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Therapeutic cancer vaccines: From biological mechanisms and engineering to ongoing clinical trials

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ABSTRACT

Therapeutic vaccines are currently at the forefront of medical innovation. Various endeavors have been made to develop more consolidated approaches to producing nucleic acid-based vaccines, both DNA and mRNA vaccines. These innovations have continued to propel therapeutic platforms forward, especially for mRNA vaccines, after the successes that drove emergency FDA approval of two mRNA vaccines against SARS-CoV-2. These vaccines use modified mRNAs and lipid nanoparticles to improve stability, antigen translation, and delivery by evading innate immune activation. Simple alterations of mRNA structure- such as non-replicating, modified, or self-amplifying mRNAs- can provide flexibility for future vaccine development. For protein vaccines, the use of long synthetic peptides of tumor antigens instead of short peptides has further enhanced antigen delivery success and peptide stability. Efforts to identify and target neoantigens instead of antigens shared between tumor cells and normal cells have also improved protein-based vaccines. Other approaches use inactivated patient-derived tumor cells to elicit immune responses, or purified tumor antigens are given to patient-derived dendritic cells that are activated *in vitro* prior to reinjection. This review will discuss recent developments in therapeutic cancer vaccines such as, mode of action and engineering new types of anticancer vaccines, in order to summarize the latest preclinical and clinical data for further discussion of ongoing clinical endeavors in the field.

Abbreviations: NSCL, non-small cell lung cancer; SCC, squamous cell cancer; TNBC, triple negative breast cancer; mCRC, metastatic colorectal cancer; mPC, metastatic pancreatic cancer; TNBC, triple negative breast cancer, SCLC, small cell lung cancer; TAA, tumor-associated antigen; LNP, lipid nanoparticle; SNL, synthetic long peptide; MHC I and MHC II, Major Histocompatibility Complex I and II; DC, Dendritic Cells; SAM, virus-derived self-amplifying mRNAs; i.d., intradermal; i.m., intramuscular; i.n., intranodal; i.v., intravenous; s.c., subcutaneous; FDA, Federal and Drug Administration; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TME, tumor micro environment; Tregs, regulatory T cells; ROS, reactive oxygen species; SAM, self-amplifying mRNAs; IVT, synthesized through *in vitro* transcription; TAAs, abnormally expressed proteins; APC, antigen-presenting cells; ORF, open reading frame; UTR, untranslated region; TLR, Toll-like receptors; TCR, T-cell receptor; HPV, human papillomavirus; VLP, targets virus-like particles; VSV, vesicular stomatitis virus; HNPCC, hereditary non-polyposis colorectal cancer; MSH-2, mouse model for the lynch syndrome; PD-1/PD-L1, programmed death protein-1/ligand1; NAP, Naproxen; CTLA-4, cytotoxic T-lymphocyte antigen 4; VEGFR, vascular endothelial growth factor receptor; MDSCs, myeloid-derived suppressor cells; DC-IL12-OVA, DC-based vaccine expressing IL-12, pulsed with OVA-peptide; CEA, carcinoembryogenic antigen.

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Background

Cancer is a significant health problem, with nearly 10 million deaths every year [1]. Besides protecting the organism from pathogens, the immune system's role is also useful for surveying the body to maintain cellular homeostasis. However, tumor cells can escape immune surveillance either by a selection of non-immunogenic tumor cell variants (immunoselection) or by actively suppressing immune response (immunesubversion)[2]. Advancements in immunotherapy have brought forth new potential therapies and prophylactic treatments that could lead to anticancer vaccines. Tumors display on their surface specific proteins generated when certain mutations occur in tumor DNA, and these proteins are called neoantigens. The body can generate an immune response against cancer cells through the help of neoantigens. Therefore, artificially triggering an immune response against tumor neoantigens constitutes the foundation for vaccines against tumors. Neoantigens are newly formed antigens that the immune system has not previously recognized. Neoantigens can arise from somatic mutation, alternative splicing, or viral proteins.

Latest vaccine-engineering strategies include administration of antigens as inactivated tumor cell extracts, purified mutated tumor proteins, or DNA and mRNA for endogenous production of tumor antigens, combined with various adjuvants and systems of delivery [3]. Two major two challenges to the development of cancer vaccines are the identification of neoantigens and the generation of new molecular epitopes recognized as foreign by the immune system to elicit a robust immune response against tumor cells. Results from ongoing clinical trials corroborate the information on therapeutic anticancer vaccine safety, with some studies indicating a potential efficacy.

This review focuses on recent advancements in therapeutic cancer vaccines, going from latest vaccine technologies, identification of neoantigens methods, to ongoing investigations in animals and humans.

Cancer vaccines: from biological mechanisms to engineering

Historically, vaccines are used to prevent diseases caused by infectious pathogens. Present-day vaccines are expanding to cancer as well. Early cancer therapeutic vaccines failed to amplify *de novo* T cell responses primarily because they targeted abnormally expressed tumor-associated antigens proteins (TAAs) or self-proteins on tumor cells [4]. Therapeutic cancer neoantigen strategies are highly advantageous since they home on an antigen while preventing central and peripheral tolerance and potential 'off-target' tissue damage observed in previous TAA-targeting strategies [5].

One critical step to developing a cancer vaccine is identifying and selecting appropriate neoantigens or neoepitope targets expressed exclusively by cancer cells. The immune system readily mounts a CD4+ and CD8+ T cell response to foreign proteins but tolerates self-proteins retained by cancer [6]. Generally, antigens with a heavy mutational burden make neoantigen identification more accessible and more likely to result in a tumor cell-specific immune response.

Another challenge lies with the tumor microenvironment (TME), which has many adverse qualities such as generation of hypoxia, nutrient depletion, low pH, an increase of reactive oxygen species (ROS), and a high number of regulatory T cells (Tregs). Also, solid tumors have other barriers such as tumor fibroblasts (fibrotic extracellular matrix), myeloid suppressor cells, and T_{regs} that further reduce the number of tumor-infiltrating T cells [7-9]. Other limitations include low tumor mutational burden, antigen escape, and antigen-presenting cells (APCs) ability. Advancements in immunotherapy have brought forth new potential therapies and prophylactic treatments that could lead to anticancer vaccines. Tumors display on their surface specific proteins generated when certain mutations occur in tumor DNA, and these proteins are called neoantigens. Vaccines with effective delivery methods, adjuvants, and appropriate antigens can potentially overcome these immunosuppressive obstacles. The most common types of therapeutic cancer vaccines that have been designed are nucleic acid vaccine (RNA or DNA), long synthetic peptide (SLP) vaccine, and cellular vaccine (tumor cell or on dendritic cell (DC)-based) (Fig. 1) [10,11].

Nucleic Acid-based vaccines

mRNA encodes target antigens expressed after administered mRNA is efficiently taken up and translated by local cells. This factor is beneficial for making mRNA available as an off-the-shelf cancer vaccine and for personalized neoantigen vaccination [12]. Another significant advantage is that mRNA encoded proteins can undergo post-translation modifications such as glycosylation, acetylation, methylation, or phosphorylation to become mature folded proteins, an essential condition to be appropriately antigenic. Previously, non-formulated or "naked" mRNA injection was shown to deliver vaccine components to the lymph nodes of mice. It also was observed that RNA directly injected into lymph node tissue effectively targets APCs and promotes high IL-12 secretion and expression of CD86 on DCs. This, in turn, promoted active proliferation and infiltration of CD8+ T and CD4+ T cells in lymph node tissue compared to controls [13]. Other methods include injecting liposome-encapsulated mRNA to increase adjuvanticity, or injecting self-adjuvanted RNA to promote cellular and humoral responses and Th1 and Th2 cell activation [14]. Self-adjuvanted mRNA molecules are mRNAs whose encoded protein expression has been enhanced by 4–5 orders of magnitude by modification of nucleotide sequences with naturally occurring nucleotides (A/G/C/U) that do not affect primary aminoacid sequence. In addition, they are complexed with protamine, thus activate immune system by the involvement of toll-like receptor (TLR) 7. Self-adjuvanted RNA vaccines induce strong, balanced immune responses comprising humoral and cellular responses, effector and memory responses, and also activate important subpopulations of immune cells such as Th1 and Th2 cells [14].

Recently, a new promising platform called the KISIMA vaccine can select tumor antigen, makes cell-penetrating peptides to improve antigen delivery and epitopes presentation and finally, it uses TLR2/4 agonist as self-adjuvant. KISIMA was used in a study to produce a vaccine against achaete-scute family bHLH transcription factor 2 (Ascl2), an antigen found in early colon cancer. The vaccine could reduce colon tumor formation by stimulation of an antitumor immune response. Combining this vaccine with anti-PD-1, significantly reduced development of colon adenomas and macroadenomas, vs. negative controls, and may be used in patients at high-risk of incurring it [15].

The United States Food and Drug Administration (US FDA) has recently approved lipid nanoparticles (LNP)-loaded mRNA for COVID-19 [12,16]. Currently, three types of LNP-loaded RNA vaccines are being studied in clinical trials for solid tumors: modified or unmodified non-replicating mRNA, and virus-derived self-amplifying mRNAs (SAM). Both non-replicating mRNA and SAM have been synthesized through *in vitro* transcription (IVT).

IVT uses a bacteriophage RNA polymerase and DNA template for target antigen sequences to synthesize RNA cell-free. This method has proven easy for large-scale production of eukaryotic-like mRNAs, which contain an open reading frame (ORF) for a target antigen, flanked by 5′ and 3′ untranslated regions (UTR) bearing a 5′ 7-methylguanosine cap and 3′ poly adenine poly(A) tail. The poly(A) tail is transcribed from DNA templates or is added post-IVT by enzymes. Non-replicating mRNAs contain optimized 3′ and 5′ UTR and the ORF target antigen sequence, but not additional sequences for RNA replication (i.e., the viral replication machinery). Therefore, these mRNA sequences cannot self-replicate [17].

SAM contains two ORFs, one encoding targeted antigen sequence, while the other machinery for viral replication is to support intracellular RNA amplification. After internalization, SAM enters the cytosol to be replicated or transcribed in mRNA and translated into proteins by ribosomes. Following translation and post-translational modification, the protein is folded and becomes functional [18].

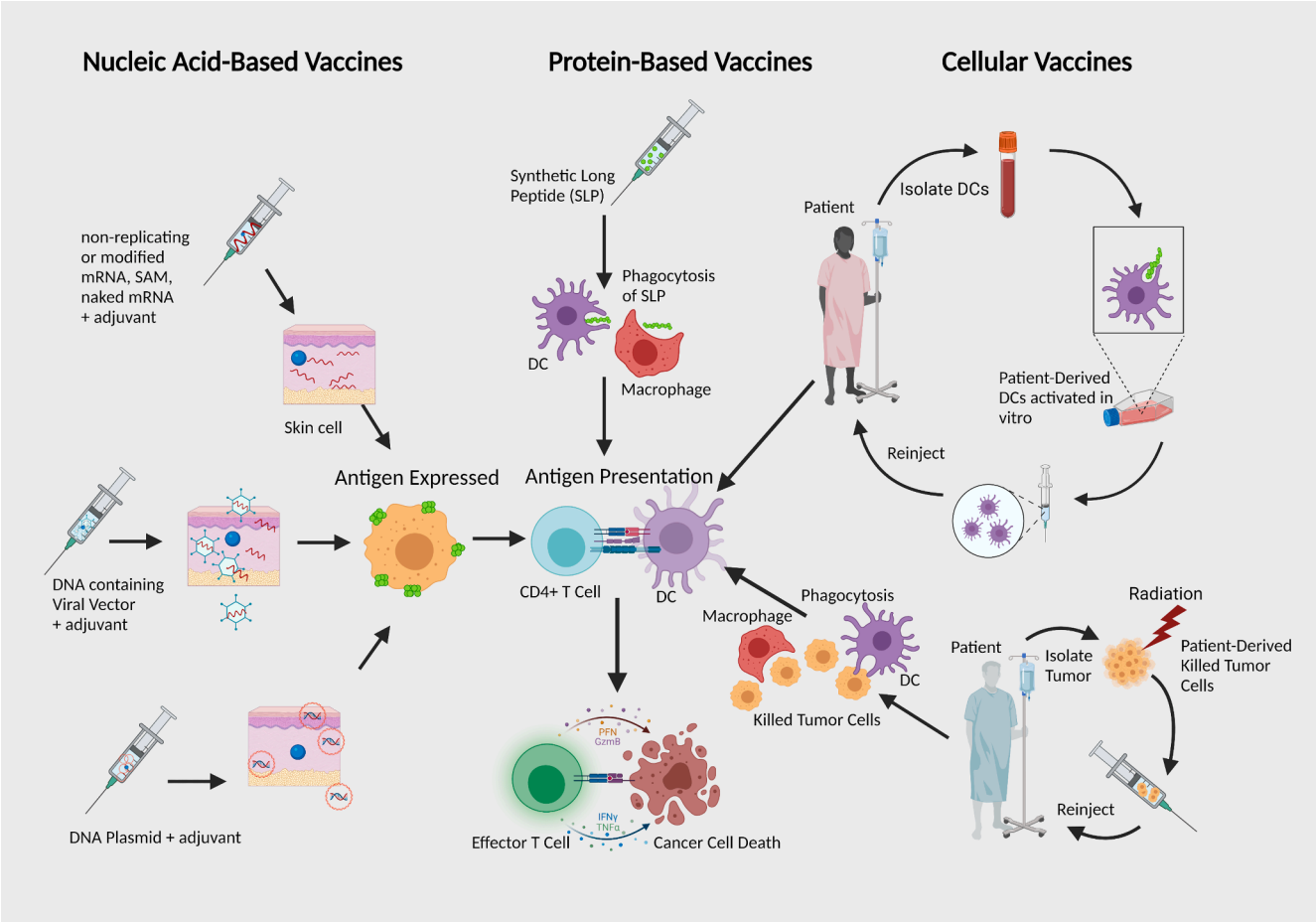

Fig. 1. The Main Types of Cancer Therapeutic Vaccines: Cancer vaccines primarily deliver antigens either nucleic acids, proteins, peptides, or patient-derived cells. Within nucleic acid-based vaccines, RNA has various approaches that differ with RNA structure manipulation and delivery compared to DNA, which is restricted by exclusively relying on plasmids to deliver antigen-encoding genetic materials. Both mRNA and DNA are taken up by cells and eventually translated into protein antigens APCs present to activate T cells. Cellular vaccines depend on patient-derived cells to deliver isolated tumor cells that are killed, or the patient's DCs are activated *in vitro* with purified tumor antigens before reinjection. All therapeutic cancer vaccine types aim for antigen presentation followed by T cell activation and tumor rejection. Abbreviations: *DC, Dendritic Cells; SAM, virus-derived self-amplifying mRNAs; SLP,* synthetic long peptide.

Modified mRNAs containing pseudo nucleotides such as, 1-methyl-pseudouridine, 5-methylcytidine, or N4-aceylcytidine to replace uridine and cytidine, improve not only translational efficiency and stability of the mRNA but also increase immune response potency [19]. Karikó *et al.* found that modified nucleoside mRNAs decreased innate immunity by reducing the activation of RNA sensors such as Toll-like receptors (TLR) and RNA-dependent protein kinase (PKR). Interestingly, it was previously observed that reduced innate immune activation prevents abolition of mRNA translation into proteins. It should be noted that the phage polymerase contained in IVT can yield short RNA contaminants, which promote innate immunity by activating intracellular PRRs. However, purification by high-performance liquid chromatography (HPLC), permits the recovery of mRNA that decreases inflammatory cytokines activating innate immunity [20-22]. In general, mRNA is an innovative and powerful cancer vaccine platform currently tested for cancer vaccination after recent advances in the field.

DNA vaccines contain closed circular DNA plasmids of bacterial origin encoding desired antigens, which are transcribed into mRNAs and translated to proteins to induce antigen-specific immune responses. Like RNA vaccines, DNA vaccines promote humoral and cellular responses specific to target antigens. In addition, bacterial DNA stimulates TLRs or membrane-bound receptors that are critical in aiding DCs, B cells, and natural killer cells in recognizing pathogen-associated molecular patterns [23]. This leads to a pro-inflammatory response cascade with cytokine production. Gardasil (Merck) is one well-known vaccine quadrivalent, HPV L1 virus-like particle (VLP) recombinant vaccine, which targets oncogenic subtypes of human papillomavirus (HPV) through the production of HPV-neutralizing antibodies. The first Gardasil vaccine protected against HPV types 6, 11 and 18. Currently, Gardasil-9 vaccine contains HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. The VLPs of Gardasil-9 are generated through the self-assembly of 360 copies of the L1 major capsid protein of the virus. Gardasil-9 VLP vaccine has been approved for use in the US against nine HPV strains and may give protection of 80% of cervical cancers [24,25]. Although the current subunit HPV vaccination provides effective prophylaxis, they do not eradicate existing infections. Therefore, other HPV vaccination strategies have begun evaluation. DNA vaccination targeting HPV proteins E6 and E7, both associated with HPV 16 and 18, results in poor immunogenicity, possibly because the virus evades host recognition [26]. To overcome this obstacle, DNA vaccination is combined with other immunotherapies. Recently, Peng et al. tested a DNA vaccine that targets E6 and E7 HPV proteins combined with PD-1 blockade in mice. Results indicated that combined therapy led to significantly prolonged survival time [26]. One type of DNA vaccine currently undergoing preclinical investigation is chimeric DNA vaccines, which encode xenogeneic antigens, homologous with the self-orthologue, but originated from a different species [27]. The xenogeneic antigens are recognized as foreign, and may help override immune tolerance for TAAs that are recognized as self-antigens. Quaglino et al. designed a xenogeneic vaccine that consisted of a plasmid encoding chimeric rat/

human ErbB2 proteins, which was administered to mice with ErbB2+ mammary tumors [28]. ErbB2 is an epidermal growth factor often highly displayed on some colorectal, pancreatic, endometrial, gastric, and breast cancers [29]. Results indicated that the chimeric/xenogeneic vaccine induced more robust antitumor responses than autologous controls [30]. Corroborating this data, in humans a phase I trial using gp100 plasmid DNA led several patients to increased levels of gp100-CD8+ T cells [31]. DNA is more stable than RNA, and its safety makes DNA vaccination an attractive strategy. However, its delivery is more complex than RNA due to its higher dimension and the need for nuclear localization. Improving transfection methods to *in vivo* delivery is necessary to enhance vaccine efficacy [32]. Ongoing clinical trials investigating nucleic-acid vaccines are summarized in Table 1.

Peptide-based vaccines

Antigens must be on the surface and be presented by the Major Histocompatibility Complex I or II (MHC I or II) molecules in order for T cells to recognize them [33]. Peptide-based vaccines are specific, safe, and rely heavily on the strong, adaptive immune response to initiate cancer-killing effects [34].

The peptide-MHC and T cell receptor interactions are highly immunogenic and result in less 'off-target' toxicity and central tolerance when neoantigens are targeted. The Synthetic Long Peptide (SLP) vaccines are subunit vaccines made from peptides that mimic epitopes of antigen that trigger direct or potent immune responses. SLPs containing class-I and class-II MHC restricted neoepitopes can induce neoantigen-reactive CD8+ T-cell responses and CD4+ T-cell responses which drive direct antitumor effects. Immunogenicity typically increases with peptides that include multiple epitopes and recognition motifs. This strategy avoids central tolerance and bolsters CD4 and CD8 T cell responses. Short peptides (~9 aminoacid residues) can be exogenously loaded onto MHC-I molecules of non-professional APCs, leading to a poor T cell response [35]. Shorter peptides are easily digested by enzymes, and thereby are quickly eliminated in the human blood serum. Longer peptides (25–35 aminoacid residues) are more readily endocytosed by professional APCs, which have the proper machinery to allow complete T cell activation and antigen presentation by MHC-II molecules [36]. Short peptides also restrict HLA-type, making broad coverage of HLA-types a greater challenge, thereby making SLP more attractive overall [37].

PTHrP plays key roles in a wide variety of solid tumors, including osteosarcoma, breast cancer, lung cancer, chondrosarcoma, anaplastic thyroid cancer, medulloblastoma, adrenocortical tumor, squamous cancer and prostate cancer cell lines. Recently, it has been shown that the combination of anti-PTHrP with zoledronic acid, which is a third-generation bisphosphonate, is promising to control bone-metastasis in immunosuppressed mice depleted of NK cells inoculated with SBC-5 human small cell lung cancer (SCLC) cells, than the agent alone, therefore suggesting that the dual-target therapy is more useful [38]. The overexpression in cancer cells of Thymidylate Synthase (TS) – a crucial enzyme for DNA repair and replication- inspired the development of a 27-mer peptide vaccine named TS poly-epitope peptide (TSPP) vaccine. TSPP contains three epitopes of HLA-A2.1-binding motifs of TS. The vaccine has shown to be safe and has antitumor activity in both preclinical studies and a clinical (phase 1) investigation [39]. To corroborate this data, another phase Ib study of 29 metastatic colorectal cancer showed that the poly-epitope vaccination to TSPP and GOLFIG chemo-immunotherapy was safe and possibly effective [40].

Since malignancy-associated hypercalcemia is characterized by excessive production of parathyroid hormone-related protein (PTHrP), a peptide vaccine was developed targeting PTHrP. The first study showed that the vaccine was effective in mice bearing transplanted human PTHrP-producing tumors, as it prolonged their survival [41]. Interestingly there is a combination of cancer peptides called TAS0313. This cancer vaccine cocktail is made of overall 12 cytotoxic T lymphocyte (CTL) epitope peptides derived from the following eight cancer-associated antigens overexpressed in various types of solid cancers: EGFR, KUA, LCK, MRP3, PTHRP, SART2, SART3, and WHSC2. A Phase I/II study showed that the TAS0313 vaccine could produce an immune response with favorable safety and tolerability in 10 Glioblastoma (GBM) patients [42]. Generally, improving adaptive immune response, effector functions, and clinical efficacy of SLP vaccines, is an ongoing process at a fast pace.

Recently, a novel proteogenomic approach has been conducted to identify tumor antigens in colorectal cancer-derived cell lines, with biopsy samples having matched tumors and normal adjacent tissues. In this study, mass spectrometry analyses identified 30,000 unique MHC-I-associated peptides. The authors identified 19 tumor-specific antigens in both microsatellites stable and unstable tumors and intriguingly, 2/3 of them were from non-coding regions. Many of such peptides were from genes involved in colorectal cancer progression. Future vaccine research could use such findings to develop T-cell-based vaccines, in which T cells are primed against these antigens to target and destroy these tumors [43].

Another recent study investigated multi-epitope-based vaccines for colorectal cancer treatment and prevention. The authors designed various vaccines targeting crucial oncogenes in this cancer, namely DC25B, COX2, RCAS1, and FASCIN1 proteins. Their peptide vaccines targeted human class II MHC for each protein and T-cells specific for both peptides and corresponding recombinant protein. Only when they immunized for both CDC25B and COX2 peptides, they were able to observe a significant tumor growth inhibition in MC38 syngeneic mice vs. control. Therefore, they suggested that immunization with CDC25B and COX2 epitopes could be a good method to suppress tumor development in both treatment and prophylactic models that they were able to evaluate [44].

Recently, a study developed a recombinant anti-mKRAS scFV-fused, mutant *Hydra* actinoporin-like-toxin 1 (mHALT-1) immunotoxin that can recognize and eradicate cells bearing K-Ras antigen from mutated codon-12. They showed high cytotoxic efficacy on SW-480 (bearing the

Table 1
Ongoing clinical trials investigating nucleic acid vaccines in cancer.

Clinical Trial Identifier Code	Investigation Plan	Vaccines, Drug/s	Clinical Setting Lines of therapy	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT03970746	64 participants, Non-Randomized, Sequential Assignment, Open label	PDC*lung01, Keytruda Injectable Product, Alimta	Wash out of 4 weeks since last cycle of chemotherapy	DLT	1/2	Recruiting
NCT02439450	121 participants, Non-Randomized, Parallel Assignment, Open label	Viagenpumatucel-L, Nivolumab, Pembrolizumab, Pemetrexed	Second or later	TEAEs, ORR, PFS	1/2	Active, not recruiting
NCT02960230	49 participants, Non-Randomized, Parallel Assignment, Open label	K27M peptide, Nivolumab	Second line	K27M peptide, Nivolumab	1/2	Recruiting
NCT01773395	123 participants, Randomized, Parallel Assignment, Triple (Participant, Care Provider, Investigator)	GVAX, Busulfan, Fludarabine, Tacrolimus, Methotrexate	First line	18/month PFS	2	Active, not recruiting

$KRAS^{G12V}$ mutation) colorectal cancer cells, whereas they spared NHDF control cells [45].

A phase II clinical study specifically showed that the combination of chemotherapy with second-line telomerase peptide vaccine (GV1001) in 56 metastatic colorectal cancer patients, was tolerable and modestly effective [46].

Viral vaccines can be designed to deliver RNA, which encode peptides antigens that are later displayed by tumor cells and other cells. Also, oncolytic viruses (OVs) are used to selectively infect, replicate in, and lyse malignant cells. In addition to killing infected malignant cells, OVs may promote the destruction of the tumor's blood cells [47]. OVs can act as an effective adjuvant and delivery platform for personalized anticancer vaccines, by using peptide antigens [48]. One OV therapy that uses an IFN-β-expressing vesicular stomatitis virus (VSV) to treat hepatocellular carcinoma (HCC), recently entered a phase I clinical trial [49]. Viral vector vaccines have shown to be a versatile strategy for promoting antitumor activity and will continue to be further developed.

Vaccines against frameshift mutations have been tested for Lynch tumors and hereditary non-polyposis colorectal cancer (HNPCC). Gebert et al. chose 10 short peptides based on frameshift modifications and immunized C57BL/6 mice, four times biweekly, to confirm the expression of the peptides through an ELISpot immunogenicity assay. In a mouse model for Lynch syndrome (MSH-2 conditional knock-out mice), vaccination improved survival and reduced tumor burden that would otherwise spontaneously develop tumors. These vaccines had to be combined with aspirin and Naproxen (NAP), two nonsteroidal anti-inflammatory drugs used for chemoprevention of HNPCC, to elicit great outcomes in the model [50]. In glioma models, current cancer vaccines needed anti-programmed death protein-1/ligand1 (PD-1/PD-L1), or anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4), to boost immune response further [51]. Ongoing clinical trials investigating peptide or viral vaccines are summarized in Tables 2 and 3, respectively.

Cellular vaccines

In DC-vaccine development, DCs are loaded with tumor antigens in the forms of mRNAs, proteins, peptides, or tumor lysates [52]. Normally, antigen delivery to the DCs occurs *ex-vivo*, where they are activated and reinjected. However, this process may impair dendritic cell trafficking to secondary lymphoid organs. An alternative strategy is to infect patient DCs with viral vectors encoding desired antigens or fuse their DCs with tumor cells [53]. Further investigation is required to standardize an effective DC-based vaccine engineering.

Another type of cellular vaccine is created using irradiated allogeneic whole tumor cells or autologous patient-derived tumor cells to induce antitumor immune responses [54]. To enhance immune response against whole tumor cells, new generations of tumor cell vaccines have been genetically modified to either produce co-stimulatory molecules, chemokines, cytokines, or reduce inhibitory molecule production [55]. For example, the FANG vaccine contains autologous-tumor cells modified with a plasmid that encodes a bi-functional short hairpin RNAi that targets furin convertase resulting in downregulation of TGF-β, an immunosuppressive transforming growth factor [56]. After tumor cells or lysate is delivered, DCs initiate T cell activation by cross-priming CD8+ T cells [55]. This strategy allows multiple tumor antigens to be targeted simultaneously without neoantigen identification prior to administration. However, neoantigen mutational burden and quality are still considered to be associated with good prognosis and are good predictors of treatment success. One study showed that neoantigen vaccine effectiveness was limited by a low mutational burden [57]. Even checkpoint inhibitor antibodies targeting PD-1 and CTLA-4 have improved clinical response when tumors have a higher mutation frequency [58,59]. Salewski et al. showed mice administered with autologous-cell line-derived tumor lysates from 328 and A7450 T1 M1 cell lines, with high-quality neoantigens, are more effective at promoting a prophylactic effect on gastrointestinal tumor formation [60]. Therefore, neoantigen identification may still add some value to tumor cell vaccine design. Regardless, improvements will need to be made to further enhance antitumor response associated with cellular vaccines.

From sequence mutations to the identification of neoantigens for vaccines

Neoantigens identification depends on several fundamental factors besides somatic mutations: translation, post-translational modifications and affinity between mutated peptide and patients' MHC molecules, and affinity between mutant peptide-MHC complex with T-cell receptor (TCR) [61]. Prediction of neoantigens needs to combine both genomic mutations and MHC information on patients, and different software has shown this conjunction to be useful, as summarized in Table 4 [62-65].

Discovery of personalized neoantigens from patients

Next-generation sequencing has advanced cancer therapy to allow patients to receive personalized therapies such as cancer vaccine, to generate a robust immune response against a patient's cancer cells based on their unique molecular profile.

Existing immunotherapies reactivating the immune system work for only 30% of patients [66]. Hence, other ways to boost antitumor immune response using a targeted vaccine for specific patient genetics and MHCs, are urgently needed [67-70]. Additionally, tumors expressing more neoantigens are associated with a stronger immune response and better survival [71-73]. Therefore, harnessing the natural ability of the immune system to detect and kill cancer cells [74].

Creating neoantigen-based vaccines

Due to the application of NGS, discovering tumor neoantigens has become a valuable tool for developing a personalized neoantigen vaccine. To manufacture a neoantigen vaccine, it is essential to compare patients' tumor cells to their normal cells using whole-exome sequencing to identify mutations uniquely present in tumor cells [74]. Neoantigens are made from mutated proteins from DNA mutations. However, not all DNA mutations are missense or nonsense to produce mutated proteins. Techniques such as RNA-seq can be used to discriminate those mutations that lead to the formation of neoantigens to target vaccines. Secondly, only some peptides from processed mutated proteins can bind to HLA class I molecules. Neural network-based algorithms were used to predict which mutant proteins are most likely to undergo this transformation, and be presented on the surface of tumor cells or APCs as neoantigens [71,75,76]. These neoantigens are most likely to be detected by T cells to produce a strong tumor-specific immune response. The number of neoantigens included in a vaccine varies by patient, but, so far, up to 20 different neoantigens have been included in a single personalized vaccine [45] (Fig. 2).

Neoantigen vaccines stimulate tumor-specific T cells

Clinical trials of personalized cancer vaccines in solid tumors have shown that neoantigen vaccines can generate tumor-specific T cells that only recognize the tumor without serious side effects [66,77-80]. Recent glioblastoma clinical trials have revealed that peripheral stimulation by vaccine-generated T-cells with neoantigen specificity could be tracked inside the tumor [78]. To overcome immune resistance, many current clinical trials have combined personalized vaccines with immunotherapies. For example, