaction with complement- and antibody-dependant cytotoxicity [135]. They have been tested in several malignancies including melanoma, pancreatic and prostate cancer [136–138] and showed encouraging results improving OS. This vaccine was further evaluated in two phase 3 clinical trials. The IMPRESS study evaluated the vaccine with or without gemcitabine/chemoradiation in resected pancreatic cancer patients but failed to achieve its primary endpoint, with no observed statistically significant difference between the treatment and control groups. The PILLAR trial for borderline resectable (stage II) and advanced unresectable (stage III) pancreatic adenocarcinoma patients, combining the vaccine with FOLFIRINOX or gemcitabine/nab-paclitaxel and chemoradiation, is currently ongoing.

2.4.4 Heat shock protein vaccines

Heat shock proteins (HSPs) are a group of intracellular protein chaperones. Their function is to protect cells from protein misfolding, dysfunction and cell apoptosis, and they have been implicated in the activation of innate and adaptive

immunity [139]. Therapeutic HSPs vaccines utilise HSPs as a source of tumour-associated antigens and involve isolation and purification of HSPs from a patient's tumour with subsequent reinfusion of the complex. The advantages of this type of vaccines are, similarly to tumour vaccines, that they do not require a pre-identification of tumour antigens and provide several targets at the same time.

GBM are natural inducers of HSP expression, making them an interesting target for HSP vaccines [139]. A phase 2 trial testing the HSPPC-96 vaccine in recurrent GMB patients showed a 90.2% 6-month OS and a 29.3% 12-month OS, with an interesting observation of an adverse effect of lymphopenia on the vaccination outcome [140]. Adjuvant vaccination following standard treatment by surgery and chemoradiation in patients with newly diagnosed GBM showed a median OS of 23.8 months [141]. Interestingly, this phase 2 trial showed better outcome (median OS: 44.7 months) in patients with low PD-L1-expressing myeloid cells than patients with high PD-L1 myeloid expression (median OS: 18 months) [141]. Nevertheless, a phase II randomised study (Alliance A071101) evaluating the combination of HSPPC-96 vaccine with bevacizumab versus bevacizumab alone in patients with recurrent GBM failed to demonstrate a survival benefit [142]. HSP-based vaccine has also been tested in various malignancies [143].

2.4.5 Viral vectors

Delivery of tumour antigens can be achieved using viral vectors. The advantage of virus-based vaccines is that human immune system has evolved to react efficiently against them with innate and adaptive responses, inducing long-lasting immunity. The most common viruses from which viral vaccines vectors have been developed are poxviruses, adenoviruses and alphaviruses [144]. A potentially restraining factor using viral vectors is the fact that the induced antiviral immune response will neutralise the vector, limiting efficacy of repeated vaccination with the same vector. In order to overcome this, heterologous prime-boost vaccination is used, where initial delivery of a tumour antigen with one virus vector is followed by a boost with the same tumour antigen delivered with another virus vector [145]. Using viral vector also offers the possibility to insert genes coding for adjuvants such as GM-CSF and IL-2.

As an example, the TRICOM vaccine platform exploits heterologous prime-boost vaccination where priming is achieved using a vaccinia vector encoding a chosen TAA and boosting using a fowlpox-derived vector encoding the same TAA. In addition, it incorporates three co-stimulatory molecules for immune activation and has been used in several trials in various malignancies. In men with CRPC, the PROSTVAC vaccine phase 3 trial, using PSA as antigen, failed to positively influence OS [146], although phase 2 trials were encouraging [147, 148]. An analysis of immune response to the PROSTVAC vaccine on pooled data from several clinical trials conducted similarly reported that 68% of the tested patients exhibited evidence of cross-priming with immune responses mounted against TAAs not found in the vaccine, for example, MUC-1, PSMA, PAP and PSCA, a phenomenon known as antigen spreading [149]. Other applications of the TRICOM vaccine in breast and ovarian cancer [150], solid carcinomas [151, 152], colorectal carcinoma [153] or advanced cancers [154] have been tested in phase 1 trials using various antigens and virus vectors, and further studies are planned.

Another example is BN-CV301, a poxvirus-based vaccine that codes for the MUC-1 and CEA TAAs. The phase 1 clinical trial showed no dose-limiting toxicity; the vaccine produced one PR in one patient and prolonged SD in multiple patients, especially in KRAS gastrointestinal cancer mutant patients [155].

