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REVIEW

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Vaccine approaches to treat urothelial cancer

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ABSTRACT

Bladder cancer (BC) accounts for about 4% of all malignancies. Non-muscle-invasive BC, 75% of cases, is treated with transurethral resection and adjuvant intravesical instillation, while muscle-invasive BC warrants cisplatin-based perioperative chemotherapy. Although immune-checkpoint inhibitors, antibody drug conjugates and targeted agents have provided dramatic advances, metastatic BC remains a generally incurable disease and clinical trials continue to vigorously evaluate novel molecules. Cancer vaccines aim at activating the patient's immune system against tumor cells. Several means of delivering neoantigens have been developed, including peptides, antigen-presenting cells, virus, or nucleic acids. Various improvements are constantly being explored, such as adjuvants use and combination strategies. Nucleic acids-based vaccines are increasingly gaining attention in recent years, with promising results in other malignancies. However, despite the recent advantages, numerous obstacles persist. This review is aimed at describing the different types of cancer vaccines, their evaluations in UC patients and the more recent innovations in this field.

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Introduction

Urothelial carcinoma (UC) represents the most frequent type of cancer developing in the urinary system, being the ninth most frequently diagnosed tumor. It may be classified into upper and lower urinary tract; bladder cancer (BC) constitutes 90% of the forms of UCs, with almost 613'800 new cases in 2022 (3.1% of all new cancer diagnoses) and about 220'000 deaths (2.3% of all cancer mortality).[1] This is a disease of the elderly with a median age at diagnosis of 73 years, which presents challenges due to comorbidities and poor performance status. Indeed, the survival of metastatic UC is suboptimal and cure is infrequent.[2] Risk factors include smoking, personal or family history of UC, occupational exposures, obesity, diabetes, chronic infection, or irritation of the urinary tract.[3]

Non-muscle-invasive (NMIBC) constitutes up to 75% of newly detected cases, while muscle-invasive (MIBC) and metastatic UC (mUC) form the rest. The treatment of choice of NMIBC is transurethral resection of bladder tumor (TURBT) followed by an adjuvant intravesical instillation of Bacillus Calmette Guérin (BCG) for high-risk disease.[4] BCG-unresponsive or -intolerant NMIBC may be directed to a radical cystectomy (RC),[4] while novel therapies have emerged including pembrolizumab[5] and gene therapy with Nadofaragene firadenovec, a non-replicant recombinant adenovirus delivering the deoxyribonucleic acid (DNA) of human interferon (IFN)-alfa-2b into the bladder epithelium.[6] MIBC is treated by neoadjuvant cisplatin-based combinations followed by RC.[7] Adjuvant nivolumab has recently showed efficacy in high-risk patients.[8] Very select patients who are ineligible for or refuse RC may receive a trimodality treatment with maximal TURBT, chemotherapy, and radiotherapy (RT).[7]

Approximately 10–15% of patients have a metastatic disease at diagnosis. The backbone treatment of advanced disease had been established as platinum-based chemotherapy followed by maintenance avelumab in 2020.[9] However, multiple advances have occurred since then.[10,11] The combination of gemcitabine-cisplatin and nivolumab improved survival, which led to the US Food and Drug Administration recent approval.[12] Thereafter, antibody drug conjugates (ADCs) are increasingly emerging in the treatment landscape of patients with UC. Enfortumab vedotin, a Nectin4 binding ADC delivering a tubulin toxin payload, was approved in salvage therapy settings and more recently in combination with pembrolizumab as first-line therapy.[13] Sacituzumab govitecan, a Trop2 targeting ADC that bears a topoisomerase-1 inhibitor payload, received accelerated approval in the United States for post-platinum and programmed cell death 1 (PD1)/ligand 1 (PD-L1) inhibitor treated patients with progressive disease.[14] Lastly, around 20% of advanced UC expresses a mutation or fusion of fibroblast growth factor receptor (FGFR)3.[15] A personalized therapy with erdafitinib, a pan-FGFR inhibitor, may be offered to these patients progressing after previous PD1/L1 inhibitor therapy.[16,17] Nevertheless, the prognosis of patients affected by advanced UC remains poor, with mostly incurable disease despite recent advances. In this context, adjuvant nivolumab or pembrolizumab have prolonged the disease-free survival, but most patients destined to recur will exhibit relapse.[8,18] Hence, understanding tumor biology and developing novel and tolerable agents are a high priority.

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Cancer vaccines are a promising strategy against tumors. They can be classified as preventive and therapeutic cancer vaccines. The preventive vaccines aim at preventing the infection from agents known to trigger cancer development, such as Human Papillomavirus (HPV).[19] In contrast, therapeutic vaccines are based on the concept of triggering the immune system against tumor cells therefore inducing tumor regression or eradicating potential minimal residual disease. They may be tailored to tumor-associated antigens, which are off-the-shelf vaccines, or tumor-specific, against the specific antigens present on the patient's cancer cells. The ideal tumor antigen should be widely exposed by tumor cells with limited expression on normal cells. Several means have been developed with the aim of delivering tumor antigens to T lymphocytes, such as peptides, dendritic or other antigen-presenting cells, virus or nucleic acids. Recently, efforts have been put into the development of personalized vaccines, built on the spectrum of neoantigens found in the malignant cells of each specific patient. In addition, combining vaccines with other treatments such as immune checkpoint inhibitors (ICIs), chemotherapy, or RT, may enhance the overall therapeutic effect. Although numerous preclinical and clinical studies have focused on vaccine cancers, in the clinic, only sipuleucel-T, a dendritic cell (DC)-based vaccine carrying a protein fusion of the antigen prostatic acid phosphatase and granulocyte-macrophage colony stimulating factor (GM-CSF) is available in the USA and Europe, for metastatic castration-resistant prostate cancer.[20] Despite the modest success, cancer vaccines have exhibited excellent tolerability and remain the focus of several experimental approaches aimed at harnessing their immunogenicity in conjunction with decreasing the immunosuppressive environment of tumor with ICIs. Several challenges remain in the development of cancer vaccines. First of all, a high heterogeneity between cancer cells may be identified, hindering the identification of universal vaccine targets[21] and not every antigen is able to induce a strong immune response in the host.[22] Moreover, tumors are able to downregulate antigen presentation and induce regulatory T (Treg) cells.[23] Lastly, manufacturing processes should be improved in efficiency and cost-effectiveness to have a global impact. Here, we conducted a review of the main cancer vaccines strategies, their challenges and developments, and their clinical implications in UC.

Methods

The present work is a descriptive review. In June 2024, we performed a literature search on PubMed and Embase using various combinations of the following keywords: ('peptide' or 'virus' or 'DC' or 'RNA' or 'DNA') and ('vaccine' or 'vaccine therapy') and ('urothelial cancer/carcinoma' or 'bladder cancer/carcinoma'). No restrictions were placed on the year of publication. We examined reviews, preclinical, and clinical trials, and selected the most relevant works based on their level of evidence.

Mechanism of action

The foundation of immune activation is antigen-presenting cells (APCs), particularly DCs. APCs capture antigens through phagocytosis, process and express them on major histocompatibility complex (MHC) molecules. Later, DCs migrate to draining lymph nodes and present the processed antigen epitopes through MHC I and MHC II molecules to CD8+ and CD4+ T cells, respectively[24] (see Figure 1). In order to increase lymphocytes activation, costimulatory receptors are expressed and costimulatory molecules are secreted, such as interleukin (IL)-12 and IFN-γ.[24] Immature DCs in the tumor microenvironment (TME) may be enhanced through Toll-like receptor (TLR) ligands.[25] After the presentation of antigens, T cells differentiate into effectors and long-lived memory T cells. The former amplify and migrate to TME where they induce cytotoxicity of tumor cells.[24] On the other hand, CD4+ T cells increase CD8+ T-lymphocyte proliferation and B-lymphocyte activation. B-cells produce antibodies, able to cause cellular cytotoxicity, directly or through the activation of complement.[26] Lysed tumor cells release antigens able to amplify the immune response.[26]

