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Therapeutic cancer vaccines

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Abstract | Therapeutic cancer vaccines have undergone a resurgence in the past decade. A better understanding of the breadth of tumour-associated antigens, the native immune response and development of novel technologies for antigen delivery has facilitated improved vaccine design. The goal of therapeutic cancer vaccines is to induce tumour regression, eradicate minimal residual disease, establish lasting antitumour memory and avoid non-specific or adverse reactions. However, tumour-induced immunosuppression and immunoresistance pose significant challenges to achieving this goal. In this Review, we deliberate on how to improve and expand the antigen repertoire for vaccines, consider developments in vaccine platforms and explore antigen-agnostic in situ vaccines. Furthermore, we summarize the reasons for failure of cancer vaccines in the past and provide an overview of various mechanisms of resistance posed by the tumour. Finally, we propose strategies for combining suitable vaccine platforms with novel immunomodulatory approaches and standard-of-care treatments for overcoming tumour resistance and enhancing clinical efficacy.

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Successful antitumour immunity requires optimal interactions between immune and non-immune constituents of the tumour microenvironment (TME). Within the TME, natural killer (NK) cells, neutrophils and macrophages of the innate immune system are essential for immediate recognition and attack of tumour cells, whereas antigen-presenting cells (APCs), such as dendritic cells (DCs), capture and cross-present the antigens released by tumour cells and activate T cells[1–3]. Tumour cells dying by immunogenic cell death (ICD) (reviewed in[4]) either naturally or following treatments, such as certain types of chemotherapy, release tumour antigens and danger associated molecular patterns. This prompts DC maturation, antigen uptake, processing and presentation on MHC class I (MHC-I) molecules (through antigen cross-presentation) and MHC-II molecules[5] (FIG. 1). These DCs travel to secondary lymphoid organs, where they interact with naive $CD4^+$ T cells and $CD8^+$ T cells via MHC–T cell receptor recognition and co-receptor engagement (FIG. 1). Migratory DCs also acquire antigens in the TME or the periphery and 'transfer' these antigens to lymph node-resident DCs for T cell priming[6–9]. It is important to note that $CD4^+$ T cell help is a crucial aspect of $CD8^+$ T cell activation and tumour immunity (BOX 1). T cell stemness is emerging as a critical regulator of tumour immunity and a determining factor for response to immunotherapy (BOX 2). The activated T cells travel back to the TME to control tumour growth by direct killing and IFNγ-mediated prevention of cancer cell proliferation, following cognate interactions[2] (FIG. 1).

Cancer vaccines typically involve exogenous administration of selected tumour antigens combined with adjuvants that activate DCs, or even DCs themselves. The aim of therapeutic cancer vaccines is to stimulate the patient's adaptive immune system against specific tumour antigens to regain control over tumour growth, induce regression of established tumours and eradicate minimal residual disease. The basic principles needed for successful therapeutic vaccination against tumours include delivery of large amounts of a high-quality antigen to DCs, optimal DC activation, induction of strong and sustained $CD4^+$ T helper cell and cytotoxic T lymphocyte (CTL) responses, infiltration of the TME and durability and maintenance of response. This may be accomplished by several methods, such as reversal of tumour-induced immune exhaustion by immune checkpoint inhibitors, activation of DCs and effector T cells by administration of tumour-associated antigens with adjuvants or vaccination with autologous DCs loaded with specific tumour antigens. Alternatively, the tumour's local immune environment can be broadly activated to induce tumour cell death[4], and it is possible to facilitate tumour antigen availability by use of in situ vaccines (ISVs). As opposed to traditional vaccines, where antigens are carefully selected, purified or prepared and injected into patients, the in situ approach generates the vaccine in the TME itself by sourcing the antigens from dead or dying tumour cells[4,10]. We have included ISVs in our profile of cancer vaccines as these fulfil the basic requirements of vaccines; that is, delivery of antigens to tumour-infiltrating DCs to provoke an adaptive T cell

Immunogenic cell death (ICD). A type of cell death that entails the release of danger-associated molecular patterns to attract and activate immune cells and the release of antigens to be acquired by activated antigen-presenting cells and presented to T cells.

response. Despite the FDA approval of a DC-focused cell-based vaccine, sipuleucel-T, more than 10 years ago[11], no other therapeutic cancer vaccine has been approved. It is now appreciated that tumour cell intrinsic resistance and local or systemic immunosuppressive (extrinsic) mechanisms substantially compromise the efficacy of cancer vaccines. The implementation of immunotherapies such as immune checkpoint inhibition (ICI) — for example, anti-CTLA-4, anti-PD1 and anti-PDL1 antibodies — to overcome resistance has effectively changed cancer care, substantially increasing response rates and even leading to potential cures. Therapeutic cancer vaccines are re-emerging as approaches to increase response rates and survival, especially in combination with ICI.

Immunotherapy for cancer was initially simply thought to be a matter of replenishing the host with

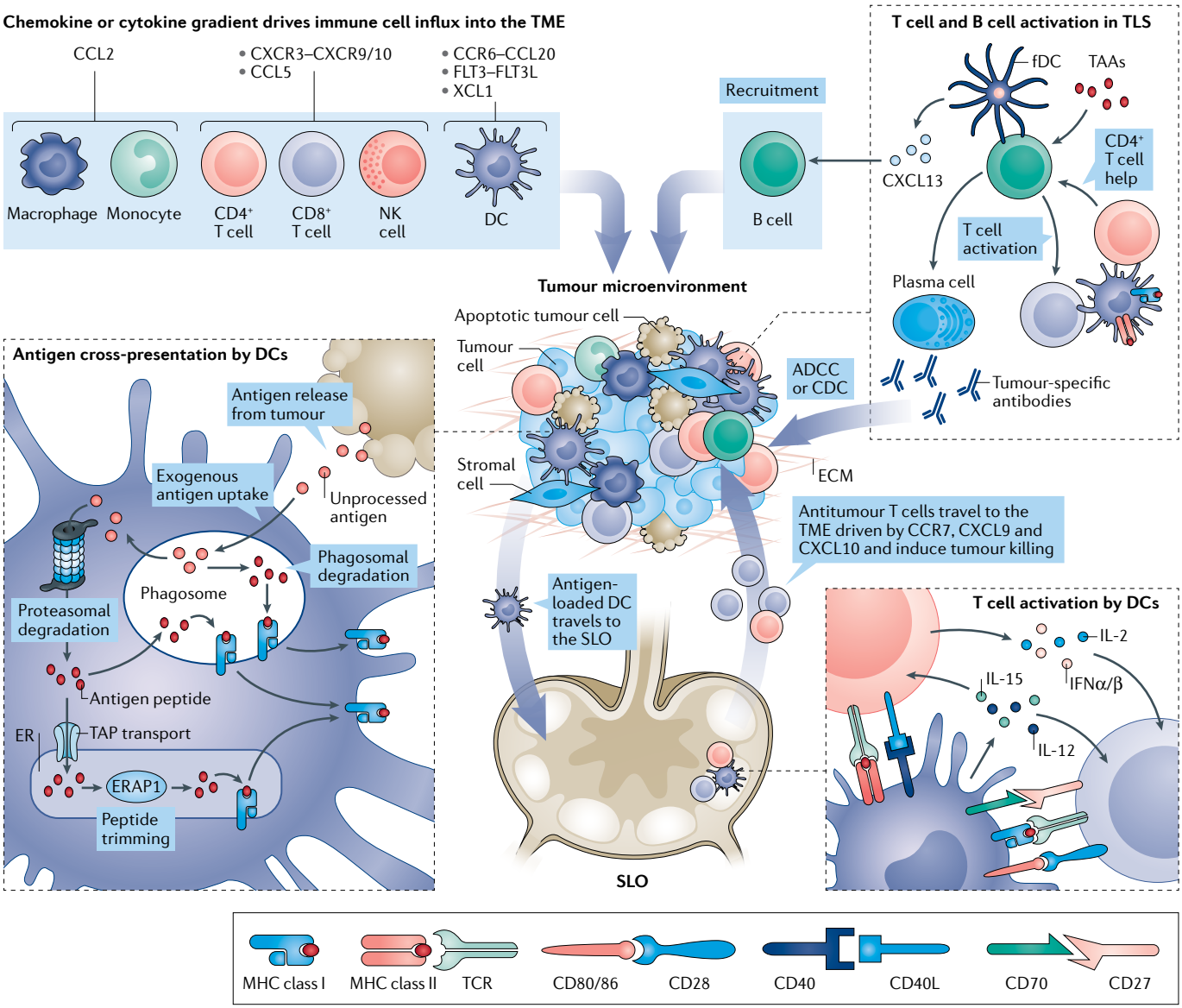

Fig. 1 | **Tumour immunity regulation.** Immune cells are driven to the tumour microenvironment (TME) via a chemokine gradient. In the TME, dendritic cells (DCs) take up and process tumour antigens and present them on MHC class II or MHC class I molecules (through cross-presentation) (bottom left). Cross-presentation may occur through the cytosolic pathway or the vacuolar pathway. In the cytosolic pathway, antigens from endosomes or phagosomes are transferred into the cytosol, proteasomally cleaved and transported to the endoplasmic reticulum (ER). Next, peptides are further edited, loaded on MHC class I molecules and transported to the cell surface. Conversely, after cytosolic proteolytic cleavage, shortened peptides may be transferred back to phagosomes, loaded on MHC class I molecules and transported to the cell surface. In the vacuolar pathway, antigens are processed and loaded onto MHC class I molecules in the phagosomes or endosomes. Antigen-loaded DCs travel to the secondary lymphoid organ (SLO) and activate T cells (bottom right). Interaction between MHC–peptide complex–T cell receptor (TCR) and cognate receptor–ligand pairs induces DCs to secrete cytokines and activate T cells. $CD8^+$ T cell responses are amplified by IL-2 secreted by $CD4^+$ T cells. Activated T cells travel to the TME and induce tumour killing. Tertiary lymphoid structures (TLS) (top right) often develop in the TME[266]. Here, antigen-loaded DCs activate T cells and follicular DCs (fDCs) facilitate the generation of memory B cells and antibody-producing plasma cells. Activated T cells, B cells and antitumour antibodies facilitate tumour cell death by direct tumour cell lysis, antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC)[267]. ECM, extracellular matrix; ERAP1, endoplasmic reticulum aminopeptidase 1; FLT3L, fms-like tyrosine kinase 3 ligand; NK, natural killer; TAA, tumour-associated antigen; TAP; transporter associated with antigen processing.