Similarly, a first-in-human trial of the LV305 vaccine, a vaccine using DCs transduced with a lentivirus expressing the NY-ESO-1 antigen, demonstrated a favourable safety profile with grade 1/2 event such as fatigue (49%), injection (46%) and myalgia (21%); induction of anti-NY-ESO-1-specific CD4+ and CD8+ responses were observed, with a DCR of 56.4% in all patients and 62% in sarcoma patients [159].

2.4.6 Oncolytic virus vaccines

Oncolytic viruses are a particular category of viruses that have the characteristic of infecting both healthy and tumour cells, but of selectively replicating only in the latter. They therefore kill tumour cells, additionally inducing activation of innate and adaptive immune responses through immunogenic tumour cell death [156]. As for viral vectors, they also offer the possibility to express cytotoxic or immunomodulatory molecules. The herpes virus vaccine called T-VEC, engineered to selectively replicate in tumour cells and to secrete GM-CSF, has been approved by the FDA for intratumoural administration for stage IIIB/C-IV melanoma based on the phase 3 OPTiM trial [51, 157], as mentioned above (see Section 2.2.1). A recently reported series of off-trial uses of T-VEC in early advanced melanoma (stages IIIB/C-IVM1a) showed a CR rate of 61.5% and a PR rate of 26.9, with a DCR of 92.3% [158].

T-VEC is also being tested in other malignancies. In HNSCC, T-VEC was used in combination with standard chemoradiation for untreated unresectable stage III/IV disease in a phase 1/2 trial. At a median follow-up of 29 months, PFS was 76%, very importantly demonstrating the safety and feasibility of this combination approach [159]. The initial design of the phase 3 trial was subsequently modified in view of the introduction of pembrolizumab in the standard of care management of HNSCC and was redesigned as a phase 1b trial randomising patients to pembrolizumab with or without T-VEC delivered to involved cervical nodes (MASTERKEY-232, NCT2626000). This trial showed a manageable safety profile, with however 24/36 (66.7%) patients experiencing serious adverse events, including one vaccine-related death. The overall response rate was 16.7%, the majority of which was in patients with PD-L1-positive tumours, and the DCR was 38.9% (again mostly in PD-L1-positive tumours) [160].

2.5 Vaccine adjuvants

Vaccination "per se" can activate antigen-specific T cells. However, when the antigen is in the form of peptides, proteins or even tumour cells, they are usually not strong enough to induce an immune response that leads to tumour eradication. The reason for this is that these antigens come without pathogen-associated molecular pattern (PAMPs) that can be recognised by innate immune cells. Most cancer vaccines are therefore combined with adjuvants, which, in addition to eliciting an innate immune response, have the role of protecting the antigen from degradation, ensuring prolonged release and promoting antigen uptake by DCs. The efficiency and choice of the adjuvant heavily influences the vaccine efficacy.

Adjuvants that act as delivery systems are classified into virosomes, liposomes, the saponin QS-21, mineral salts and the water-in-oil emulsion Montanide (an incomplete Freund's adjuvant analogue). Montanide is used in many trials of peptide vaccines and is generally well tolerated [61]. Aluminium is mostly used for antiviral vaccines such as the HPV vaccine as it promotes humoral rather than cellular responses [61]. Immunostimulatory complexes (ISCOMs) are ring-like structures containing lipids and saponin and can incorporate the antigen for optimal presentation for DCs. GM-CSF, which is employed to recruit and activate DCs at the injection site, is also being used in a large number of trials [61].

Innate immune stimulatory adjuvants are dominated by TLR ligands, but STING ligands, C-type lectin receptor (CLR) ligands and RIG-like receptor (RLR) ligands are also being tested [161]. TLR ligands induce a strong activation of DCs, and currently tested molecules include agonists to TLR2, TRL3 (e.g. the dsRNA analogue poly-ICLC), TLR7/8 (e.g. imiquimod) and TLR9 (e.g. the bacterial dinucleotide DNA CpGs). Many trials using CpGs have demonstrated its potential to improve T-cell responses, but it is now difficult to have access to it. Imiquimod is approved for the treatment of basal cell carcinoma and is used in combination with vaccines in several trials [61]. The TLR4 agonist glucopyranosyl lipid A (GLA) is currently used as adjuvants in peptide vaccines, such as with the NY-ESO-1 antigen [166]. Use of poly-ICLC is increasing, mostly for GBM vaccine trials, at it has proposed to favour T-cell homing to the brain [162].

Although many of the above-mentioned adjuvants are promising, the fear that using them alone would not induce strong enough immune response has led to development of combination strategies. Montanide is commonly used to protect the antigen in combination with a TLR ligand to promote inflammation. However, combining several immunostimulatory adjuvants such as two or more TLR ligands is being tested. Many more combination can be envisaged as long as safety is preserved.

2.6 Vaccine combinations

Vaccines, when efficiently designed, have the ability to induce strong T-cell responses. However, this does not imply that these T cells will be allowed to function at the tumour site, for several reasons. These include, among others, the immunosuppressive tumour microenvironment and the induction of immune checkpoint molecules on T cells. In an attempt to target these mechanisms, many combinations of vaccines with other immunotherapeutic strategies are currently in development. Checkpoint inhibitors, agonist antibodies and immunostimulatory cytokines can increase tumour cell immune destruction. Moreover, combining with radiotherapy, hormonotherapy and chemotherapy may also be synergistic.

2.6.1 Vaccines + checkpoint inhibitors

2.6.1.1 Vaccine + anti-CTLA-4 antibodies

CTLA-4 is expressed on T cells after activation as part of the normal regulation process of immune responses. However, in the case of antitumour responses, function of T cells need to be sustained, which is prevented by CTLA-4 expression [163]. To prevent that, two anti-CTLA-4 monoclonal antibodies, ipilimumab and tremelimumab, are currently in various stages of clinical development in combination with vaccines.

As examples, the PROSTVAC vaccine was tested with ipilimumab in mCRPC in a phase 1 escalation clinical trial. As a result, 14 of the 24 chemotherapy-naïve patients had reduction in PSA. Median OS was 31.3 months, which was longer than PROSTVAC alone [164]. This vaccine is currently being tested in combination with other checkpoint inhibitors (NCT2506114, NCT02933255, and NCT03532217).

GVAX was studied in combination with ipilimumab in 28 mCRPC patients in a phase 1 trial. Around 39% grade 3/4 irAEs were seen (most common: hypophysitis, alveolitis and hepatitis). About 25% had >50% decline in PSA, while 53.5% had SD radiologically [165]. GVAX has also been combined with ipilimumab in 30

pancreatic adenocarcinoma patients, versus ipilimumab alone [166]. The combination arm showed that three patients had extended SD and seven patients had a reduction in their tumour marker.

2.6.1.2 Vaccines + PD-1/PD-L1 inhibitors

PD-1 is a protein expressed on T cells, some B cells and NK cells, and binding of its ligands PD-L1 and PD-L2 results in cell inhibition [163]. PD1 ligands can be expressed not only by tumour cells but also by other cells of the tumour microenvironment, and PD-L1 has been shown to be induced as a result of T-cell activity [167]. The blocking of this interaction is being tested with the aim to allow prolonged T-cell activity to take place, and several anti-PD1 (pembrolizumab and nivolumab, among others) and anti-PD-L1 (atezolizumab, avelumab and durvalumab) antibodies have been developed.

Among others, combination of nivolumab with a multipeptide vaccine has been evaluated for the adjuvant treatment of high-risk melanoma. Results were promising, showing a median PFS of 47.1 months compared to historical median of 5–7.2 months with other approaches [168].

Pembrolizumab has been combined with a DNA vaccine encoding PAP in mCRPC patients. PSA responses were more important in the cohort receiving concurrent than sequential treatment. PSA declines were associated with the development of PAP-specific Th1-biased T-cell immunity and $CD8^+$ T-cell infiltration in metastatic tumour biopsy specimens. No confirmed CR or PR was observed; however, 4/5 patients treated concurrently had measurable decreases in tumour volume at 12 weeks [169].

A multitude of studies are currently testing vaccines combinations with checkpoint inhibitors for different malignancies.

2.6.2 Vaccines + tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKIs) have been used for the treatment of several solid tumours and haematological malignancies. There is preclinical and clinical data proposing that TKIs have an "off-target" effect on immune cells that restraint and/or intensify the antitumour response [170].

A phase 3 trial evaluating the combination of sunitinib with a modified vaccinia Ankara-based vaccine encoding the tumour-associated antigen 5T4 (MVA-5T4) was not able to demonstrate benefit in OS, although patients with good-risk tumours responded better to the combination [171].

Based on the positive results of a phase 3 trial evaluating the epidermal growth factor (EGF) vaccine CIMAvax-EGF as switch maintenance therapy versus placebo for previously chemo-treated advanced NSCLC patients [172], a phase 1b study evaluating the CIMAvax-EGF vaccine in combination with *EGFR* TKI in *EGFR*-mutated NSCLC tumours (EPICAL trial) is currently ongoing (NCT03623750).

2.6.3 Vaccines + endocrine treatment

Endocrine treatment is important in hormonally driven tumours like prostate and breast cancer. Patients treated with letrozole, an aromatase inhibitor used for the adjuvant treatment of hormone-responsive breast cancer, were found to have less Tregs in the tumour microenvironment [172]. In addition, androgen deprivation therapy in prostate cancer patients generates an immunostimulatory microenvironment increasing the number of effector T cells [173, 174].

A post hoc analysis of a phase 3 randomised trial of the Sialyl Tn-KLH vaccine in women with metastatic breast cancer indicated an improved clinical outcome with the addition of concomitant endocrine therapy, with prolonged time to progression and OS [175]. The order of sequential treatment seemed to be important; a combination crossover study of nilutamide with a PSA-encoding poxvirus-based vaccine in non-metastatic CRPC suggested improved OS when the vaccine was administered before the hormonotherapy [176].

These combinations are attractive therapy options for hormonosensitive cancers because vaccines are minimally toxic and can easily be incorporated into standard of care regimens.

2.6.4 Vaccines + chemotherapy

Chemotherapy agents are known to induce reduction in both $CD4^+$ and $CD8^+$ T cells, however still allowing for immune responses to occur [177]. Several chemotherapeutic agents such as gemcitabine, taxanes, topoisomerase inhibitors, platinum compounds and 5-FU have been shown to produce immunomodulatory effects [177, 178].

The OPT-822 vaccine in combination with cyclophosphamide was tested in a phase 2/3 study in metastatic breast cancer versus cyclophosphamide plus placebo. The vaccination arm failed to show a PFS or interim OS benefit in the overall study population; however, they were significantly improved in the 50% of patients that developed an immune response to the vaccination [179].

IMA950 is a multipeptide GBM-specific vaccine composed of tumour-associated MHC class I- and II-restricted peptides [179]. The vaccine has been combined with standard chemoradiotherapy and adjuvant temozolomide in patients with newly diagnosed GBM in two reported trials. A phase 1 study of IMA950 adjuvanted with GM-CSF showed that the primary immunogenicity endpoint of observing multi-antigen responses in at least 30% of patients was reached. PFS was 74% at 6 months and 31% at 9 months [180]. The second clinical trial was a phase 1/2 trial of the IMA950 vaccine adjuvanted with poly-ICLC in high-grade gliomas; CD8 T-cell responses to a single or multiple peptides were observed in 63.2% and 36.8% of patients, respectively, while median OS was 19 months, comparing favourably to classical chemoradiation results [181]. A phase 1/2 trial evaluating the combination of the IMA950 vaccine with pembrolizumab in recurrent GBM is currently ongoing (NCT03665545).

2.6.5 Vaccines + radiotherapy

The concept of synergy between vaccines and radiotherapy attracts growing interest in cancer therapy. One of the hypotheses to explain this is that radiation can not only elicit a tumour-specific immune response locally but also at distant sites, therefore acting as an in situ vaccine, eliciting both local and systemic responses [182]. Many trials have tested and are currently testing vaccines and radiotherapy, and hope is that they will provide important information on how to optimise cancer vaccines.

3. Conclusions

Vaccine immunotherapy currently shows a prolific activity in early phase trials and an expanding pipeline, with however few successes in late phase trials, despite encouraging or promising early results, resulting in a limited number of approved drugs with modest therapeutic benefit. Furthermore, there have been therapeutic

vaccine studies reported in the early or mid-2000s, without further translation or progression to later trial phases.

As our understanding of the potential of immunotherapy expands so does the list of research questions that will need to be answered before this approach can be translated for effective clinical use. Can the thus far limited success, reflected by the very few approved drugs, be attributed to suboptimal or inadequate trial design? What is the optimal endpoint for vaccine trials? How long would we need to treat patients with immune modulatory therapies? What is the best combination of approaches? What is the optimal sequence strategy?

It is evident that, in order to proceed in the next stage of therapeutic vaccine development, paradigm changes ought to probably be made towards more optimal utilisation of resources and therapeutic potential. We need a clearly defined clinical readout for therapeutic response, and we need a blueprint for successful translation. We might need to consider that the concept of using vaccines in stage IV disease is not the correct way forward, but rather bringing vaccines in earlier disease stages and developing adjuvant or maintenance strategies. In this context, OS might not be the correct endpoint to use, but disease-free or relapse-free survival might be more appropriate. Our understanding of the evolution of immune escape is still incomplete, and additional work must be done to identify those patients who will benefit most from immunotherapy and to develop novel strategies.

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