The backbone of the efficacy of cancer vaccination is the recognition of tumor antigens by T lymphocytes. The optimal tumor antigen should have an extensive expression on tumor cells but minimal expression on normal cells. Moreover, they should present a strong affinity to human leukocyte antigen (HLA), to be effectively presented to T cells.[27] Tumor antigens are classified in tumor-associated antigen (TAA) and tumor-specific antigen (TSA). TAAs, also defined as tumor-shared, are self-antigens, generally expressed in healthy tissues but overexpressed in tumor cells, often tissue-specific. For this reason, they are usually versatile and able to address different patients. However, since they are physiologically expressed antigens, the risk of immune-tolerance, or, on the contrary, the induction of autoimmunity, is high.[28] Differently, TSAs, also known as neoantigens, are only present on tumor cells, and arise as a consequence of a mutational process. They can be "shared neoantigens," identifiable in different kind of tumors, or "personalized neoantigens," related to a specific patient.[29] They are defined based on the mutation type leading to their transcription into antigens, such as single mutation (single nucleotide variants, insertion, deletions), fusions or rearrangements, copy number duplications or loss, transcriptional abnormalities, or microsatellite instability.[27] Inducing an immune reaction against these antigens could personalize the treatment, with limited autoimmunity priming, but tumor loss of their specific antigens may occur, nullifying the process; in conclusion not every TSA is able to establish an effective immune response.[22] The TAAs and TSAs identification is possible through performing next generation sequencing (NGS), usually whole-exome sequencing (WES), ribonucleic acid (RNA)-sequencing and confirmed by mass spectrometry techniques.[27] A neoantigen vaccine is usually composed by several different neoantigens, in order to improve its immunogenicity. Even when adequate neoantigens are identified, efforts to guarantee effective peptide procession, MHC presentation and binding and subsequent T cell recognition, should be provided. Specifically, HLA typing is a crucial step

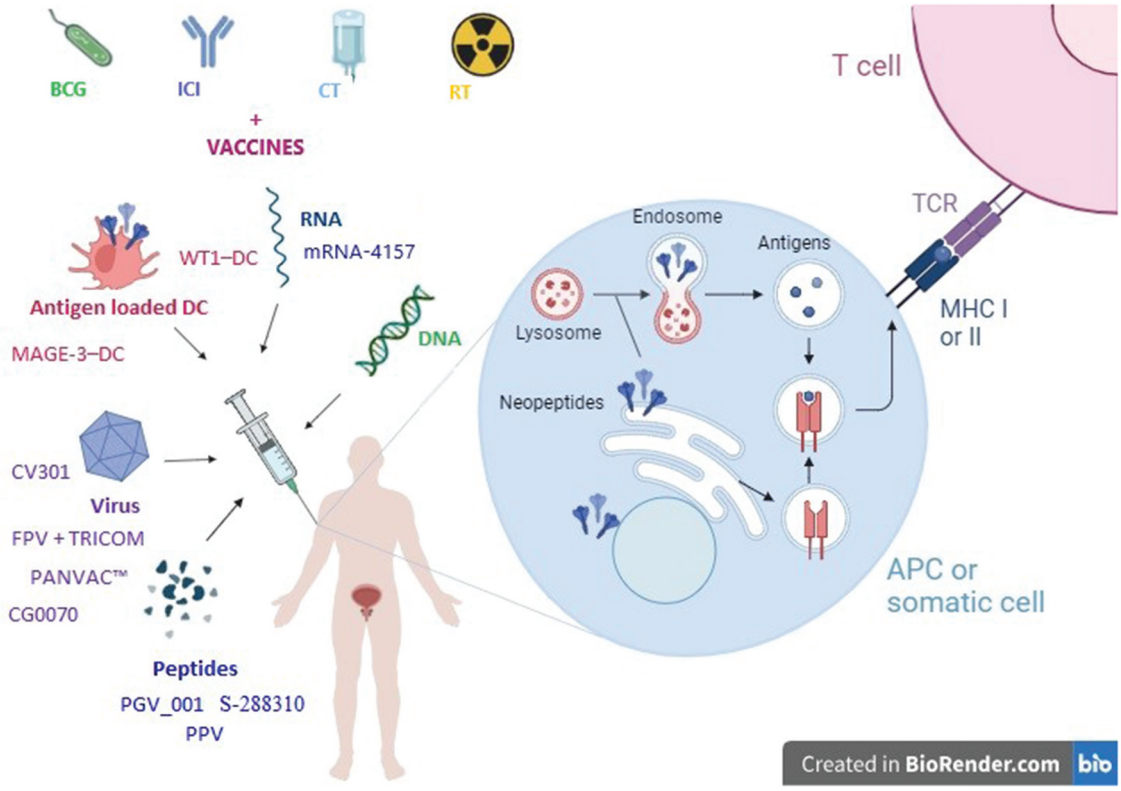

Figure 1. Approaches for the use of cancer vaccines in the treatment of urothelial carcinoma and their mechanism of action. Legend: APC = antigen-presenting cell, BCG = Bacillus Calmette-Guerin, CT = chemotherapy, DC = dendritic cell, DNA = deoxyribonucleic acid, FPV = fowlpox virus, ICI = immune checkpoint inhibitor, MAGE = melanoma-associated antigens, MHC = major histocompatibility complex, PGC = peptide-based personalized genome vaccine, PPV = personalized peptide vaccination, RNA = ribonucleic acid, RT = radiotherapy, TCR = T-cell receptor, TRICOM = three immune costimulatory molecules: B7.1, ICAM-1 LFA-3, WT1 = Wilms' tumor 1.

in the process. Consequently, several potent prediction algorithms have been developed.[30]

Cancer-based vaccines

Neoantigens may be transported and presented to T lymphocytes through several mechanisms of vaccination, based on: dendritic or other antigen-presenting cells, virus, RNA, DNA, or peptides (see Figure 1).

Administration route is also a crucial key, since different delivery methods may activate different immune-cell types. UC is a unique type of cancer, suitable for multiple administration routes beside the typical intradermal, intramuscular, subcutaneous, or intravenous, including intravesical and intratumoral. T-cells may be enhanced by intradermal and subcutaneous injection, when Langerhans and mesenchymal cells are mostly solicited,[30] therefore vaccination may require lower doses, although absorption may be variable and local skin reactions might occur. A major and prompt antibody production has been reported with intravenous injection, with a spread distribution, even though a rapid liver clearance may limit its efficacy.[31] Furthermore, systemic adverse events (AEs) are more frequent than with other routes. Intramuscular injection is convenient and avoids liver first-pass metabolism, although, taking into account the scarce immune cells in this tissue, adjuvants able to solicit an inflammatory response are usually needed.[31,32] An oral administration is noninvasive and convenient, albeit limited by gastrointestinal barriers, gastric acid pH and digestive enzymes.[31] Direct intra-tumor injection may promptly modify the TME, with limited systemic side effects showing better T cell induction, with respect to intranodal injection,[33] however not every patient has accessible sites.[34] Intravesical administration offers a unique route for NMIBC, with uncontested advantages of direct activity with high doses and limited or no systemic AEs. Conversely, its efficacy may be limited to bladder, not being feasible for advanced UC. Ultimately, combining different routes of administration may enhance immune responses.[35] Here, we review the most relevant trials investigating cancer-based vaccines for urothelial carcinoma. The phase II trials are summarized in Table 1.

Peptide-based vaccines

Peptide-based vaccines may be composed of TAAs or TSAs. At first, they were created as single-antigen short peptides, formed by <15 antigens, directly binding MHC class I and activating CD8+ response.[36] In the absence of adequate co-stimulatory signals, they were often not effective enough to stimulate a strong T cell response and lead to tolerance.[22] For this reason, synthetic long peptides (SLP) have been created. SLPs comprise about 30 antigens, that are not directly active and need to be processed by APCs and later loaded to MHC class I and II molecules, thus generating both effective CD4+ and CD8+ responses.[36] Nevertheless, despite demonstrating immune-responses, clinical trials with peptide-based vaccines have led to little efficacy, probably due to the MHC restriction of epitopes.[37]

Table 1. Phase II clinical trials evaluating cancer vaccines in patients with urothelial cancer.