Box 1 | **Role of CD4⁺ T cells in tumour immunity**

CD4⁺ T cells provide 'help' to promote effector and memory responses of CD8⁺ T cells. Non-helped CD8⁺ T cells differ in the expression of about 950 genes from helped T cells, indicating extensive reprogramming of CD8⁺ T cells by CD4⁺ T cell help[268]. CD4⁺ T cells also exert antitumour effector function themselves, through cognate recognition of HLA class II-positive tumour cells[269], activation of tumour-associated macrophages[270] or direct cytotoxic antitumour functions[271]. Dendritic cells (DCs) are an important messenger between CD4⁺ T helper cells and CD8⁺ T cells. CD4⁺ T cells that have recognized an antigen through MHC class II interaction increase expression of CD40 ligand (CD40L) trimer, triggering interaction with CD40 on DCs[133] (FIG. 1). This causes activation of the DCs, associated with increased expression of CD80, CD86 and CD70. CD80 and CD86 interact with CD28, and CD70 engages CD27 on the T cells. The latter interaction imprints CD8⁺ T cell effector and memory functions and can be mimicked by agonistic antibody to CD27 (REF.[126]). Agonistic anti-CD40 antibody activates and modulates myeloid cells in the tumour microenvironment, causing secretion of the chemokine CCL5, which subsequently attracts more CD4⁺ T cells to the tumour microenvironment[272]. In herpes simplex virus infection in mice, CD8⁺ T cell priming depends on CD4⁺ T cell-driven amplification of IL-15 by DCs in response to type I interferons. Additionally, increased innate stimulation by type I interferons decreased the helper dependence of CD8⁺ T cell priming[273]. T cell help therefore becomes more important when danger signals are lacking, as often seen in cancers.

The location of cancer-specific CD4⁺ T cells can profoundly affect CD4⁺ T helper cell polarization. In a mouse model, CD4⁺ T cells in the TGFβ-rich bone marrow differentiated into a T helper cell 17 phenotype that prevented tumour eradication by immune checkpoint inhibition. In contrast, in the same model, T helper cell 1 differentiation from CD4⁺ T cells in the subcutaneous site promoted tumour eradication by immune checkpoint inhibition[274]. Collaboration between CD4⁺ T cells and CD8⁺ T cells is essential in the protection against virus-induced murine tumours[132], as well as in immune checkpoint inhibition therapy for neoepitope-bearing carcinogen-induced murine tumours[156]. CD4⁺ T cell help is important both during priming of T cell responses and at the effector cell level within tumours. For example, production of IL-2 by tumour-resident CD4⁺ T cells enhanced recruitment of CD8⁺ T cells into the tumour, and promoted their proliferation and upregulation of granzyme B expression[275].

Danger-associated molecular patterns
Non-microbial endogenous factors released from dead or distressed cells that serve as activating ligands for innate immune receptors on antigen-presenting cells, prompting activation of antigen-presenting cells and secretion of cytokines and chemokines to recruit other immune cells.

Antigen cross-presentation
A specialized mechanism that allows select cells such as type 1 dendritic cells to process and present internalized exogenous antigens on MHC class I molecules to activate CD8⁺ T cells.

Sipuleucil-T
Dendritic cell-focused cell-based vaccine for hormone-refractory prostate cancer consisting of a prostate acid phosphatase, fused with GM-CSF, loaded ex vivo on blood cells partially enriched for dendritic cells.

tumour-reactive T cells. This led to a general disappointment in cancer vaccines after it was found that treatment with sipuleucel-T conferred only a small survival advantage in patients with prostate cancer[11]. Moreover, a series of large phase III cancer vaccine studies in advanced disease reported negative outcomes[12–17]. In hindsight, the complex set of host, tumour and environmental factors was not appreciated to have such a direct impact on tumour-specific immunity[18], and the design of cancer vaccines did not take into account all the mechanisms governing immune ignorance, exclusion, suppression and escape[12–14,17,19–24]. Consequently, these vaccines failed to generate the numbers of T cells or the durability of the T cell response required to elicit long-lasting immunity, making it evident that new strategies are required to overhaul the field's approach to vaccine therapy. The emergence of new technologies to analyse and define the TME and the tumour's immunogenic antigen profile (determined by imaging mass cytometry (IMC), cytometry by time of flight (CyTOF), multispectral flow, single-cell RNA sequencing, whole-exome sequencing and antigen prediction algorithms), the appreciation that mutations and the resulting mutated tumour antigens can be drivers of protective immunity and that combinations with standard-of-care therapies can increase immunogenicity, have radically altered our approach towards vaccine-based immunotherapy.

An obvious reason for the failure of cancer vaccines appears to be the choice of less immunogenic vaccine platforms or antigens, on the basis of the low percentage (11–50%) of patients showing a response to vaccination or the induction of non-effective allo-HLA-specific immune responses when allogeneic cell vaccines were used[12–14,17,21–24]. Another explanation is the vaccination of patients with essentially 'cold' tumours. The concept of 'cold' versus 'hot' tumours is only now becoming clear. Evidence shows that 'cold' tumours are refractory to immunotherapy and 'hot' tumours, characterized by the pre-existing tumour infiltration with tumour-specific immune cells and a proinflammatory milieu[18], are more responsive to standard-of-care therapy, ICI and therapeutic vaccination[17,25–27]. The choice of combination therapy can also significantly affect the therapy outcome. For example, chemotherapy agents such as gemcitabine, sunitinib and cyclophosphamide were used in cancer vaccine trials because of their reported effects on eliminating immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs)[28–35] and regulatory CD4⁺FOXP3⁺CD25⁺ T cells (T_{reg} cells)[31,36,37] in preclinical models[38–40]. But in the clinic, these drugs failed to efficiently deplete the immunosuppressive cells[41–45] and may have inadvertently promoted immune evasion[15,46–48] resulting in suboptimal vaccine efficacy.

There have also been a series of encouraging studies which indicate that cancer vaccines can be effective when (1) disease burden is low, (2) systemic and local immunosuppression is limited or reduced as with certain chemotherapies or ICI and (3) a strong sustainable CD4⁺ T_H1 cell and/or cytotoxic CD8⁺ T cell (CTL) response is mounted[28,49–71]. These trials demonstrate that given the optimal setting, therapeutic vaccines can be successful. However, several factors need to be considered to design reliable anticancer vaccines with reproducible clinical benefits. Here we review strategies for improving vaccine design through expansion of the antigen repertoire and improving cancer vaccine platforms. Furthermore, we describe TME-mediated regulation of immunoresistance that compromises immunotherapy and vaccine efficacy and discuss combination therapies that can help overcome such resistance.

Increasing and expanding tumour specificity of the vaccines

The success of antigen-specific therapeutic vaccines rests heavily on the nature of antigens in the vaccine. Technological advances have radically changed our approach to choosing the optimal target antigens for each vaccine platform. In this section we provide an overview of various classes of tumour antigens, discuss properties of optimal neoantigens and explore ways to improve current target discovery platforms.

For many years shared tumour antigens were the focus for vaccine targets. These include 'self-antigens' such as the cancer testis antigens, differentiation antigens and overexpressed antigens[72,73] and 'non-self' antigens of viral origin such as the E6 and E7 proteins[74–76] of high-risk human papillomavirus (HPV). For examples of different classes of tumour antigens, see TABLE 1. More recently, attention has turned to 'neoantigens', a broadly encompassing term that comprises of antigens that arise in tumours through mechanisms such as non-synonymous somatic mutations in the

Box 2 | **T cell stemness in the context of antitumour immunity**

T cell 'stemness' is a measure of the T cells' capacity for self-renewal, differentiation, proliferation and persistence, and is guided by changes in T cell metabolism upon encounter with their MHC-restricted antigens and environmental cues[276]. The transition from naive, to stem effector memory, to effector memory, to effector T cells and finally to terminally differentiated T cells follows a gradient where T cells gain immune and cytolytic functions and lose stemness[277]. In the context of tumour immunotherapy, T cells with increased signatures of stemness, such as expression of the transcription factor TCF7, have proven to display improved antitumour functions and are a strong predictor of response to immune checkpoint inhibition and patient survival[278–280]. Several tumour-related factors have been reported to affect the stemness of tumour-infiltrating lymphocytes[281]. Abundance of potassium in the tumour microenvironment released from dying tumour cells was shown to hinder nutrient uptake, thus altering metabolic pathways and epigenetically reprograming tumour-infiltrating lymphocytes to repress expression of effector genes[282]. A relatively slower metabolic rate during the in vitro expansion phase and a high metabolic phase at the tumour site has been shown to optimize anti-tumour T cell response when in vitro T cell expansion is used for adoptive T cell transfer[283]. Indicators of metabolic fitness such as mitochondrial integrity, fatty acid oxidation and glycolytic potential all contribute to T cell antitumour activity[284]. Lately, co-stimulation by 4-1BB agonism and manipulation of PPARγ coactivator 1α (PGC1α) expression in T cells has been shown to skew T cell metabolism to overcome tumour microenvironment-induced suppression and restore response to immune checkpoint inhibition[285,286].

coding regions (such mutations are collectively also referred to as the 'mutanome'), human endogenous retroviruses[77], frameshifts as occurring in microsatellite-instability-high tumours[78] or post-translational modifications such as phosphorylation[79], citrullination[80] and glycosylation[81] (TABLE 1). Here we use the term 'neoantigen' to refer to mutated tumour neoantigens only. Neoantigens are generally not germ line encoded. Thus, in most cases, the host theoretically harbours no central tolerance towards these antigens. Therefore, it is possible to rouse a robust T cell response against these antigens, both spontaneously and upon immunotherapy.

Neoantigen vaccines

To a large extent the success of the neoantigen platform depends upon the tumour mutational burden (TMB); that is, the number of mutations, of any nature, per megabase in the tumour tissue[82,83]. It is reasonable to assume that tumours with a high TMB are likely to have a correspondingly high number of tumour-rejection neoantigens for vaccine targeting and a better response to ICI[82–84]. However, the occurrence of high TMB does not always coincide with response to ICI[85,86]. Aside from tumour intrinsic mechanisms of resistance (discussed later), other reasons for this disparity could be directly related to the 'quality' of the neoantigen (that is, the capacity of a neoantigen to elicit a definitive T_H1 cell and/or CTL response against the tumour), which is based on properties such as foreignness, clonal distribution, biological property of the neoantigen to be processed and presentation on MHC-I and MHC-II molecules, whether the mutation is a driver mutation, and T cell receptor avidity[87–92] (FIG. 2). Moreover, patients' HLA class I genotype has also been shown to influence therapy outcomes, with higher heterozygosity in HLA class I loci correlating with increased survival after ICI[93]. Studies thus far indicate that only a subset of neoantigens elicit a T cell response, so precision in neoantigen prediction and ranking is crucial for clinical success. In addition to using tools to predict neoantigen 'qualities', prediction accuracy can be improved by using high-quality starting tissue, minimizing loss of coverage, accurate HLA typing, identifying antigens' serological activity and protein sequence, and inclusion of MHC-II antigens[94–97]. Consortia such as the Tumor Neoantigen Selection Alliance that aim at unifying the neoantigen prediction process through large-scale downstream T cell validation assays are required to increase reproducibility and move the field forward towards discovery of maximally immunogenic tumour neoantigens.

Several recent clinical trials using neoantigen vaccines have proven their immunogenicity (induction of $CD4^+$ T cell and $CD8^+$ T cell antigen-specific responses, albeit with higher proportions of $CD4^+$ T cells) with indications of increasing patient survival, especially when ICI therapy is delivered afterwards. Some notable examples include a single-arm study where patients with melanoma received monocyte-derived DCs loaded with personalized neoantigen short peptides[98]. This first study demonstrated that neoantigen vaccines could induce a T cell-specific immune response resulting in antigen spreading[98]. An mRNA lipoplexed vaccine delivering neoantigens into draining lymph nodes elicited T cell responses (60% of neoantigens induced $CD4^+$ T cell responses, while 25% induced $CD8^+$ T cell responses), induced responses against endogenously expressed tumour antigens and was associated with lack of development of new metastatic lesions and increased progression-free survival in patients with melanoma[99]. Similarly, peptide neoantigen vaccines have been demonstrated to induce T cell responses and T cell infiltration into the TME in patients with high-risk melanoma (60% of neoantigens induced $CD4^+$ T cell responses, while 16% induced $CD8^+$ T cell responses)[100] and glioblastoma[101]. Although these are small single-arm studies, their results indicate that (1) neoantigen vaccines can safely induce antitumour responses and infiltration of T cells into the TME; (2) $CD4^+$ T cell responses are preferentially elicited over $CD8^+$ T cell responses, consistent with the requirement of $CD4^+$ T cells to provide help for eliciting neoantigen-specific $CD8^+$ T cells (BOX 1) — this argues in favour of systematically including MHC-II-restricted neoantigens in vaccinations[102]; (3) there is a need to improve accurate prediction of MHC-I-restricted neoantigens; and (4) while most of the patients in the studies mentioned above showed increased progression-free survival, the patients who did progress responded exceptionally well to anti-PD1 treatment[99,100], suggesting a synergy between neoantigen vaccination and ICI. Currently several studies are exploring the efficacy of neoantigen vaccines in the form of peptides (NCT03639714, NCT03223103 and NCT02721043), mRNA (NCT04163094) and DNA (NCT04015700 and NCT04251117)[103] in combination with ICI in clinical trials. Additional issues regarding neoantigen vaccines include screening antigens for maximal clinical efficacy before vaccination, the number of neoepitopes to include in each vaccine and at what dosage, minimizing the lengthy vaccine production pipeline (currently around 3 months), the delivery platform, combination with ICI or other modalities and overcoming secondary mechanisms of resistance.

'Cold' tumours
Non-inflamed tumours lacking the presence of immune cells, particularly cytotoxic T cells, in the tumour bed and the invasive margin.

Antigen spreading
A phenomenon where endogenous cellular immune responses develop against epitopes not targeted by the vaccine or immunotherapy.

Table 1 | **Target antigens for therapeutic vaccines**

Tumour antigens	Associated cancers	Type of antigen
HER2/NEU	Breast cancer	Overexpression
Human TERT	Multiple cancers	
p53 WT	Multiple cancers	
Survivin	Multiple cancers	
TPD52	Multiple cancers	
CD19	Haematological malignancies	
Folate receptor-α	Ovarian cancer	
MAGE-A3	Melanoma	
MUC1	Multiple cancers	Overexpression and post-translationally modified (glycosylation)
Vimentin	Multiple cancers	Post-translationally modified (citrullinated)
ENO1	HCC	
BCAR3	Breast cancer and melanoma	Post-translationally modified (phosphorylated)
ISR2	Melanoma	
AIM2	Colorectal cancer	Frameshift mutations in MSI-high tumours
HT001	Colorectal cancer	
TAF1B	Colorectal cancer	
Micoryx	Colorectal cancer	
TGFβRII mutant	Colorectal cancer	
ERVE-4	CCRCC	hERV (NCT03354390)
Mutant p53	Multiple cancers	Mutations
Mutant Ras	Multiple cancers	Mutations
NY-ESO-1	Melanoma	CTA
BAGE	Melanoma and other cancers	CTA
PRAME	Melanoma and other cancers	CTA
XAGE1B	Multiple cancers	CTA
WT1	Multiple cancers	Stem cell antigen
Mesothelin	Multiple cancers	Differentiation antigen
Melan A (also known as MART1)	Melanoma	Differentiation antigen
gp100	Melanoma	
Tyrosinase	Melanoma	
TRP1	Melanoma	
TRP2	Melanoma	
PAP	Prostate cancer	
PSA	Prostate cancer	
PSMA	Prostate cancer	
Immunoglobulin idiotype	B cell leukaemia/lymphoma	B cell differentiation antigen
Immunoglobulin κ-chain	B cell leukaemia/lymphoma	B cell differentiation antigen
Immunoglobulin λ-chain	B cell leukaemia/lymphoma	B cell differentiation antigen
LMP1	Nasopharyngeal carcinoma B cell lymphoma Hodgkin disease	Viral antigen; EBV (herpesvirus)
LMP2	Nasopharyngeal carcinoma, B cell lymphoma Hodgkin disease	Viral antigen; EBV (herpesvirus)
Tax protein	Adult T cell leukaemia	Viral antigen; HTLV1 (retrovirus)

Table 1 (cont.) | **Target antigens for therapeutic vaccines**

Tumour antigens	Associated cancers	Type of antigen
All viral proteins	HCC	Viral antigen; HBV (hepadnavirus) and HCV (RNA flavivirus)
E6	Anogenital cancer	Viral antigen; HPV (papillomavirus)
	Head and neck cancer	
E7	Anogenital cancer	Viral antigen; HPV (papillomavirus)
	Head and neck cancer	
Large T protein	Skin cancer	Viral antigen; Merkel polyomavirus
Small T protein	Skin cancer	

CCRCC, clear cell renal cell carcinoma; CTA, cancer testis antigen; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; hERV, human endogenous retrovirus; HPV, human papillomavirus; HTLV-I, human T-lymphotrophic virus type 1; LMP, latent membrane protein; MSI, microsatellite instability; WT, wild type.

Shared-antigen vaccines

Despite the many advantages of neoantigen vaccines, shared-antigen vaccines may be a more suitable option for candidates with low TMB, who are often poor candidates for neoantigen vaccines, although the inclusion of immunodominant epitopes may alleviate this concern[104]. Use of sophisticated vaccine platforms and appropriate combination therapy may prove shared-antigen vaccines to be overall more feasible. Indeed, an mRNA vaccine encoding four shared tumour-associated antigens in combination with anti-PDL1 therapy resulted in induction of type I IFN, promoting T cell recruitment. More than 75% of the patients responded to at least one of the vaccine antigens with high immunogenicity[105]. Several groups are attempting to include neoantigens alongside shared antigens to expand the antigen repertoire for vaccinations. For example, the APVAC1 and APVAC2 vaccines for treating glioblastoma contain a library of precurated and preprepared shared tumour antigens and patient-specific neoantigens, respectively. The vaccine is delivered as long peptides with polyribinosinic-polyribocytidylic acid, polylysine complex (poly-ICLC; a synthetic double-stranded RNA preparation) and GM-CSF as adjuvants. Results from a phase I study revealed a predominant $CD8^+$ T cell response and $CD4^+$ T cell response to APVAC1 and APVAC2, respectively[106]. Similarly, the TNBC-MERIT trial for treatment of breast cancer will use mRNA vaccine encoding shared antigens (IVAC WAREHOUSE concept) and individualized neoantigens (IVAC MUTANOME concept) (NCT02316457). In other approaches, CpG-activated tumour cells[107], DCs fused with tumour cells (NCT03679650) or DCs loaded with whole-tumour lysates[59,108] are being explored as therapeutic vaccinations to leverage the full range of tumour antigens.

Developments in vaccine platforms

A big challenge in the field of therapeutic cancer vaccinology is ensuring the delivery of vaccine components to the appropriate compartment, be it the tumour-proximal secondary lymphoid organ or the TME. The anatomic location of the tumour, the baseline TME immune landscape, the mechanism of action of the vaccine and the biochemical properties of the vaccine components all need to be cumulatively considered before the best-suited delivery platform can be determined.

Direct administration of antigen

A concentrated source of an antigen, injected by various routes (intravenous, intramuscular, subcutaneous or intracutaneous) introduces the antigen into the antigen-processing pathways of DCs in vaccine-draining lymph nodes. Key determinants of antigen vaccine success appear to be (1) the type of antigens (MHC-I or MHC-II restricted), (2) the antigen dosing, (3) the adjuvant used and (4) the administration route. So far, satisfactory sources of tumour antigen delivery have included DNA, RNA and synthetic long peptides (SLPs)[74,75,109–111]. Each of these platforms, discussed in the following sections, can generate robust T cell responses and therapeutic effects against established disease (FIG. 3a).

DNA vaccines. DNA vaccines are easy to manufacture, carry built-in adjuvants and, like RNA and peptide vaccines, represent a concentrated form of tumour-associated antigens but require extra steps of transcription and translation before cross-presentation by DCs[5,112] (FIG. 3a). DNA vaccines are most effective in driving sufficient antigen processing and presentation for induction of $CD4^+$ T cell and $CD8^+$ T cell responses when administered at relatively high doses via intramuscular injection in combination with electroporation[28,61–63,66,67]. A DNA vaccine against HPV-16/HPV-18 E6 and E7 oncogenes has demonstrated clinical efficacy in patients with premalignant high-grade cervical intraepithelial neoplasia. Among non-vaccinated, placebo-controlled patients, 30.6% experienced spontaneous regression of lesions, and 49.5% of vaccinated patients showed complete regression of lesions[61]. Recently, a synthetic DNA multineoantigen vaccine was shown to elicit predominant $CD8^+$ T cell responses in association with antitumour immunity in mouse tumour models[113]. For $CD8^+$ T cell induction, a further interesting innovation uses fusion of DNA, encoding the antigen of choice, and the chemokine CCL3, inducing excellent $CD8^+$ T cell responses against HIV-1 (REF.[114]). A further extension of this idea is to have the DNA encode chemokine genetic information to enhance targeting to subsets of DCs to promote T cell and antibody responses of choice, a technique which has recently been licensed for application in neoepitope-specific vaccines[115].

RNA vaccines. Like DNA vaccines, RNA vaccines are relatively straightforward to manufacture and have built-in adjuvants[110]. However, unlike DNA vaccines, RNA vaccines do not require transcription and are thus closer to protein antigen expression and processing and presentation on MHC molecules. RNA vaccines have been injected directly into lymph nodes[99] and also as lipoplex nanoparticles intravenously[116]. Patients with melanoma, when intranodally administered mRNA vaccine, encoding ten personalized neoantigens, displayed a remarkable vaccine-specific antitumour immune response and the number of metastatic episodes after vaccination was markedly reduced compared with before vaccination[99]. Moreover, upon recurrence of disease, one patient showed complete regression of all lesions following anti-PD1 therapy. A second patient who relapsed did not respond to anti-PD1 therapy, but turned out to have complete loss of cancer cell HLA class I expression due to a β_2M mutation[99]. Lipoplex RNA-loaded particles, upon intravenous injection, have the interesting property of selectively entering DCs in the spleen and in lymph nodes throughout the entire body, inducing a more powerful immune response than just loading of a single lymph node station after subcutaneous, intracutaneous or intramuscular injections. The first clinical results for this type of vaccination in three patients with late-stage melanoma were promising[116]. Recently an intravenously administered RNA lipoplex vaccine consisting of four melanoma-associated antigens (NY-ESO-1, MAGE-A3, tyrosinase and transmembrane phosphatase with tensin homology (TPTE)) induced strong T cell responses associated with durable partial clinical responses in combination with anti-PD1 treatment in patients with anti-PD1 resistance before the combination treatment[105]. Incorporation into the RNA of a RIG-I immunostimulatory sequence, combined with CTLA-4 antibody, increased antitumour efficacy in murine melanoma models[117]. Recently an RNA vaccine directed against the target of chimeric antigen receptor (CAR)-specific T cells proved to be capable of expanding CAR T cells directed against claudin 6, a target expressed on certain solid cancers[118]. These results demonstrate that appropriate vaccination can overcome immunological tolerance to tumour antigens and even increase the potency of other cell therapies. Clinical benefit must now be confirmed in randomized clinical trials.

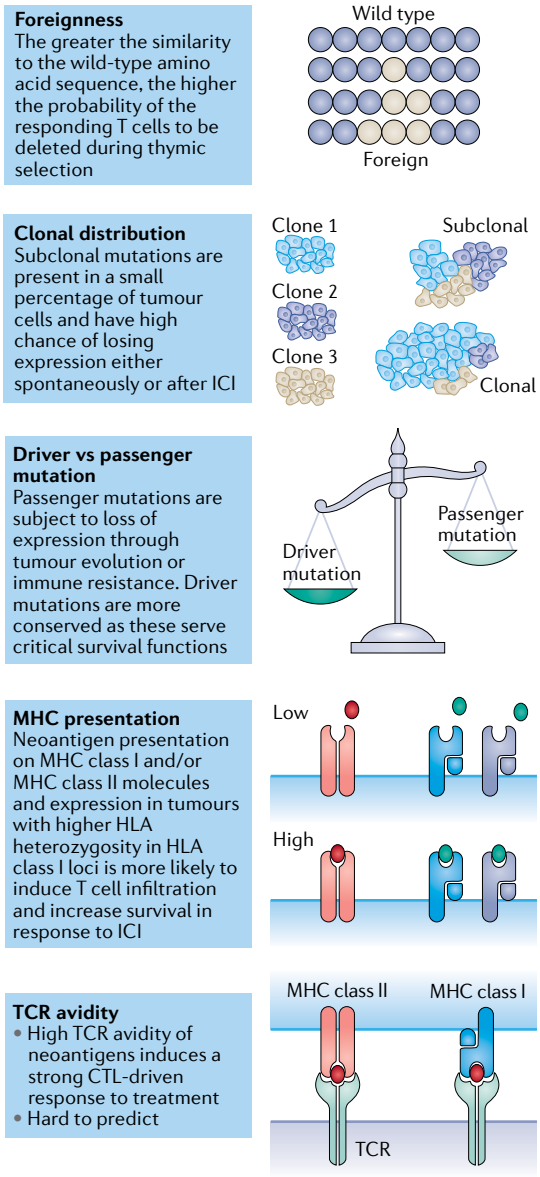

Fig. 2 | **Qualities of neoantigens.** The 'quality' is a cumulative assessment of the neoantigen repertoire based on the following characteristics: (1) 'foreignness', or the measure of how novel the new antigen is as compared with the wild-type protein; (2) clonal distribution — clonal mutations lead to the expression of neoantigens in most tumour cells and are correlated with a positive response to immune checkpoint inhibition (ICI), whereas subclonal mutations are more likely to lose their expression under the selection pressure of ICI; (3) driver status of the mutation as driver mutations are less likely to develop escape variants as compared with passenger mutations; (4) the likelihood of the neoantigen to be appropriately processed and presented on MHC class I and MHC class II molecules with high binding affinity and expression; and (5) the T cell receptor (TCR) avidity of the MHC–epitope complex. CTL, cytotoxic T lymphocyte.

SLP vaccines. Initially, peptide-based cancer vaccines often consisted of exact MHC-I-binding short peptides. Although these vaccines generated robust T cell responses, when they were used with a combination of mineral oil adjuvant, Montanide ISA-51 VG and Toll-like receptor 9 (TLR9) ligand, CpG 7909 (REFS[119–122]), the T cell responses were suboptimal for several reasons. Short peptides bind exogenously to MHC-I molecules on all cells that express MHC-I molecules (all nucleated cells), while only DCs express co-stimulatory molecules needed for a proper T cell response. In mice this was found to induce antigen presentation throughout the body without proper co-stimulation and adjuvanticity. In contrast, SLP vaccination leads to DC-focused antigen presentation only in vaccine-draining lymph nodes[123–125]. Indeed, 25–35 amino acid long SLPs have to pass through a processing step which only professional APCs, such as DCs, can accomplish[75,123–125] (FIG. 1), thus ensuring optimal antigen presentation on MHC-I molecules.

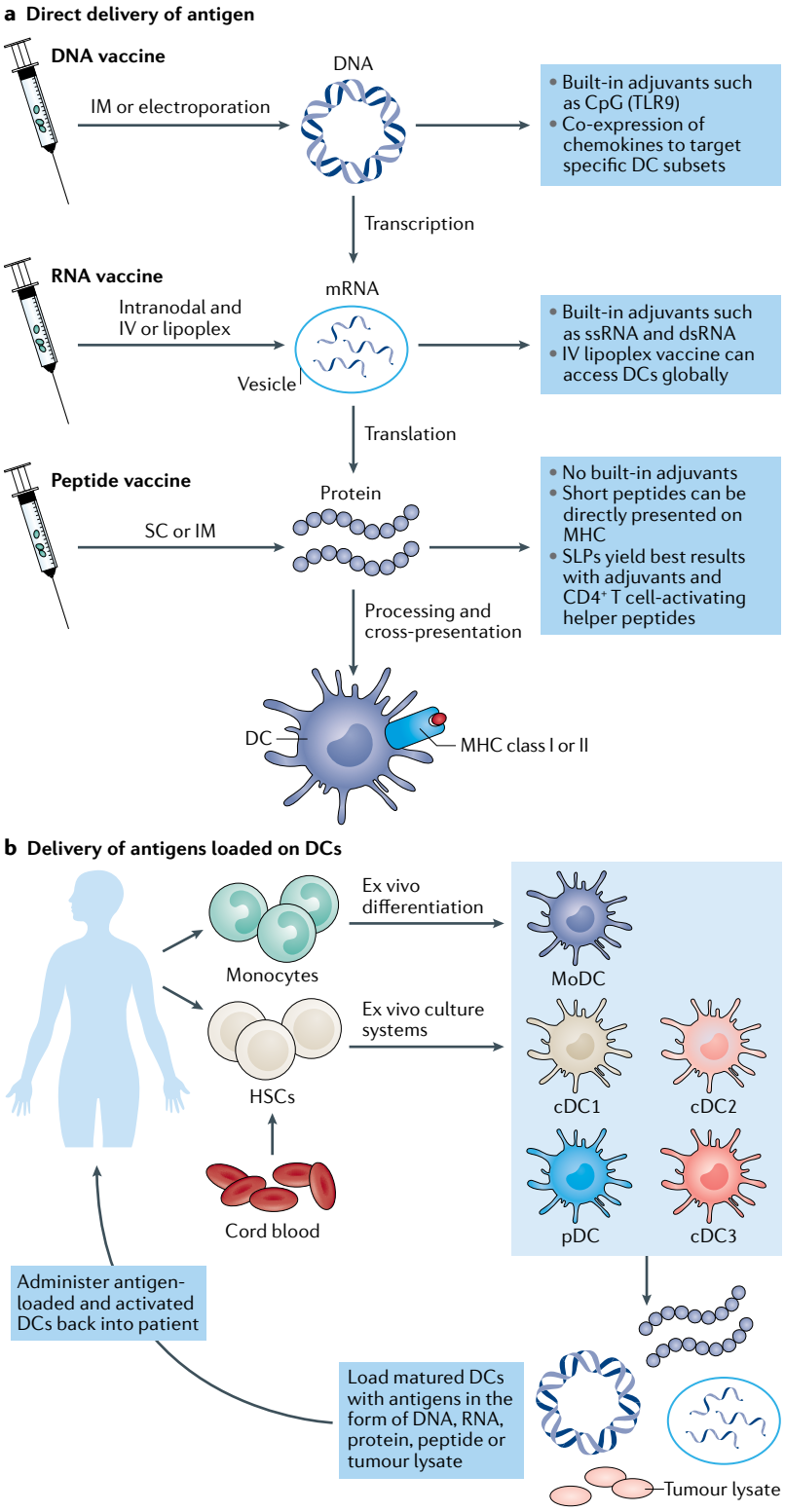

Fig. 3 | **Simplified depiction of cancer vaccine delivery platforms. a** | Antigens, either shared antigens or neoantigens, can be delivered directly via antigen-based cancer vaccines, formulated with the desired adjuvant and administered directly into the patient subcutaneously, intramuscularly, intranodally (in the lymph nodes) or intravenously. DNA vaccines, compared with RNA vaccines, require more processing steps before being presented on dendritic cells (DCs), while peptide vaccines have the shortest processing route. However, DNA and RNA vaccines are more suited as compared with peptide vaccines to deliver the antigen for MHC class I presentation. Moreover, DNA vaccines can be electroporated directly at the injection site, while RNA vaccines maybe delivered intravenously with newly developed nanoparticles such as lipoplexes, which facilitate delivery of the vaccine to lymph node-resident DCs. **b** | Monocyte-derived DCs (MoDCs) can be generated from patient-derived monocytes or specific DC subsets such as conventional DC1 (cDC1), cDC2, cDC3 subsets and plasmacytoid DCs (pDCs), differentiated from haematopoietic stem cells (HSCs), or isolated from patient's peripheral blood or from umbilical cord blood. These are loaded with tumour-associated antigens and reinfused into the patient. dsDNA, double-stranded DNA; IM; intramuscular; IV, intravenous; SC, subcutaneous; SLP, synthetic long peptide; ssRNA, single-stranded RNA; TLR9, Toll-like receptor 9.

Efficient induction of CD8$^+$ T cell effector and memory responses requires specific CD4$^+$ T cell help[126] (BOX 1; FIG. 1). Presentation of short exact MHC-I-binding peptides, but not SLPs, formulated at a high dose in incomplete Freund's adjuvant (IFA) may lead to accumulation of T cells at the vaccine site, where they appear to die because they are deprived of co-stimulation and CD4$^+$ T cell help[127]. However, robust and durable T cell responses and promising clinical results have been obtained with SLP vaccines in IFA (Montanide ISA-51 VG generates an emulsion like IFA) in patients with both premalignant[64,65] and malignant[28,128] HPV-16-induced disease[123,125,127]. Similarly, SLP vaccines directed against NY-ESO-1 in IFA emulsions with either poly-ICLC or CpG have induced robust CD4$^+$ T cell and CD8$^+$ T cell responses[129,130]. Interestingly, in a recent clinical trial in patients with melanoma, vaccination with short peptides in Montanide ISA-51 mixed with lipopolysaccharide (TLR4 ligand) or poly-ICLC and tetanus-derived T cell helper peptide did not lead to T cell death in the vaccine depot[131]. The reason for this unexpected observation, as compared with the prior mouse study[127], is hypothesized to stem from a difference in vaccine dosing, TLR ligand-driven immune activation and the induction of CD4$^+$ T_H cells by the tetanus peptide[126,132,133]. These results not only highlight the importance of choosing the right adjuvant but also the importance of CD4$^+$ T cell help for generation of CD8$^+$ T cell responses[102] (BOX 2). SLP vaccines, like RNA vaccines, have been successfully used to generate CD4$^+$ T cell and CD8$^+$ T cell responses to mutation-based neoantigens and shared tumour-associated antigens in cancers with a hint of clinical activity[28,66,100,101]. In treatment of premalignant HPV-16-induced lesions, SLP vaccination against the oncogenic proteins E6 and E7 has shown clinical efficacy as monotherapy[64,65]. Finally, although few clinical studies have systematically investigated different delivery sites and doses, effective delivery of peptides in Montanide adjuvant has involved both the subcutaneous route and the intramuscular route[74,75].

Delivery of antigens loaded into DCs

DCs that have been isolated or cultured from blood, adjuvant activated and loaded with antigens through direct pulsing with peptides corresponding to TAA or

neoantigens, mRNA electroporation (TriMix DCs), lentiviral transduction (for example, self-differentiated myeloid-derived antigen-presenting cells reactive against tumours-DCs (SMART-DCs), fusion with tumour cells[134] or incubating with whole tumour lysate ex vivo[59,108,135–140] (FIG. 3b), known as DC vaccines, have been tested in several trials. DC vaccines are injected intracutaneously or subcutaneously or even intravenously. Peptide-pulsed DCs were also used to prime neoantigen-specific $CD8^+$ T cells to broaden the breadth and diversity of melanoma neoantigen-specific T cells[98].

Importantly, in murine studies, along with directly priming T cells as APCs, injected DCs also serve as antigen donor cells that transfer antigens to endogenous cross-presenting DCs[6,7]. Most clinical trials use ex vivo differentiated monocyte-derived DCs for vaccinations, primarily because until now it has not been feasible to acquire other, more relevant, DC subsets in sufficient numbers for vaccination. However, monocyte-derived DCs do not possess the full repertoire of co-stimulatory molecules and antigen cross-presentation mechanisms available to other DC subsets. Various DC subsets are specialized to perform unique functions. While these functions are not mutually exclusive, the conventional type 1 DC (cDC1) subset has been shown to be superior at cross-presentation and $CD8^+$ T cell activation, while the cDC2 subset is recognized for priming $CD4^+$ T cells[3]. Thus, it is not known whether use of other DC subsets (DC1s, DC2s, DC3s and plasmacytoid DCs)[3,141–143] alone or in combination will shift the role of DCs in the vaccine towards APCs or antigen donor cells and how this will affect the vaccine outcome[8]. Important improvements in culture systems will allow the generation of large quantities of desired DC subsets from umbilical cord blood and adult peripheral blood mononuclear cell-derived stem cells for use in clinical studies[144] (FIG. 3b). In the future, DC subsets derived from these culture systems will help determine the true physiological potential of DC vaccines in eliciting a therapeutic antitumour response.

Novel platforms for antigen delivery

Non-cellular particles, characteristically smaller than DCs and usually referred to as 'nanoparticles' (size range 20–100 nm), are being explored as carriers for improving the non-cellular protein or peptide-based therapeutic cancer vaccine platforms which may not efficiently travel to or target lymph node DCs. Such approaches include synthetic particle delivery by lipoplexes[99], amphiphile vaccine[145], liposomes[146] and the novel self-assembling nanoparticles comprising TLR7/8–SLP neoantigen conjugate (SNP-7/8a)[147,148]. Furthermore, a polyethyleneimine silica microrod vaccine has been shown to enhance the immunogenicity of HPV-16 E7 and several neoantigen-bearing peptides, with excellent results when treating established tumours in mice[149], and use of specialized high-density lipoprotein nanodiscs carrying CpG oligodeoxynucleotides and peptide tumour antigens was shown to vastly enhance antigen delivery to the draining lymph nodes and improve antigen-specific CTL responses[150].

Non-antigen-specific ISVs

Despite some success, select antigen-based vaccines, especially neoantigen vaccines, suffer from disadvantages such as resource-intensive production processes, inability to encompass the entire gamut of immunogenic epitopes and probability of being ineffective against emerging tumour epitopes. ISVs, on the other hand, are antigen-agnostic agents, not tailor-made to individual mutanomes but instead aiming to improve endogenous antitumour responses. The mechanism of action of ISVs entails activating immune cells in situ through stimulation of innate immune pattern recognition receptors (PRRs) or other activating receptors on APCs, induction of ICD[10], enhancement of antigen presentation and enabling T cell priming and/or memory T cell activation[151]. In addition, ISVs may target tumour cells locally and at distal sites (abscopal effect). Often ISVs themselves act as agents that activate PRRs such as TLRs[72,152–156] and stimulator of interferon genes protein (STING)[157–159]. Approved PRR-activating ISVs include bacillus Calmette–Guérin (BCG) vaccine, which activates TLR2 and TLR4, for non-muscle-invasive bladder cancer[160]. Imiquimod, a synthetic TLR7 and TLR8 agonist used to treat superficial basal cell carcinoma[161], has been successfully used off label to treat in transit melanomas[162]. Agents such as fms-like tyrosine kinase 3 ligand (FLT3L)[163,164] and agonists of CD40 receptor[165] or activators of CD40 may or may not be administered intratumourally but function as ISVs by improving DC mobilization and increasing proliferation, and improving effector functions, respectively, to eradicate tumours and restore sensitivity to ICI[166–168]. While monotherapy with FLT3L failed to rouse a clinical response, combining FLT3L with other platforms has yielded promising outcomes[169].

Oncolytic viruses are recently introduced ISV agents that induce both local and abscopal immunity. They can be manipulated genetically or chemically to express immunomodulators such as cytokines, antibodies and co-stimulatory factors[170]. ICD induced by oncolytic viruses releases tumour-associated antigens, including neoantigens, and facilitates activation of neoantigen-specific T cells[171]. Talimogene laherparepvec (TVec) is the first and only oncolytic virus therapy approved by the FDA for the treatment of advanced melanoma[172] and is being investigated for increased clinical efficacy as a combination therapy. Other viruses being developed for oncolytic virus therapy include coxsackie virus, Newcastle disease virus, adenovirus, poliovirus type 1, reovirus, vaccinia, measles virus and flu viruses[173]. Stereotactic radiotherapy is another ISV approach that allows precise focusing of hypofractionated radio beams at tumour targets to induce ICD and promote antitumour responses[174]. A list of clinical studies investigating the use of TLR and STING agonists, recombinant human FLT3L, anti-CD40 antibody, TVec and radiotherapy as ISVs is provided in TABLE 2.

Immune-activating cytokines such as GM-CSF, IL-12, IL-15 and IL-2, which are directly or indirectly important for mounting effective antitumour T cell and NK cell responses, serve as promising ISVs, particularly when administered intratumourally[172,175,176]. Especially

Stimulator of interferon genes protein
(STING). An intracellular pattern recognition receptor activated by double-stranded DNA, cyclic GMP–AMP and microbial cyclic dinucleotides to induce a strong interferon response.

Talimogene laherparepvec
(TVec). An oncolytic virus therapy composed of herpes simplex virus type 1 modified to infect tumour cells and express GM-CSF, a cytokine known for promoting differentiation of dendritic cells.

Table 2 | **In situ vaccine candidates under clinical consideration as monotherapies or in combination**

Receptor	Agonist	Clinical trial identifier	Route[a]	Treatment	Condition
TLR and STING agonists					
RIG-I/MDA5 and TLR3	Poly-ICLC	NCT02423863	IT + IM	Poly-ICLC + anti-PD1 or anti-PDL1	Melanoma, H&N cancer, sarcoma, non-melanoma skin cancers
		NCT02643303	IT + IM	Poly-ICLC + anti-CTLA-4 and anti-PDL1	Advanced, measurable, biopsy-accessible cancers
TLR4	Glucopyranosyl lipid A (G100)	NCT02501473	IT	G100 + pembrolizumab	Follicular low-grade NHL
		NCT03915678	IT	G100 + atezolizumab + radiotherapy	Multiple solid tumours
		NCT02406781	IT	G100 + pembrolizumab + cyclophosphamide	Sarcoma
TLR7/8	NKTR-262	NCT03435640	IT	NKTR-262 + NKTR-214 (CD122 agonist) + nivolumab	Multiple cancers
TLR9	CpG ODN SD-101	NCT02927964	IT	Radiotherapy + SD-101 + ibrutinib	Lymphoma
		NCT02521870	IT	SD-101 + pembrolizumab	Melanoma and H&N cancer
	(VLP) encapsulated-TLR9 agonist CMP-001	NCT03084640	SC	CMP-001 + pembrolizumab	Melanoma
		NCT03618641	SC + IT	CMP-001 + nivolumab	Melanoma
		NCT02680184	IT	CMP-001 ± pembrolizumab	Melanoma
		NCT03983668	IT	CMP-001 ± pembrolizumab	R/R lymphoma
		NCT03438318	SC + IT	CMP-001 + atezolizumab ± radiotherapy	NSCLC
		NCT03507699	SC + IT	CMP-001 + nivolumab + ipilimumab ± radiotherapy	Metastatic CRC with liver metastases
STING	MK-1454	NCT03010176	IT	MK-1454 ± pembrolizumab	Solid tumours and lymphoma
	E7766	NCT04109092	IT	Monotherapy	Bladder cancer
	ADU-S100	NCT03937141	IV	ADU-S100 + pembrolizumab	H&N cancer
		NCT03172936	IT	ADU-S100 + anti-PD1	Solid tumours and lymphoma
		NCT02675439	IT	ADU-S100 + ipilimumab	Solid tumours and lymphoma
	BMS-986301	NCT03956680	ND	BMS-986301 + nivolumab + ipilimumab	Advanced solid cancers
	SB-11285	NCT04096638	IV	SB-11285 ± nivolumab	Advanced solid cancers
FLT3L and CD40 agonists					
rhFLT3L	CDX-301	NCT02129075	SC	Poly-ICLC + CDX-1401 ± CDX-301	Stage IIB–IV melanoma
		NCT03789097	ND	CDX-301 + poly-ICLC + pembrolizumab + radiotherapy	NHL, metastatic breast cancer, H&N squamous cell carcinoma
		NCT01976585	IT	CDX-301 + poly-ICLC	Low-grade BCL
		NCT02839265	SC	CDX-301 + SBRT	NSCLC
Agonistic anti-CD40 antibody	APX005M	NCT02482168	IV	Monotherapy	Multiple solid cancers
	CDX-1140	NCT03329950	ND	CDX-1140 ± CDX-301 (rhFLT3L) ± pembrolizumab	Multiple cancers
	SEA-CD40	NCT02376699	IV or SC	SEA-CD40 + pembrolizumab + chemotherapy	Solid tumours and Lymphoma
Oncolytic virus, TVec					
Modified HSV-1	TVec	NCT02263508	IT	Tvec ± pembrolizumab	Melanoma
		NCT03802604	IT	Tvec + atezolizumab	Breast cancer
		NCT03256344	IT	Tvec + atezolizumab	Breast cancer and CRC
		NCT02509507	IT	Tvec + pembrolizumab	Multiple cancers
		NCT04185311	IT	Tvec + nivolumab + ipilimumab	Breast cancer

Table 2 (cont.) | **In situ vaccine candidates under clinical consideration as monotherapies or in combination**

Receptor	Agonist	Clinical trial identifier	Route[a]	Treatment	Condition
Stereotactic radiotherapy					
SRS	NA	NCT03483012	NA	SRS + atezolizumab	Metastatic breast cancer with metastasis to brain
	NA	NCT03807765	NA	SRS + nivolumab	Metastatic breast cancer with metastasis to brain
SBRT	NA	NCT01896271	NA	SBRT + IL-2	CCRCC

Source: ClinicalTrials.gov as of June 2020. BCL, B cell lymphoma; CCRCC, clear cell renal cell carcinoma; CDX-1401, the fusion product of DEC205; CRC, colorectal cancer; FLT3L, fms-like tyrosine kinase 3 ligand; H&N, head and neck; HSV-1, herpes simplex virus type 1; IM, intramuscular; IT, intratumoural; IV, intravenous; NA, not applicable; ND, not declared on ClinicalTrials.gov; NHL, non Hodgkin lymphoma; NSCLC, non-small cell lung carcinoma; ODN, oligodeoxynucleotide; poly-ICLC, polyribinosinic-polyribocytidylic acid, polylysine complex; rhFLT3L, recombinant human fms-like tyrosine kinase 3 ligand; R/R, relapsed or refractory; SBRT, stereotactic ablative body radiotherapy; SC, subcutaneous; SRS, stereotactic radiosurgery; STING, stimulator of interferon genes protein; TLR, Toll-like receptor; Tvec, talimogene laherparepvec; VLP, viral-like particle. [a]The route of administration of the in situ vaccine.

designed agonists that selectively target IL-2R on anti-tumour lymphocytes (NKTR-214 (REFS[177,178]) and ALKS 4230 (REFS[179])) are in clinical trials. Delivery of these cytokines intratumourally[180] (IL-2), using plasmid DNA electroporation[181,182] (IL-12 and GM-CSF), by encapsulation in biodegradable microspheres[183] (IL-2 and IL-12) or through adenoviral vectors[184] is being explored. Intratumoural mRNA vaccines encoding IL-12 (MEDI1191)[185] and OX40L (a co-stimulatory protein that facilitates T cell activation) alone (NCT03323398) or together with mRNA-encoded IL-23 and IL-36γ (NCT03739931) are being tested in solid tumours. Overall, ISVs may be safe off-the-shelf non-specific modalities with low off-target toxicity. They have the potential to spark a spontaneous immune response (autovaccination) while increasing responsiveness to other immunotherapies, such as ICI.

Mechanisms compromising vaccine efficacy

The resistance to clinically active forms of immunotherapy, including therapeutic vaccines, may present itself as a direct lack of response to therapy (primary resistance) or may develop following initial responsiveness of tumours to treatment (secondary resistance). Many underlying causes may foster this immune escape. These can be grouped into either tumour cell 'intrinsic' mechanisms, determined by the traits of the tumour cell itself, or tumour cell 'extrinsic' mechanisms, involving the tumour stromal components (FIG. 4). It is important to realize that the same factors determining initial resistance to immunotherapy may also drive the occurrence of secondary resistance.

Tumour intrinsic resistance

The tumour-intrinsic factors of resistance to T cell-based therapies, predominantly ICI, adoptive cell transfer and/or therapeutic vaccination (FIG. 4; reviewed in[186]), include the downregulation or lack of tumour antigen expression[82,83,88,89,99–101,187], alterations in the antigen processing pathway[92,188–194] and soft-wired and hard-wired loss of HLA expression[84,92,188–192,195,196], all of which prevent recognition of tumour cells by T cells. Indeed, the loss of HLA class I expression was found to cause primary resistance to a therapeutic vaccine composed of autologous tumour cells and BCG in melanoma[197] and led to secondary resistance to BCG vaccine in bladder cancer[198] and to autologous virus-specific T cell transfer in Merkel cell carcinoma[199]. The constitutive expression of the ligands for immune checkpoints (for example, PDL1) interferes with the activation of effector functions in T cells[192]. Resistance to TNF and IFNγ signalling affects the immediate antitumour effects of immunotherapy-activated T cells[86,200–202]. For instance, the expression of multiple immune checkpoints expressed by neoantigen-specific $CD4^+$ T cells and $CD8^+$ T cells was associated with non-responsiveness to a combination of treatment with a neoantigen vaccine and PD1 checkpoint blockade in patients with melanoma, non-small cell lung cancer and bladder cancer[103]. In addition, the expression of the mouse homologue of HLA-E ($Qa\text{-}1^b$), a non-classical MHC-I molecule which interacts with the co-inhibitory receptor NKG2A expressed by NK cells and cytotoxic T cells[203,204], was recently implicated as a mechanism for driving primary resistance through repression of NK cell and $CD8^+$ T cell responses to a combination therapy of anti-PD1 with a GM-CSF-secreting tumour cell vaccine in a mouse model of melanoma[205] as well as a mechanism for secondary resistance to therapeutic vaccination in several other mouse tumour models for HPV-induced cancers, melanoma and leukaemia[206]. Here, treatment led to local production of IFNγ and expression of PD1 and NKG2A on activated tumour-infiltrating $CD8^+$ T cells, as well as upregulation of the respective ligands on tumour cells[206].

Data from gene expression analysis of patient biopsy samples, after treatment with ICI, and mouse studies indicate that active tumour-intrinsic WNT–β-catenin signalling or PTEN loss is associated with increased expression of immunosuppressive cytokines, downregulation of chemokines (CCL3, CXCL1, CXCL2, and CCL4 in the tumours and CCR5 on the intratumoural DCs) and lack of $CD8^+$ T cell and DC1 infiltration into the tumour[207,208]. Increased expression of β-catenin, which is known to result in T cell exclusion[209], in a patient with metastatic melanoma was found to mediate secondary resistance in response to vaccination with melanoma peptides and IL-12 by preventing the vaccine-activated T cells from infiltrating the melanoma[210]. While therapeutic vaccines are reported to drive T cell infiltration in tumours with existing T cell infiltration[103,211] they seem not able to do so in T cell-excluded lesions[27]. Finally, a tumour-intrinsic incapacity to attract myeloid effector

cells was demonstrated to mediate primary and secondary resistance to therapeutic peptide vaccination (targeting HPV-16 E6 and E7 proteins) despite a strong vaccine-mediated T cell infiltration in tumour-bearing mice and patients with cancer[27], presumably because the necessary programmed cell removal following T cell-initiated programmed cell death did not ensue[212].

Tumour extrinsic resistance

Resistance may also derive from 'extrinsic' factors (FIG. 4). Systemic and local accumulation of immunosuppressive cells such as T_{reg} cells, MDSCs, tumour-associated macrophages (TAMs) with a tumour-promoting phenotype, cancer-associated fibroblasts and pro-tumour N2 neutrophils are extrinsic factors linked to immunoresistance[128,213,214]. These cells can interfere with T cell activation, proliferation and effector functions by expression of inhibitory receptors (for example, PD1 and/or CTLA-4); production of immunosuppressive cytokines (IL-10, TGFβ and VEGF)[142,215,216], arginase 1, inducible nitric oxide synthase (iNOS) and reactive oxygen species[217,218]; inhibition of systemic and local T cell activation[219–222]; polarization of local $CD4^+$ T cells, neutrophils and monocytes towards a protumorigenic phenotype[223–226]; and suppression of DC function[227,228]. An increase in the numbers of T_{reg} cells and MDSCs has been shown to inhibit the efficacy of ICI[229,230] and also to induce primary resistance, limiting the efficacy of DC vaccine-induced antitumour T cell responses in mice[231]. Furthermore, the influx of intratumoural T_{reg} cells and macrophages induced by NY-ESO-1 vaccination-driven inflammation and tumour cell apoptosis was associated with melanoma progression[232]. In addition, cancer-associated fibroblasts may interfere with vaccine efficacy as they remodel the extracellular matrix to construct a dense fibrotic stroma that inhibits DC

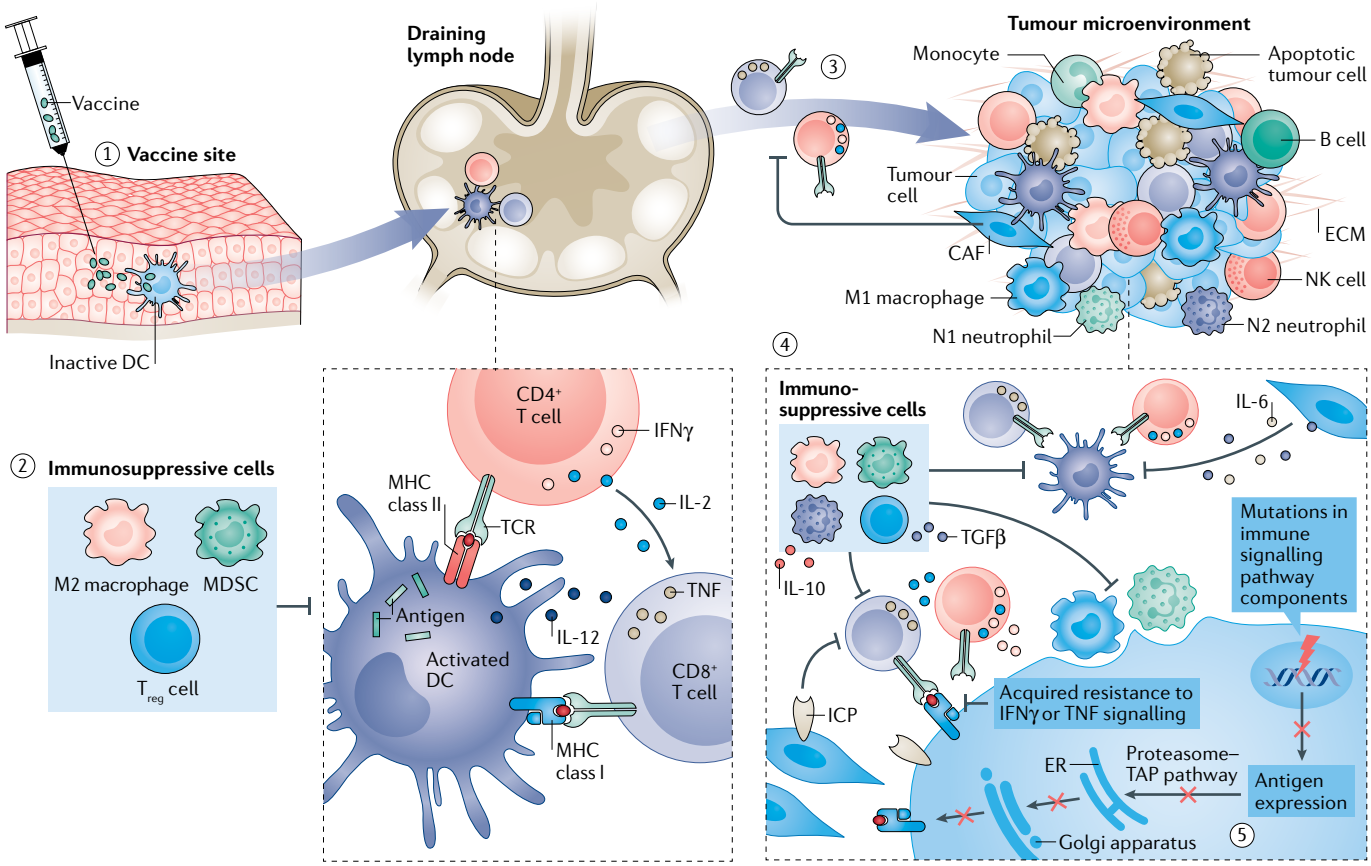

Fig. 4 | **Mechanisms of resistance to vaccine therapy.** The priming of tumour-reactive T cells requires antigens to be taken up at the vaccine site (1) for presentation to T cells in the draining lymph nodes (2). The tumour induces abnormal levels of immunosuppressive type 2 macrophages (M2 macrophages), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (T_{reg} cells) that impair activation or alter the quality of tumour-reactive T cells. Activated T cells from the lymph nodes may be blocked in their immigration to tumours (3) due to chemokine-gradient and cytokine-gradient regulation by cancer-associated fibroblasts (CAFs) and a dense extracellular matrix (ECM). In the tumour cell bed (4) CAFs may impair dendritic cell (DC) trafficking via IL-6 and TGFβ. M2 macrophages, MDSCs, T_{reg} cells and type 2 neutrophils (N2 neutrophils) secrete IL-6 and/or IL-10, which impair DC-mediated local T cell activation and block development of tumouricidal M1 macrophages and N1 neutrophils. Production of arginase 1, inducible nitric oxide synthase (iNOS) and reactive oxygen species and the expression of immune checkpoint (ICP) molecules inhibits tumour-resident T cells from exerting their function. In addition to these tumour-extrinsic resistance mechanisms, tumour cells (5) exploit tumour-intrinsic mechanisms, including mutations in signalling pathways known to support immune control of tumours as well as lowering or loss of tumour antigen expression, alterations in antigen processing pathways or loss of HLA expression, all resulting in suboptimal recognition of tumour cells by T cells. Moreover, constitutive expression of the ligands for ICPs and acquired resistance to TNF and IFNγ signalling interfere with the activation and impact, respectively, of T cell function to control tumour cell death and growth. ECM, extracellular matrix; ER, endoplasmic reticulum; NK, natural killer; TAP, transporter associated with antigen processing; TCR, T cell receptor.

proliferation and migration[215,223,233–237], prevents T cell infiltration and recruits MDSCs.

Macrophages are pragmatically characterized as M1-like or M2-like depending upon their role in promoting or inhibiting inflammation, respectively. In the tumour setting, TAMs are classified as antitumorigenic M1-like or protumorigenic M2-like macrophages, with the M2-like phenotype being more abundant than the M1-like phenotype[238]. TAMs may localize to the TME either by travelling via chemotactic gradients regulated by factors such as CCL2, IL-1β and macrophage colony-stimulating factor 1 (CSF1), differentiating from monocytes in the TME or by repolarization of tissue-resident macrophages[239]. Despite the role of TAMs in immunosuppression, the same cells are also known for their capacity to support the adaptive immune system and to exert direct tumouricidal activities, provided that local cues in the TME support TAMs to acquire these functions[240]. A few recent publications showed that the efficacy of vaccine-induced T cell responses in mediating tumour regression and cure in mice depended on the attraction of macrophages[241,242] and/or neutrophils[145,243]. Current knowledge of how to manipulate these cells for optimal therapeutic vaccination is limited, in particular because of the lack of robust cell surface markers. However, our knowledge will likely be extended now that high-dimensional techniques can be used to pinpoint which cells provide clinical benefit. This information will promote the development of therapeutic pipelines and products to increase the efficacy of cancer vaccines. Currently, there are only a handful of therapeutic vaccines with demonstrable clinical efficacy[28,49–71], making it difficult to pinpoint how primary and secondary resistance mechanisms regulate the response to therapeutic cancer vaccines. The intricacy of these mechanisms can be illustrated by using the example of therapeutic vaccination against HPV-associated malignancies. A pre-existing exclusion of T cells and/or inflammatory myeloid cells was associated with a lack of regression in patients with a vulvar high-grade squamous intraepithelial lesion despite strong vaccine-induced HPV-specific T cell responses[27]. The presence of immunosuppressive myeloid cells was associated with decreased immunogenicity of the therapeutic HPV vaccine in patients with cervical cancer[128]. Interestingly, recent data suggest that such resistance can be overcome by removing these suppressive cells[28]. The modest progression-free survival and response rate associated with PD1 blockade in patients with platinum-resistant recurrent HPV-positive head and neck squamous cell carcinoma[244] was later traced to a profound lack of pre-existing HPV-specific T cells in the TME[245]. Furthermore, the response could be partially restored by vaccination before ICI[66], thus underscoring the importance of pre-existing tumour-specific T cells in immunotherapy[246,247].

Overall, the mechanisms of tumour resistance to vaccines and, more generally, immunotherapy are multifaceted and complex, suggesting that multiple modalities may be required to overcome a suppressive TME. As illustrated in the example above, identification of the resistance mechanisms that play a role in specific groups of patients is required to guide the use of potential combinations.

Combination therapies

While ICI with anti-PD1, anti-PDL1 and anti-CTLA-4 has shown unprecedented success in treating significant proportions of patients in more than a dozen cancer types, most patients either do not respond or eventually relapse. Keeping in mind the unique biology of individual tumours, the resistance mechanisms of each tumour type and the pros and cons of each vaccination platform, a combination of therapies designed to counter the specific challenges of individual tumour types will be necessary to ensure clinical impact.

In the case of advanced tumours, which are generally more resistant to ICI, we envisage a multiphasic approach, wherein the first phase involves tumour debulking with surgery, chemotherapy or radiotherapy (which may also reduce immunosuppressive pathways)[28], while the second phase would involve vaccination that helps reduce the number of residual cancer cells and establishes immunological memory to prevent remission. The vaccine could be based on viral antigens, shared tumour antigens or personalized neoantigens, administered as peptides, RNA or DNA[142]. This module should be combined with chemotherapy, ICI or other immunomodulatory treatment concomitantly or after vaccination to reinvigorate T cells rendered exhausted by the TME. For example, a chemotherapy doublet of carboplatin and paclitaxel reduced the numbers of immunosuppressive MDSCs in patients with late stage HPV-16-induced cancers, increased spontaneous HPV-16-specific T cell immunity in 30% of the patients, induced a stronger type 1 T cell response (than previously observed[30]) and was associated with increased survival in response to HPV-16 SLP vaccine[28]. Recently a therapeutic HPV-16 SLP vaccine was shown to overcome the weak or absent HPV-specific T cell response and lead to a doubling of the clinical response rate associated with PD1 blockade in patients with HPV-16-positive oropharyngeal squamous cell carcinomas[66]. Similarly, robust HPV-16/18-specific immune responses were observed in patients with HPV-associated head and neck cancer who were vaccinated with a DNA vaccine targeting the HPV-16/18 E6 and E7 proteins. One of these patients was subsequently treated with ICI (anti-PD1) because of progressive disease and showed complete tumour regression[67] (NCT03162224 and NCT03444376). However, subsets of patients succumbed to the disease perhaps due to secondary resistance caused by expression of HLA-E and NKG2A[67]. Many of the HPV-positive head and neck squamous cell carcinomas express the HLA-E ligand and up to 50% of tumour-infiltrating $CD8^+$ T cells from different regions in the head and neck express NKG2A. High transcript levels of the CD8 gene correlated with good prognosis but high co-expression of either the gene encoding NKG2A or the gene encoding HLA-E neutralized this benefit[206]. These tumours were also infiltrated with highly active T_{reg} cells[248–250] and MDSCs[251]. This example indicates the complexity of tumour resistance to vaccines and immunotherapy and emphasizes how therapeutic vaccines or other immunomodulatory therapies will require multiple modalities to overcome a suppressive TME. Furthermore, mice lacking PDL1 expression specifically in mature DCs were

able to control tumour growth with comparable efficacy to mice lacking global PDL1 expression. Further investigation revealed that PDL1 expression was hindering DC-driven antitumour immunity as eliminating PDL1 expression on DCs enhanced cross-presentation and improved expansion of stem-like T cells and consequent tumour control[252]. Interestingly, in tumour-bearing mice resistant to anti-PD1 blockade, administration of ICI therapy before antitumour vaccination resulted in differentiation of suboptimally primed $CD8^+$ T cells into $PD1^+CD38^{hi}CD8^+$ T cells that were unable to respond to the vaccine. On the other hand, the mice that received the vaccination first, before ICI, demonstrated a strong vaccine-specific T cell response and concomitant reversal of anti-PD1 resistance. These data indicate that administering anti-PD1 antibodies before vaccination could adversely affect the functionality of vaccine-targeted CTLs[253]. However, pending further investigation, it is unclear at which stage of vaccination PD1 blocking would most benefit the vaccine response.

Combinations with ISV agents such as TLR and STING ligands, FLT3L or CD40 antibodies can further enhance recruitment and activation of DCs (TABLE 2). Hammerich et al. combined intratumoural FLT3L, poly-ICLC and local radiotherapy, which led to clinical responses with $CD8^+$ T cell infiltration in patients with indolent non-Hodgkin lymphoma and loss of the tumour, suggesting an antigen-specific response. This vaccine also restored responsiveness to anti-PD1 therapy in mice with lymphoma[167]. Oncolytic virus therapy with TVec, which yielded modest results as a monotherapy, is now being investigated in combination with immune checkpoint inhibitors (TABLE 2). Similarly, stereotactic radiotherapy (TABLE 2) or inhibitors of DNA damage response (such as poly(ADP-ribose) polymerase inhibitors)[254] can be part of this arsenal because they are designed to release tumour antigens, introduce genomic instability to enhance mutational burden and improve overall immune stimulation[10,151,167,170,174,255]. Several other immunomodulatory agents can likely synergize with therapeutic vaccines, immune checkpoint inhibitors or other treatment platforms to neutralize or prevent tumour resistance to therapy. For example, in a randomized three-arm study, the combination with all-*trans*-retinoic acid successfully decreased the levels of $CD33^+$ (immunosuppressive) MDSCs and doubled the rate of immunological response to a DC-based p53 vaccine[58]. Inhibitors of TGFβ, drugs that control immunosuppressive cells in the TME (such as cancer-associated fibroblasts, MDSCs and TAMs) and inhibitors of hypoxia are also promising in this regard.

To directly introduce a targeted effector T cell response, vaccination approaches may be combined with adoptive cell therapy. In this regard CAR T cell therapy is particularly interesting due to its remarkable success in treating haematological malignancies[256]. Combination with cancer vaccines may increase the durability of CAR T cell therapy and even increase its efficacy against solid tumours. Towards this end, several groups are exploring combinations of therapeutic CAR T cell therapy with DC vaccines, RNA vaccines or novel approaches such as co-delivering CAR-specific ligands that decorate the APCs in the lymph node and provide critical priming signals to the CAR T cells[118,257,258].

ICI in the neoadjuvant setting (that is, before surgical debulking) has led to impressive pathological responses and induction of neoantigen-specific T cells in blood and tumour. In separate studies, in non-small-cell lung cancer[259,260], Merkel cell carcinoma[261], HER2-negative stage II/III breast cancer[262] and mismatch repair-proficient and mismatch repair-deficient colon cancers[263], neoadjuvant treatment with ICI with or without combination with chemotherapy induced a major or complete pathological response in 37–85% of the resected tumours. Similarly, neoadjuvant treatment with PROSTVAC vaccine for treatment of prostate cancer (consisting of priming with a recombinant vaccinia vector and boosting with a recombinant fowlpox vector, both expressing PSA and T cell stimulatory molecules) was reported to induce T cell infiltration in the TME and systemic antitumour T cell responses[264]. It is possible that clinical responses may be further sustained with maintenance vaccination following tumour resection. Indeed treatment with ipilimumab was shown induce a neoantigen-specific T cell response that correlated with favourable prognosis even in patients with low TMB[265]. Overall, it is apparent that finding the right combination of therapies for each patient and each tumour type will define the next generation of clinical advancement for tumour vaccines.

Conclusion

The basis of all immunotherapy and cancer vaccines is a comprehensive understanding of the tumour's immune evasion mechanisms. Emerging technology has made it possible to dissect the TME in depth and draw significant conclusions regarding the mechanisms of intrinsic and extrinsic resistance governing the response to therapy at various stages of disease. Mechanistic insights gleaned from previously unsuccessful and some clinically impactful vaccine trials can further inform the design of future therapeutic platforms. Breakthrough advances in antigen prediction platforms have increased our capacity to personalize vaccines. However, further developments to enhance the quality of neoepitopes are required to maximize their impact. Shared-antigen vaccines, whole-tumour lysate vaccines and ISVs are being explored in new formats to maximize the antigen repertoire in the vaccine. The mode of delivery of antigen-based vaccines is a major determinant of a vaccine's success. Antigens may be delivered directly in the form of peptide, mRNA or DNA vaccines or loaded on DCs ex vivo. The development of new culture systems to generate large numbers of desired DC subsets, while still in the preclinical stage, will make it possible to vaccinate with the most relevant DCs. To thwart the numerous mechanisms of tumour resistance, different vaccine platforms, concomitant immunomodulation and supporting traditional cancer therapies (chemotherapy and radiotherapy) will need to be administered in combinations. However, the timing, sequence and dosing of each component need to be carefully determined and tailored to individual patients to ensure success.

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Author contributions
All authors contributed equally to the manuscript.

Competing interests
C.J.M.M. is Chief Scientific Officer of ISA Pharmaceuticals in Leiden, Netherlands, a biotechology company aiming at commercial development of synthetic peptide-based therapeutic vaccines against cancers caused by high-risk human papillomaviruses and against non-viral cancers. He receives a salary as a full-time employee at ISA Pharmaceuticals and is a beneficiary of a management participation plan that goes into effect if the company reaches a predefined value inflection point in the future. C.J.M.M. is an inventor on several patents regarding the use of synthetic long peptides as therapeutic vaccines for treatment of premalignant and malignant lesions. S.H.v.d.B.is named as an inventor on a patent for the use of synthetic long peptides as a vaccine, serves as a paid member of the strategy board of ISA Pharmaceuticals and received honoraria as a consultant for PCI Biotech, IO Biotech and DC prime, which develop cancer vaccines. N.B. serves as an advisor/board member for Neon Therapeutics, Novartis, Avidea, Boehringer Ingelheim, Rome Therapeutics, Roswell Park Comprehensive Cancer Center, MD Anderson Cancer Center, BreakBio, Carisma Therapeutics, CureVac, Genotwin, BioNTech, Gilead and Tempest Therapeutics. N.B. is an extramural member of the Parker Institute of Cancer Immunotherapy. M.S. declares no competing interests.

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