Reference	Study type	Vaccine name	Vaccine type	Vaccine target	Administration route	Schedule	Setting	Population (number of patients)	Results
Non-Muscle Invasive Bladder Cancer									
Obara W, et al. Cancer Immunol Immunother. 2018	Phase II, non randomized	S288310	Peptide-based	DEPDC1 + MPHOSPH1	Subcutaneous	Combined with BCG	Untreated NMIBC	127	2-year RFS 74%
Ogasawara M, et al. Ther Apher Dial. 2018	Phase II, non randomized	NA	Dendritic cell-based	Wilms' tumor 1 + adjuvant OK-432	Intradermal	Monotherapy (2 NMIBC), combined with chemotherapy (UC, 1 NMIBC) or TKI (RCC)	NMIBC, mUC or metastatic RCC	3 + 2 + 5	NMIBC: 1 relapse at 26.8 months, 2 did not relapse with a follow-up of 84.8 months and 33.4 months mUC: ORR 0.0%
Packiam VT, et al. Urol Oncol Semin Orig Investig. 2018	Phase II, open-label	CG0070	Virus-based	GM-CSF encoding adenovirus + dodecyl matoside	Intravesical	Monotherapy	BCG-resistant NMIBC	45	Overall population: 6-month CR rate 47%, 6-month SD rate 27% Pure carcinoma in situ patients: 6-month CR rate 58%, 6-month SD rate 13% Pure Ta/T1 patients: 6-month CR rate 33%, 6-month SD rate 33%
Li R, et al. J Clin Oncol. 2022	Phase II, open-label	CG0070	Virus-based	GM-CSF encoding adenovirus + dodecyl matoside	Intravesical	Combined with pembrolizumab	BCG-resistant NMIBC	35	3-month CR rate 87.5%
Saoud R, et al. J Urol. 2021	Phase II, randomized	PANVAC™	Virus-based	Epithelial mucin 1 and CEA encoding fowlpox + vaccinia + TRICOM	Subcutaneous	Combined with BCG vs. BCG alone	BCG-resistant NMIBC	30	12-month RFS rate 44.4% (CI 24.8–79.7) vs. 42.9% (22.9–80.0), $p = .971$ Median TTR 9.9 vs. 11.7 months, $p = .77$ 12-month PFS rate 78.6% (95% CI 59.8–100) vs. 79.6% (CI 56.9–100)
Metastatic Urothelial Carcinoma									
Obara W, et al. Ann Oncol. 2017	Phase I-II, non randomized	S288310	Peptide-based	DEPDC1 + MPHOSPH1	Subcutaneous	Monotherapy	Pre-treated mUC	32	DCR 56.3% ORR 6.3% mPFS 1.9 (90% CI: 1.2–2.2) months mOS 9.4 (90% CI: 4.2–11.9) months
Suekane S, et al. Eur Urol Suppl. 2012	Phase II, non randomized	NA	Peptide-based	PPV	Subcutaneous	Monotherapy	Pre-treated mUC	25	mOS 11.3 months
Noguchi M, et al. Clin Cancer Res. 2016	Phase II, randomized, open label	NA	Peptide-based	PPV	Subcutaneous	Monotherapy vs. best supportive care	Pre-treated mUC	80	ORR 23.0% vs. 0.0% mPFS 2.0 (95% CI, 1.8–3.4) vs. 1.8 (95% CI, 1.3–2.3) months, HR 0.7, $p = .17$ mOS 7.9 (95% CI, 3.5–12.0) vs. 4.1 (95% CI, 2.8–6.9) months HR 0.58, $p = .049$
Suekane S, et al. Cancer Sci. 2017	Phase II, open label	NA	Peptide-based	PPV	Subcutaneous	Monotherapy or combined with chemotherapy	Pre-treated metastatic UTUC	48	PPV monotherapy: mOS 4.5 (95%CI, 1.7–10.1) PPV + chemotherapy: mOS 13.0 (95% CI, 5.7–17.5) months
Nishiyama T, et al. Clin Cancer Res. 2001	Phase I-II	NA	Dendritic cell-based	Melanoma Antigen – 3	Subcutaneous	Monotherapy	mUC	4	DCR 75%

(*Continued*)

Table 1. (Continued).

Reference	Study type	Vaccine name	Vaccine type	Vaccine target	Administration route	Schedule	Setting	Population (number of patients)	Results
Sonpavde GP, et al. J Clin Oncol. 2022	Phase II, single arm	CV301	Virus-based	Epithelial mucin 1 and CEA encoding fowlpox + modified vaccinia ankara + TRICOM	Subcutaneous	Combined with atezolizumab	Cohort 1: cisplatin-ineligible mUC Cohort 2: Pre-treated mUC	19 + 24	Halted for futility Cohort 1: ORR 5.3%, (90%CI 0.3–22.6) Cohort 2: ORR 8.3% (90%CI 1.5–24.0).
Matsumoto R, et al. J Clin Oncol. 2021	Phase I-II, single arm	TAS0313	Peptide-based	Three long peptides and 12 CTL epitopes	Subcutaneous.	Combined with pembrolizumab	Cohort 1: cisplatin-pre-treated mUC Cohort 2: ICI-pre-treated mUC	36 + 10	Cohort 1: ORR 33% DCR 67% mPFS 5 months 1-year OS rate 74.3% Cohort 2: DCR 50.0%

BCG = Bacillus Calmette-Guerin, CI = confidence interval, CR = complete response, DCR = disease control rate, DEPDC1 = DEP domain-containing 1, GM-CSF = granulocyte-macrophage colony stimulating factor; HR = hazard ratio, ICI = immune checkpoint inhibitor, mOS = median OS, mPFS = median PFS, MPHOSPH1 = M-phase phosphoprotein 1, mUC = metastatic UC, NA = not available, NMIBC = non-muscle invasive bladder cancer, ORR = objective response rate, OS = overall survival, PFS = progression-free survival, PPV = personalized peptide vaccination, RCC = renal cell carcinoma, RFS = relapse-free survival, SD = stable disease, TRICOM = three immune costimulatory molecules: B7.1, ICAM-1 LFA-3, TKI = tyrosine kinase inhibitors, TTF = time to recurrence, UC = urothelial cancer, UTUC = upper tract urothelial carcinoma.

To enhance their activity, several improvements have been proposed. For instance, preclinical models showed how fusion with carriers proteins, i.e. keyhole limpet hemocyanin (KLH) or heat shock proteins (HSP), may enhance APC's activation.[38] Moreover, virus-like particles (VLP) or bacteria may be an effective platform for peptide-based vaccines, due to their ability to induce APCs activation and transportation to lymph nodes.[30,39] In addition, modifications of the peptide structure, that may include cytotoxic T lymphocytes (CTL) and T helper peptides, could improve responses.[40] Finally, adjuvants have been tested to reach the purpose, since they are able to enhance the leukocytes' recruitment, expansion and activation and their later migration to tumor sites.[41] The most evaluated adjuvants are AS04, an aluminum salt associated with a detoxified form of lipopolysaccharide, incomplete Freund's adjuvant (IFA),[42] molecules targeting TLRs and liponanomaterials.[30] Recently, data supported the use of gold nanoparticles as vaccine adjuvants in preclinical mouse models. Gold nanoparticles loaded with the bacterial peptide 91–99 of the listeriolysin O toxin (GNP-LLO91–99 nanovaccines), showed a systemic T helper 1-type immune response in TME: increased percentages of CD4+ and CD8+ T cells, B cells, DCs and reduced levels of myeloid-derived suppressor cells and suppressor T cells.[43] In closing, cytokines given as adjuvants, such as IL-2, GM-CSF or IFN, are gaining attention for their immunomodulatory properties.[44] Peptide-based vaccines are versatile, easy to engineer with a low probability of AEs and contamination; however, the adjuvant necessity and the MHC restriction limit its immunogenicity.[30,37,41]

Several trials have demonstrated promising results with improved survival rates and reduced tumor recurrence in patients affected by UC, treated with peptide-based vaccines. Here, we summarized the most relevant ones. A subcutaneous peptide-based vaccine, S-288310, was developed uniting two tumor-associated antigens, identified through expression profile analysis and singularly tested to be active in patients with UC: DEP domain-containing (DEPDC1) and M-phase phosphoprotein 1 (MPHOSPH1). In a phase I-II study, S-288310 demonstrated tolerability and activity in inducing peptide-specific CTL, in 32 patients with UC, showing a disease control rate (DCR) of 56.3% and a 2 years overall survival (OS) rate of 32%.[45] A subsequent non-randomized phase II trial associated subcutaneous MPHOSPH1- and DEPDC1-peptide vaccine to intravesical BCG therapy, showing a remarkable 2-year relapse-free survival (RFS) of 74%, with peptide-specific cytotoxic T lymphocyte response of 75.8–77.5%[46] (see Table 1). These noteworthy results, with 2-year prolonged response, deserve further and broader evaluations to be validated.

CDX-1307, an intradermal peptide vaccine composed by a human monoclonal antibody against the mannose receptor fused to the human chorionic gonadotropin-β chain (β-HCG), a frequently expressed tumor antigen, showed safety in a phase I study; unfortunately the phase II trial in patients with UC was stopped early because of slow enrollment.[47]

A peptide-based vaccine against the NY-ESO-1 tumor antigen, combined with a novel delivery system, cholesteryl pullulan, together with the adjuvant MIS416, activating NOD-2 and TLR9 pathways, was tested with subcutaneous administration in a phase II trial on 16 patients, four of whom affected by UC. Although patients with prostate cancer exhibited tumor response from the treatment, the vaccine did not show activity in UC.[48]

Another interesting regimen of vaccination was based on the subcutaneous administration of the most reactive peptides chosen from a large cohort of candidate antigens: personalized peptide vaccination (PPV).[49] In a phase I trial, a population of 10 pre-treated patients with UC received PPV, achieving one complete response (CR), one partial response (PR), two stable disease (SD) and six progressive disease (PD), with a median OS of 8.9 months, and it is noteworthy that duration of response extended to 24 months in the responders.[49] Its safety and activity were confirmed in a phase II trial on 25 pre-treated patients with UC, reaching a median OS of 11.3 months[50] (see Table 1). PPV was later compared to best supportive care in a randomized phase II study in 80 cisplatin-refractory patients affected by UC. PPV did not show benefit on progression-free survival (PFS), but appeared to be safe and to prolong OS with a hazard ratio (HR) of 0.58, $p = .049$[51] (see Table 1). Successively, the PPV strategy was investigated in 48 pre-treated patients affected by metastatic upper tract urothelial cancer (UTUC), in a single arm phase II trial. PPV was well tolerated without severe AEs, and induced a specific T-cells response in 46% of the patients, related to a longer OS with respect to patients with negative response (HR 0.37; 95%CI, 0.16–0.85; $p = .019$). Median OS was 4.5 months, but remarkably it was extended to 13 months in patients receiving combined PPV and chemotherapy (60% of patients)[52] (see Table 1). PPV is one of the most promising peptide-based vaccine strategies; the benefit was observed for only a minority of patients, but the encouraging responses were enduring. Alternative forms of personalized neoantigens peptide-vaccines are being evaluated (see Table 2). Further analyses are needed to identify the subgroup of patients who may mainly benefit.

Survivin-2B80–88 is a HLA-A24 restricted antigenic apoptosis inhibitor, recognized by CTLs. After demonstrating its safety and activity in a phase I trial,[53] the subcutaneous vaccine was tested in association with IFA and IFNα in 12 patients with metastatic UC, achieving a significantly longer OS with respect to a control group ($p = .0009$).[42] Subsequent evaluations are likely anticipated, ideally confirming these results.

Promising results were reported combining peptide-based vaccines and BCG. The association of NY-ESO-1 intradermal peptide-based vaccine with GM-CSF and BCG, demonstrated safety and ability to induce the generation of antibodies and a predominant CD4+ T-cell response in a cohort of six patients with UC.[54] In a non-randomized phase I open-label exploratory study, a recombinant melanoma-associated antigens (MAGE)-A3 protein vaccine was tested with the adjuvant AS15, alone or added to BCG instillations, in 24 patients with NMIBC. The treatment was safe and lead to the identification in blood of vaccine-specific T cells, in half of the patients, although no oncological outcomes were available.[55] Unfortunately the following phase II trial, the MAGNOLIA

Table 2. Selected ongoing clinical trials evaluating cancer vaccines on patients affected by urothelial cancer (2016–2024).

Clinicaltrials.gov Registration number	Study type	Vaccine name	Vaccine target	Regime	Population	Setting	Enrollment	Endpoints	State
NMIBC									
Peptide-based vaccines									
NCT05843448	Phase I	IO102-IO103	Indoleamine-pyrrole 2,3-dioxygenase + PD-L1	Combined with pembrolizumab	NMIBC	BCG unresponsive or intolerant disease	30	AEs, CR, event-free survival, cystectomy-free survival, DOR	Active, recruiting
NCT04106115	Phase Ib/II, multicenter	S-488210/S-488211	5-peptide + montanide adjuvant	Combined with durvalumab	NMIBC	BCG unresponsive or intolerant disease	64	DLT, DFS	Active, recruiting
Virus-based vaccines									
NCT04387461	Phase II, single arm, open label	CG007 + n-dodecyl-B-D-maltoside adjuvant	Adenovirus, tumor-selective GM-CSF synthesis	Combined with pembrolizumab	NMIBC	BCG-unresponsive disease	35	CRR, AEs, DOR, OS, PFS	Active, not recruiting
NCT04452591	Phase III, single arm, open label	CG007 + n-dodecyl-B-D-maltoside adjuvant	Adenovirus, tumor-selective GM-CSF synthesis	Monotherapy	NMIBC	BCG-unresponsive disease	110	CRR, DOR, PFS, TTP, AEs	Active, recruiting
MIBC									
Peptide-based vaccines									
NCT03359239	Proof-of-concept study, single arm	PGV001	Multipeptide personalized neoantigen + Poly-ICLC adjuvant	Combined with atezolizumab	UC	– Adjuvant therapy after surgery in localized disease - Second-line for cisplatin-resistant disease	10	ORR, DOR, TTP, OS	Active, recruiting
mRNA-based vaccines									
NCT03313778	Phase I, open label, non-randomized	mRNA-4157	Personalized neoantigens	Monotherapy or combined with pembrolizumab	Solid tumors, including UC: resected localized disease or advanced or metastatic disease	– Adjuvant monotherapy for resected disease - First-line combined with ICI in metastatic disease	108	AEs, DLT, change from baseline of biomarker levels, Antigen-specific T-cell responses in peripheral blood, ORR, DOR, PFS, OS	Active, not recruiting
NCT06305767	Phase II, randomized, double-blind, placebo-controlled, multicenter	V940 (mRNA-4157)	Personalized neoantigens	Combined with pembrolizumab *vs* pembrolizumab plus placebo	UC	Adjuvant for high-risk resected disease	200	DFS, OS, DMFS, AEs	Active, recruiting
Metastatic disease									
Peptide-based vaccines									
NCT05077709	Phase II, multiarm (basket)	IO102-IO103	Indoleamine-pyrrole 2,3-dioxygenase + PD-L1	Combined with pembrolizumab	Metastatic NSCLC, metastatic SCCHN, metastatic BC	First-line	90	ORR, PFS, DOR, CRR, DCR, OS, TTR, safety	Active, recruiting

(*Continued*)

Table 2. (Continued).

Clinicaltrials.gov Registration number	Study type	Vaccine name	Vaccine target	Regime	Population	Setting	Enrollment	Endpoints	State
NCT03715985	Phase I-II	NeoPepVac	5–15 personalized neoantigens + CAF09b adjuvant	Combined with ICIs	Metastatic UC, NSCLC, melanoma	NA	25	AEs, OS, ORR, PFS	Active, not recruiting
NCT05269381	Phase I	NeoVCA	Personalized neoantigens + GM-CSF	Combined with cyclophosphamide and pembrolizumab	Advanced or metastatic solid tumors, including UC	NA	36	AEs, feasibility, ORR	Active, recruiting
NCT03639714	Phase I-II	GRT-C901 and GRT-R902	Personalized neoantigens	Combined with nivolumab and ipilimumab	Metastatic NSCLC, colon cancer, UC, gastroesophageal cancer	NA	29	AEs, DLT, ORR, DOR, CBR, PFS, OS	Completed
NCT04864379	Phase I, open label	iNeo-Vac-P01	Personalized neoantigens + GM-CSF	Combined with ICI and radio-frequency ablation	Advanced solid tumors, including UC	NA	30	AEs, ORR, CD4/CD8 T lymphocyte subsets, IFN-γ T cells responses, OSS, PFS, peripheral blood T cell receptor sequencing analysis	Active, recruiting
NCT03502785	Phase I/IIA, open label, multicenter	INO 5401 + INO 9012	- INO 5401: Wilms tumor gene-1 antigene, PSMA, human telomerase reverse transcriptase - INO 9012: IL-12	Combined with atezolizumab	Advanced or metastatic UC	- First-line for cisplatin-ineligible disease - Second-line for cisplatin-resistant disease	35	AEs, Antigen-Specific Cellular Immune Response, ORR DOR, OS, PFS	Active, not recruiting
NCT03359239	Proof-of-concept study, single arm	PGV001	Multipeptide personalized neoantigen + Poly-ICLC adjuvant	Combined with atezolizumab	UC	- Second-line for cisplatin-resistant disease – Adjuvant therapy after surgery in localized disease	10	ORR, DOR, TTP, OS	Active, recruiting
DC-based vaccines									
NCT05749627	Single group assignment	Neoantigen-based DC immune preparation or neoantigen peptide vaccine	Personalized tumor neoantigen DC or peptide	Monotherapy	Advanced or metastatic solid tumors, including UC	Second-line	20	PFS, ORR, tumor markers, OS, DCR, tumor imaging, changes in peripheral blood cytokines, ECOG-PS.	Active, recruiting
Virus-based vaccines									
NCT02432963	Phase I	MVA-p53	AnkarA virus expressing p53	Combined with pembrolizumab	Advanced or metastatic solid tumors, including UC	Second-line	11	AEs	Active, not recruiting
NCT04610671	Phase I	CG007 + n-dodecyl-B-D-maltoside adjuvant	Adenovirus, tumor-selective GM-CSF synthesis	Combined with nivolumab	BC	Cisplatin-ineligible disease	21	AEs, Change in PD-L1 expression on tumor and immune cells, change in intraepithelial CD8+ T cell density	Active, not recruiting
mRNA-based vaccines									
NCT03739931	Phase I, open label, multicenter	mRNA-2752	Human OX40L, IL-23, IL-36γ	Monotherapy or combined with durvalumab	Advanced or metastatic solid tumors, including UC	- First-line in platinum-ineligible disease - Later lines in platinum-resistant disease	264	DLTs, AEs, ORR, Cmax	Active, recruiting

(*Continued*)

Table 2. (Continued).

Clinicaltrials.gov Registration number	Study type	Vaccine name	Vaccine target	Regime	Population	Setting	Enrollment	Endpoints	State
NCT03289962	Phase Ia/Ib, openlabel, multicenter	Autogene Cevumeran (RO7198457)	Personalized neoantigens	Monotherapy or combined with atezolizumab	Advanced or metastatic solid tumors, including UC	First-line in standard therapy – ineligible – Later lines in relapsed disease	272	DLT, AEs, CRR, PRR, DOR, ORR, OS, PFS	Active, not recruiting
NCT03313778	Phase I, open label, non-randomized	mRNA-4157	Personalized neoantigens	Monotherapy or combined with pembrolizumab	Solid tumors, including UC: resected localized disease or advanced or metastatic disease	– Adjuvant monotherapy for resected disease - First-line combined with ICI in metastatic disease	108	AEs, DLT, change from baseline of biomarker levels, Antigen-specific T-cell responses in peripheral blood, ORR, DOR, PFS, OS	Active, not recruiting

AEs = adverse events, BC = bladder cancer, BCG = Bacillus Calmette-Guerin, CBR = Clinical benefit rate, Cmax = maximum observed concentration, CRR = complete response rate, CT = chemotherapy, DC = dendritic cell, DCR = disease control rate, DFS = disease-free survival, DLTs = dose limiting toxicities, DMFS = distant metastasis-free survival, DOR = duration of response, ECOG-PS = Eastern Cooperative Oncology Group – performance status, ICIs = immune checkpoint inhibitors, IL = interleukin, NA = not available, NMIBC = non-muscle invasive bladder cancer, NSCLC = non-small cell lung cancer, ORR = objective response rate, OS = overall survival, PD-L1 = programmed cell death ligand 1, PFS = progression-free survival, PRR = partial response rate, PSMA = prostate-specific membrane antigen, SCCHN = squamous cell carcinoma of head or neck, TTP = time to progression, TTR = time to response, UC = urothelial cancer.

trial, that was aiming to assess the activity of adjuvant recombinant MAGE-A3 and AS15 after RC for MIBC, was stopped prematurely because of negative outcomes in parallel phase III trials in other cancer types.[56]

Apart from NGS, neoantigens' identification is feasible through platforms such as ATLAS™, an autologous immune assay that uses ex vivo screening of all patient-specific mutations to select neoantigens. For instance, this technique showed promising results identifying up to 20 TSAs, leading to a subcutaneous peptide vaccine, GEN-009, that was successfully tested in combination with PD1 inhibition in a phase 1 trial in patients affected by solid tumors, including UC. Preliminarily, broad neoantigen-specific immune responses and epitope spreading was observed.[57]

Recently, a subcutaneous and intradermal peptide-based personalized genome vaccine (PGV_001) platform was developed employing the OpenVax computational pipeline applied to genomic and transcriptomic sequencing of tumor aimed at generating personalized neoantigen vaccines.[58] In patients with various solid tumors, including UC, its safety and efficacy as a monotherapy or in combination with atezolizumab in the adjuvant or metastatic setting was evaluated, finding a neoantigen-specific immune response in all patients.[59,60] Further trials are ongoing (see Table 2).

These trials and additional ones in other malignancies are demonstrating the induction of persistent memory T cell responses and epitopes, spreading showcase the promise of personalized neoantigen-based peptide vaccines patients.[59,60]

Cell-based vaccines

A cellular vaccine is a method of delivering TAAs, based on tumor cells or patient-derived immune cells loaded with antigens. Different types of whole cells or fragments have been tested, such as tumor cells and modified autologous cancer or immune cells. Tumor cell vaccines are killed tumor cells injected into the patient in order to stimulate a response against numerous TAAs. Nevertheless, their immunogenicity is limited, probably due to the concurrence of immunosuppressor factors.[61] Various ways of improving it have been tried, facilitating cell death, such as adding IFN genes-activating nanoparticles,[30] or utilizing emulsions, liposomes, or polymeric particulate as carriers.[62] Moreover, cells can be engineered to secrete co-stimulatory molecules or receptors, such as IL-21, IL-7 and chemokine receptor-7, to enhance lymphoid-homing and antigen's presentation and generate a more efficacious immune response.[63,64]

Usually, the immune cell vaccines of choice are DCs-based. Monocytes and hematopoietic stem cells are isolated from patient's peripheral blood or from umbilical cord blood, they are activated in monocyte-derived DCs, loaded with TAAs and injected into the patient, intravenously, subcutaneously or intradermally. The loading may be by direct pulsing,[65] messenger RNA (mRNA) electroporation,[66] viral transduction,[67] fusion with tumor cells[68] or incubation with tumor lysate.[69] DC vaccines are able to present TAAs to T cells and may also be able to transfer antigens to endogenous cross-presenting DCs.[70] Their efficacy may depend on the DC subset. In fact, it has been shown that conventional DCs and myeloid/plasmacytoid DCs act on different T-activation pathways,[71] and their combined use may improve the vaccine's immunogenicity.[72]

Due to the interactions between DCs and the different types of T cells in lymph nodes involved at different sites, the administration route may influence the type of immune response and combining different routes of DC administration may enhance immune responses.[35] Several potential adjuvants have been evaluated to improve DC-based vaccines efficacy. The most widely promoted have been IL-2,[73] GM-CSF,[47] IL-4-secreting autologous fibroblasts,[74] and KLH.[75]

An emerging strategy of DCs vaccination is in situ vaccine. The rationale is based on an attempt of enhancing DCs intratumoral infiltration and activation causing a local extensive antigen release. In detail, it consists of injection of FMS-like tyrosine kinase 3 ligand and TLR agonist into the tumor lesion, aimed at causing infiltration of DCs in the tumor and their activation, respectively; a local irradiation is provided to generate the TAAs release. A phase I trial on patients affected by lymphoma reported promising anti-tumor T cell responses and cancer remission.[76]

So far, DCs have had the greatest success in efficacy, among the different types of cancer vaccines, being safe and able to generate strong immune responses, although they may be limited by the complexity and the cost of the implementation process, since the vaccine needs to be customized and engineered specifically for each patient.[77] In order to overcome this limit, recently DCs have been developed from induced pluripotent stem cells, with promising results.[78]

Described below are the most interesting clinical trials on cell-based cancer vaccines.

A phase I-II trial, assessed a DCs-based vaccine loaded with Wilms' tumor 1 (WT1), in association with the adjuvant OK-432, a penicillin-killed and lyophilized preparation of Streptococcus pyogenes, able to enhance DC's activity through the TLR-4 link.[65] Patients affected by advanced UC ($n = 2$), NMIBC ($n = 3$), or renal cell carcinoma (RCC) ($n = 5$) received the adjuvant and the intradermal vaccine in addition to standard of care therapy, either chemotherapy or tyrosine kinase inhibitors (TKI). The treatment was tolerable and active. Seventy percent of patients with RCC reached SD, no patient with advanced UC had benefits, although all three patients with NMIBC achieved long-term RFS, two of whom avoiding cystectomy (RFS of the three patients: 26.8, 84.8, and 33.4 months after the initiation of DC)[65] (see Table 1). Albeit limited by the small sample size, these results are promising and hopefully will be validated in additional and wider trials in patients with NMIBC.

A DC-based intradermal vaccine loaded with human epidermal growth factor receptor 2 (HER-2) was developed. DCs were transduced with an adenovirus targeting HER-2.[67] A phase I study was held, evaluating 21 patients with advanced pre-treated UC expressing HER-2. Preliminary results showed safety and promising activity with a clinical benefit rate of 33.3%.[79]

The efficacy of a subcutaneous DC-vaccine loaded with MAGE-3 was evaluated in preclinical studies[80] and in a phase I/II trial on four patients with advanced UC. Three

PR were noted, without significant toxicity[81] (see Table 1). To our knowledge, no subsequent evaluations are currently ongoing, hopefully further trials will affirm these data.

A DC-based intradermal or intracutaneous vaccine pulsed with CDX-1307 was evaluated in a phase I trial in combination with several adjuvants: GM-CSF, the TLR3 agonist Poly-ICLC and/or the TLR7/8 agonist resiquimod. The experimental treatment was well tolerated, generating a significant hCG-β–specific cellular and promising results.[47] Unfortunately, a randomized phase II trial on patients with newly diagnosed, resectable UC, was ended early due to scarce accrual.[82]

To our knowledge, the only allogeneic whole cell intradermal vaccine being tested in UC is Vesigenurtacel-L. It uses HSP technology to chaperone TSAs for presentation to T-cells. A phase I-II trial assessed Vesigenurtacel-L as an adjuvant treatment for 10 patients affected by NMIBC, considered at high risk or relapse (see Table 1). The 1-year RFS rate was 70%. Unluckily, the following phase II trial comparing Vesigenurtacel-L in combination with BCG *vs* BCG alone, was terminated early because of accrual inability due to evolution of the treatment landscape.[83] Allogeneic natural killer (NK) cell-based vaccines and chimeric antigen receptor (CAR)-T cell therapies are being vigorously developed for solid tumors but have not been evaluated in UC yet.[84]

Virus-based vaccines

The virus-based vaccine is composed of a virus whose encoded genes are modified to include TAA sequences. Viruses have a high efficiency of cell infection and replication in the cytoplasm. They are able to transport a large amount of recombinant genetic material without losing infectivity and may activate both cellular and humoral immune responses.[26] In addition, oncolytic virus can directly lyse the tumor, enhancing antigens and virus release.[85] The virus may be delivered in live attenuated or inactivated, although, more frequently, VLP are used.[39] The most used virus species are adenovirus,[86] poxvirus,[87] HSV,[88] measles,[89] reovirus,[90] and coxsackievirus.[91] Particularly, adenovirus is considered one of the best vectors, because of its stability, its broad tropism, the facility of its production and the manipulability of its genic material.[92] As well, poxviruses are able to carry large antigens and have a strong immunogenicity mediated by TLR-dependent and -independent cytokines.[93] To reduce the replication in normal cells viruses are usually genetically modified to be tumor cell selective.[94]

A major limitation of virus-based vaccines is the host's natural immune response, which is able to neutralize the viral vector. In order to avoid this effect, different types of viral vectors with the same tumor antigen may be sequential injected.[94] In addition, the TME inhibitory pathways may limit efficacy; hence various strategies have been proposed. First of all, combining the vaccine with ICI,[95] RT,[96] or chemotherapy[97] showed promising results. Secondly, other promising approaches include targeting immunosuppressor molecules such as yes-associated protein, a coactivator of the immunosuppressive environment secreted by Tregs,[98] or transforming growth factor (TGF)-β.[99] In conclusion, virus's genetic code may be modified with immune-regulating genes, such as human mucin-1, IL-12, and GM-CSF.[86]

We now summarize the most relevant results of trials on virus-based vaccines in patients with UC.

An adenovirus-based vaccine encoding for GM-CSF, CG0070, was developed.[86] It is an oncolytic virus selectively replicating in retinoblastoma-deficient cells of UC. Moreover, the transcription of GM-CSF is controlled by the E2F–1 promoter, selectively active in UC cells with a retinoblastoma pathway defect. Therefore, the GM-CSF production is tumor-selective. In a phase I trial, the BOND trial, intravesical CG0070 was safe and active with a CR rate of 48.6%.[86] In the following phase II trial, BONDII, CG0070 was administered intravesically, after dodecyl maltoside, an agent used to improve transduction, on 45 patients with NMIBC relapsed after BCG. It is noteworthy that the 6-month CR rate was 47% in the overall population, 58% in patients with pure carcinoma in situ (CIS), 33% in pure Ta/T1 forms and 50% in patients with both[100] (see Table 1). This promising results led to the design of a phase II trial in patients with NMIBC, that tested CG0070 associated with pembrolizumab, with preliminary interesting results, with a durable CR rate of 87.5%[101] (see Table 1). This encouraging strategy is being evaluated in further ongoing trials (see Table 2).

A Coxsackievirus A21 (CVA21)-based vaccine, targeting an intercellular adhesion molecule-1 (ICAM-1), was evaluated in a phase I trial, the CANON trial, on 15 patients with NMIBC; CVA21 was administered intravesically alone or in combination with mitomycin-C, prior to surgery. The treatment was well tolerated and active, with 1 CR and evidence of marked inflammatory changes in tissue biopsies, toward a more "immunological hot."[91] Intravenous CVA21-based vaccine was tested in combination with pembrolizumab in a phase I-II on patients with metastatic non-small cell lung cancer (NSCLC) or UC. The 43 patients affected by UC showed an objective response rate (ORR) of 20%, similar to the efficacy of pembrolizumab alone[102] (see Table 1). Consequently, this strategy was not subsequently investigated in patients with UC.

Following the positive results of the vaccination with vaccinia virus in patients with melanoma metastases,[103] a phase I trial was held, on four patients with NMIBC. They received a neoadjuvant treatment with intravesically Dryvax vaccinia virus before RC. The histological report described significant mucosal and submucosal inflammatory infiltration and evidence of viral infection. The treatment showed no severe systemic side effects. At a median follow-up of 4 years, three patients out of four were free of recurrence.[104] Expectantly, deeper and broader trials will probably assess the vaccine, with the hope of confirming these encouraging findings.

TRICOM is the combination of three immune costimulatory molecules: B7.1, ICAM-1, and leukocyte function-associated antigen-3 (LFA-3). It has been evaluated in multiple trials. A virus-based vaccine encoding GM-CSF or TRICOM, using the viral vector fowlpox (FPV), a recombinant poxvirus, was administered intravesically in 20 patients affected by BCG unresponsive NMIBC, scheduled for surgery. The histological reports of the cystectomies showed proof of effective infection/transfection. The treatment was safe and able to generate

immune responses, testified by the detection of serum antibodies.[87]

PANVAC™ is a vaccine composed by an association of two viral vectors, vaccinia and FPV, encoding epithelial mucin 1 (MUC1) and carcinoembryonic antigen (CEA), administered with TRICOM. In a randomized phase II trial, subcutaneous PANVAC™ in association with BCG was compared to BCG alone, in 30 patients affected by NMIBC relapsed after BCG. The treatment was safe, with comparable outcomes to those with BCG alone[105] (see Table 1). A second generation of PANVAC, CV301, was then developed. CV301 contains two recombinant poxviruses, Modified Vaccinia Ankara (MVA), used for priming, and FPV, for booster doses; the viruses encode CEA and MUC1, along with TRICOM. The association of CV301 and ICI showed potential activity in a phase I trial[106]; unfortunately the following phase II trial testing the combination of subcutaneous CV301 and intravenous atezolizumab on 43 cisplatin-refractory or cisplatin-unfit patients with advanced UC, was halted at the interim analysis for poor efficacy[95] (see Table 1).

Nucleic acids-based vaccines

The rationale of generating a vaccine based on nucleic acids, lies on the ubiquity and sharing of the transcriptional and translational systems. The host is able to translate the information encoded into the nucleic acids-based vaccine generating antigens able to induce immune responses. Both mRNA and DNA vaccines have been developed.

mRNA-based vaccines

mRNA is formed by an exogenous plasmid which is translated directly inside the cytoplasm, instantly generating peptides. The TAAs are then expressed by MHC molecules to APCs.[26] mRNA vaccines usually contain several TAAs able to induce a wide cellular immunity.[26] Humoral immunity is frequently increased by the co-administration of adjuvants.[107] Moreover mRNA itself has immunogenic features.[108]

mRNA-based vaccines are efficient, easy to produce and safe, without the risk of insertion in the host's genome, although the molecule is not very stable and the delivery efficiency may be challenging.[109] In addition, when the required mRNA sequence information is determined, rapid and large-scale production can be achieved. Ultimately, the molecule is intracellular degraded by natural pathways.[109]

Two types of mRNA-based vaccines exist: non-replicating mRNA and self-amplifying RNA (SAM). The structure of the former comprises 7-methylguanosine 5′ cap, 5'-untranslated region, open reading frame (OFR), 3'-untranslated region, and 3'poly(A) tail.[110,111] SAMs contain two OFRs, one encoding TAAs and one including genes necessary for viral replication and amplification, thus producing numerous copies of TAAs.[112] Both are complexed with protamine, able to link with TLR and increase immune response.[113]

SAM-based vaccines on cancer have not yet reached trials on human, non-replicating mRNA-vaccines are the most studied. Both types of mRNA-based vaccines are synthesized through in vitro transcription, a method that uses bacteriophage RNA polymerase and DNA template.[114] This method is also able to enhance innate immunity due to short RNA contaminants, with consequent high levels of IFN secretion; the subsequent purification process by high-performance liquid chromatography or cellulose, decreases the excessive immune activation.[115] New production techniques enhancing stability and nanotechnologies improving delivery, together with acting on the untranslated regions of mRNA, are the key to increasing vaccine efficacy. For instance, crucial elements include regulating the GC content, modifying the transcript with alternative nucleotides,[116] correcting hairpin loops, replacing rare codons[117] and encapsulating mRNA into positively charged liposomal particles.[30,118] Moreover, epigenomic modifications of post-transcriptional RNA may regulate translation, efficiency, and immunogenicity.[119] 5' cap may be engineered to link translation factors[120] and the poly(A) sequence is crucial to stability, translation start, and molecule degradation.[121] To conclude, the liponanoparticles platform can be induced to target specific cells.[122]

mRNA-4157, a lipid-encapsulated personalized mRNA-based intramuscular vaccine that codes for up to 34 neoantigens, was assessed in a phase I dose-escalation trial enrolling patients affected by various solid tumors. The vaccine was administered alone as an adjuvant therapy ($n = 16$) or in combination with pembrolizumab ($n = 63$), in patients with advanced disease. The treatment showed no grade 3 AEs. At a median follow-up of 8 months, 92% of patients who received adjuvant therapy was free from recurrence. Whereas among the 20 patients who were treated with the combination (6 UC), 3 CR and 2 PR were reported. The mRNA-4157-based vaccine showed good anti-tumor activity.[122] Adjuvant intramuscular mRNA-4157 plus intravenous pembrolizumab prolonged RFS versus pembrolizumab monotherapy (HR for recurrence or death, 0.561 [95% CI 0.309–1.017]; two-sided $p = .053$) in a randomized phase II trial of 157 patients with resected high-risk melanoma. The safety profile was excellent with grade ≥3 treatment-related AE in 25% of patients in the combination group and 18% of patients in the monotherapy group, with no mRNA-4157-related grade 4–5 events and similar rates of immune-mediated adverse events.[123] These promising data have led to a phase III trial ongoing in the melanoma setting (NCT05933577). A randomized phase II trial (InterPath-005, NCT06305767) is evaluating adjuvant mRNA-4157 combined to pembrolizumab in high-risk resected UC (see Table 2). Thus, the customized mRNA-based neoantigen platform appears highly promising preliminarily.

DNA-based vaccines

DNA-based vaccines derive from bacterial plasmids engineered to encode TAAs.[124] They are more stable and durable in the human body than mRNA. The DNA plasmid needs to enter inside the cell nucleus to be transcripted and translated into peptides; from a single DNA plasmid numerous mRNA copies and peptides may derive. The vaccine may be activated inside a somatic cell, where peptides may be directly presented by MHC-I, or they may be released and subsequently processed by APCs. The double-stranded DNA structure is also

able to directly improve responses, activating innate immunity.[125] In addition, DNA may be encoded inside APCs, through intradermal delivery. Thus, DNA-based vaccines are able to activate both humoral and cellular immune responses.[126] Moreover, they may prime the direct activation of signaling pathways leading to GM-CSF and cytokines production,[30] or they may be modified to express immune agonists in the form of CpG motifs, polymers, liposomes, or small molecules.[127] Nano-carriers protect DNA vaccines from degradation, they may be based on lipids, proteins, inorganic materials, or polymeric nano-carriers.[128] DNA-based vaccines are selective and safe. The main concern is the rare but potential risk of DNA insertion into the genome, with the consequent possible activation of oncogenes.[129]

In order to improve the DNA-based vaccine efficacy, powerful promoter sequences may be added. The Kozak sequence[130] and cytomegalovirus (CMV) promoters[131] were proven to be effective. Multi-antigens DNA vaccines are constructed to diversify the responses,[132] while nanodevice vaccines are able to assemble antigen and adjuvants inside tumor cells.[30] In order to ease the delivery through extra and intracellular barriers, electroporation, gene gun, and sonoporation strategies have been developed.[30] Ultimately, chimeric antigens, such as xenoantigens, including homologous and heterologous antigens, are able to bypass immune tolerance.[133]

A DNA vaccine (pTVG-HP [MVI-816]) encoding prostatic acid phosphatase (PAP) appeared to improve outcomes in a subgroup of non-metastatic prostate cancer with rapidly progressive disease.[134] Additional trials using pTVG-HP in combination with PD-1 blockade are under way.

To our knowledge, there are no data on DNA-based vaccines trials focused on patients with UC. Here, we reported the most promising developments from preclinical studies. It has been proved that antisense oligonucleotide-based therapy encoding TGF-beta may be able to arrest tumor cells growth in vitro and in UC mouse models.[135] A recombinant BCG DNA-based vaccine proved the ability of generating immune responses, CR and prolonging survival in preclinical models of UC.[136] The association of BCG DNA-based and murine IL-12-based vaccines showed promising preclinical activity as well.[137]

Histone deacetylases (HDAC) are enzymes that catalyze the removal of acetyl groups from histone, inhibiting DNA transcription. Adding an HDAC inhibitor could improve DNA-based vaccine efficacy. The combination of an HDAC inhibitor with a CMV promoter driven-DNA vaccine against HER-2 was able to increase lymphocyte infiltration and specific CTL enhancing the antitumor effects.[131] DNA-based vaccines encoding Mycobacterium tuberculosis's antigens Ag85A and Ag85B showed anti-tumor effects in UC mice models.[138]

A DNA-based vaccine encoding Flk-1 extracellular domain and the complement 3d component was developed and assessed in UC mice models, with tumor growth inhibition and survival improvements.[139] The combination of a tumor cell-based vaccine secreting IL-2, IL-4 and GM-CSF and a DNA vaccine against HER-2 showed antitumor activity and an increase of CD4+ T lymphocytes and anti-HER-2 antibodies, in UC mice models.[140]

Combination strategies

The promising results of cancer vaccines appear to be related to a limited group of patients. Several mechanisms of resistance have been pointed out. Firstly, TAAs expression varies in the tissue because of tumor heterogeneity. The immunogenicity of TAAs may be not effective enough to induce a strong immune response. Moreover, the tumor may generate immune escape strategies.[141] In detail the prolonged exposure to antigens lead to the increase of inhibitory molecules on T-cells, such as programmed cell death protein 1, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and lymphocyte-activation gene (LAG)-3. This leads to T-cells exhaustion.[141] Based on these findings, the combination of cancer vaccination and immune-checkpoint inhibitors (ICIs) may overcome resistance mechanisms.[142] In addition, cancer vaccines seem to increase TME susceptibility to ICIs.[143] Other combination strategies include treatment with chemotherapy, RT or CAR-T cell therapy. Chemotherapy may enhance responses to cancer vaccines increasing TAAs release, reducing or weakening Treg and modulating DCs and T cells activity.[30] Occasionally, a systemic response after stereotactic RT has been reported, in addition to local response. This finding, defined abscopal effect, firstly suggested a relationship between response to RT and host's immune system. Subsequent research confirmed the RT ability of enhancing immune activation, to the point of being considered as an in situ vaccine.[144] Additionally, RT is able to improve cytokines release, APC activity and MHC expression.[145] Therefore, the combination of cancer vaccines and RT was tested in preclinical models, showing promising activity.[144] Ultimately, a mRNA-based vaccine targeting CLDN6 was proved to enhance the outcomes of claudin-CAR-T cell therapy, in hematological and solid tumors.[146]

To our knowledge, no clinical trials evaluating chemotherapy or RT combined with cancer vaccines have been conducted in patients with UC. Therefore, we discuss the major evidence on ICIs and cancer vaccines combination strategies.

The treatment with NEO-PV-01, a personalized neoantigen peptide subcutaneous vaccine, combined with nivolumab, was shown to be safe and effective in a phase Ib clinical trial, that evaluated 60 patients with melanoma, NSCLC and UC. At a median follow-up of 12 months, the following results were reported in the cohort of patients with UC: ORR 27%, response conversion rate 7%, median PFS 5.8 months, median OS 20.7 months and 1-year OS rates 67%.[147] Results in UC patients were similar to the benefit conferred by ICI alone, consequently further studies focused on melanoma and NSCLC.

A phase I/II study evaluated 46 patients with chemotherapy-resistant UC treated with an association of TAS0313 and pembrolizumab. TAS0313 is a subcutaneous vaccine formed by three long peptides and 12 CTL epitopes. The combination was safe and active, with an ORR of 33%, a DCR of 67%, a median PFS of 5 months and 1-year OS rate of 74.3%, in ICI-naïve patients, while in ICI-resistant patients the DCR was 50%.[148] Given the promising results in extending survival, larger studies in ICI-naïve patients are expected.

A fascinating vaccine strategy against immunosuppressive molecules has been developed. IO102-IO103 is an immune-modulatory subcutaneous peptide vaccine targeting indoleamine-

pyrrole 2,3-dioxygenase (IDO) and PD-L1. T-cells against tumor or immune cells expressing IDO and PD-L1 in TME, may enhance ICIs efficacy.[149] Trials evaluating the combination of IO102-IO103 vaccine and ICI are ongoing (see Table 2).

Safety

Safety and feasibility are critical aspects of drug development. Cancer vaccines appear extremely safe in general, with injection site reaction being the most common AE. Almost every clinical trial described in this review reported injection site reactions, including pain, erythema, swelling, and induration, typically mild and self-limiting. Sometimes, influenza-like illness, fever, myalgia, or fatigue were reported with a full recovery within few days[42,45,93,100,102,147,49,51–53,55,56,65,83]. In addition, gastrointestinal alterations were described, such as nausea, diarrhea, abdominal pain, and appetite loss.[83,93,147] In the event of intravesical administration, irritation symptoms may occur like dysuria, frequency, and hematuria.[55,100] Grade 3 or higher AEs are rare, as confirmed in an analysis that described only six severe AEs in 500 patients receiving subcutaneous peptide-based cancer vaccines (1%).[150] These included cellulitis or ulceration around the injection site, edemas of the head and neck regions, diarrhea and rectal bleeding, ulcerative colitis, and bladder-vaginal fistulae.[150] Infrequently, allergic reactions have been reported, mainly mild (rash, itching), exceptionally severe (anaphylaxis).[83] While vaccines are generally perceived as safe, their combination with other therapies, may increase toxicity: rarely, myelotoxicity, increased liver enzymes, acute kidney injury, or urological AEs have been reported, mainly in combination with chemotherapy or ICIs.[51,52,55,65,93]

Future perspectives and conclusions

Several preclinical and clinical trials on cancer vaccines are ongoing, exploring novel strategies to overcome the major obstacles to vaccination response. Particularly new antigens, adjuvants, platforms, and combination approaches are under investigation.

The novel technologies of genetic sequencing, advanced genomics and proteomics, artificial intelligence and computational assessment of the mutations, may help us to better define the immunogenicity and expression of TAAs, identifying the ideal candidate.[57] Furthermore, a crucial element of efficacious vaccine production is the prediction of HLA typing and neoantigen-MHC binding and modern tools like OptiType[151] and HLAscan,[152] NetMHCpan-4.0,[153] and MHCflurry 2.0,[154] may be useful. In addition, beyond TAAs, cancer vaccine may encode cytokines and molecules able to stimulate APCs and T cells activity.[40]

Adjuvants are an important component of the immunogenicity of a vaccine and developments are rapidly increasing the potentiality of these means.[41] Furthermore, novel materials are being explored, able to enhance APCs' activity, antigens presentation, and immune cells' activation. For instance, photothermal nanomaterials are gaining interest, for their direct photothermal effects and their synergic immune activation. Consequently, a photothermal nano-vaccine was developed, showing potent antitumor activity in melanoma mice models.[155]

While UC treatment is rapidly evolving, most cancer vaccines struggle to gain their spot in the current therapeutic algorithm.

Positive preclinical studies on UC cells or UC mice models focused on new antigens such as B7-H1,[156] antigen 85A and α-syn,[157] an element of the p53/p21 signaling pathway.[158] Promising responses were detected in UC models treated with autologous DCs loaded with an allogeneic UC cell line;[159] also irradiated allogeneic whole apoptotic tumor cells or autologous patient-derived tumor cells were able to induce antitumor immune responses.[160]

A promising approach is to utilize different kinds of vectors targeting the same TAA: one vector as a priming and the following as a boosting.[94] A synergistic activity of vaccine and ICI or cytokine therapy may be a successful strategy to overcome the immune suppressive environment of tumor.[93] Moreover, vaccines targeting immune receptors or molecules may induce a change in TME.[149]

The neoantigen coding mRNA agents appear highly promising preliminarily and might be evaluated for the adjuvant therapy of high-risk UC. This customized neoantigen-based approach incorporates the principles of precision medicine. Additionally, the adjuvant setting might be well suited to develop vaccines in the context of low burden of microscopic disease. Precision medicine needs to be developed in concert to select patients with molecular residual disease.

Recent human trials evaluating patients with UC are displayed in Table 2. The most frequently assessed strategy is a combination therapy with ICI; personalized neoantigens associated with various adjuvants are often the target of choice and peptide- or adenovirus-based the most tested platforms. To our knowledge, only one phase III trial is currently ongoing on patients with UC, evaluating CG007, in BCG-unresponsive NMIBC.

Of all the ongoing strategies to improve vaccines' efficacy, the combination with common cancer treatment seems the most appealing. Both ICIs and vaccines may influence TME, decreasing the immune-suppressive features and thus enhancing host's immune responses.[142,143] Chemotherapy and RT can directly kill tumor cells, increasing the neoantigen burden released in the bloodstream. Moreover, chemotherapy might alter the bone marrow cell composition, ultimately enhancing antigen-specific T-cell responses, when combined to SLP vaccine.[161] In contrast, RT is sometimes able to elicit systemic immune responses, and the abscopal effect is considered a form of in situ vaccination. Its combination with cancer vaccine is thought to be capable of improving immune activation.[144] Intriguingly, the intravesical administration of cancer vaccine, may be combined to BCG, with the dual aim of directly targeting UC cells and synergizing the immune responses.[46] Lastly, cancer vaccines may both prime the immune system and later sustain the activity and persistence of CAR-T cells, leading to enduring responses.[146]

Current UC treatment comprehends both local therapies, such as intravesical instillation and RT, and systemic treatments, including ICIs, chemotherapy, and target therapy. Hence, combining cancer vaccines with current UC therapies offers a promising approach to improve efficacy, overcoming resistance and ultimately increasing patients' outcomes.

In conclusion, a deeper understanding of the interactions between vaccines, tumor and immune system, combined with innovative manufacturing strategies and combination with current treatments, will help us define the role of vaccines in the UC treatment.

Abbreviations

ADC	antibody drug conjugate
APC	antigen-presenting cell
AE	adverse event
β-HCG	human chorionic gonadotropin-β
BC	bladder cancer
BCG	Bacillus Calmette Guérin
CAR	chimeric antigen receptor
CIS	carcinoma in situ
CEA	carcinoembryonic antigen
CMV	cytomegalovirus
CR	complete response
CTL	cytotoxic T lymphocytes
CTLA4	cytotoxic T-lymphocyte-associated protein 4
CVA21	Coxsackievirus A21
DC	dendritic cell
DCR	disease control rate
DEPDC1	DEP domain-containing 1
DNA	deoxyribonucleic acid
FGFR	fibroblast growth factor receptor
FPV	fowlpox virus
GM-CSF	granulocyte-macrophage colony stimulating factor
HDAC	histone deacetylases
HER-2	human epidermal growth factor receptor 2
HLA	human leukocyte antigen
HR	hazard ratio
HSP	heat shock proteins
HSV	herpes simplex virus
ICAM-1	intercellular adhesion molecule-1
ICI	immune checkpoint inhibitors
IDO	indoleamine-pyrrole 2,3-dioxygenase
IFA	incomplete Freund's adjuvant
IFN	interferon
IL	interleukin
KLH	keyhole limpet hemocyanin
LAG	lymphocyte-activation gene
LFA-3	leukocyte function-associated antigen-3
MAGE	melanoma-associated antigens
MHC	major histocompatibility complex
MIBC	muscle-invasive BC
MPHOSPH1	M-phase phosphoprotein 1
Mrna	messenger RNA
mUC	metastatic UC
MUC1	epithelial mucin 1
MVA	modified vaccinia ankara
NGS	next generation sequencing
NK	natural killer
NMIBC	non-muscle-invasive BC
NSCLC	non-small cell lung cancer
OFR	open reading frame
ORR	objective response rate
OS	overall survival
PAP	prostatic acid phosphatase
PD	progressive disease
PD1	programmed cell death protein 1
PD-L1	programmed cell death ligand 1
PFS	progression-free survival
PGV	peptide-based personalized genome vaccine
PPV	personalized peptide vaccination
PR	partial response
RC	radical cystectomy
RCC	renal cell carcinoma
RFS	relapse free survival
RNA	ribonucleic acid
RT	radiotherapy
SAM	self-amplifying RNA
SD	stable disease
SLP	synthetic long peptides
TAA	tumor-associated antigen
TGF	tissue growth factor
TLR	toll like receptor
TKI	tyrosin kinase inhibitor
TME	tumor microenvironment
TRICOM	three immune costimulatory molecules
Treg	regulatory T cells
TSA	tumor-specific antigen
TURBT	transurethral resection of bladder tumorUC: urothelial cancer
UTUC	upper tract urothelial cancer
VLP	virus-like particles
WT1	Wilms' tumor 1

Disclosure statement

Guru Sonpavde, MD:

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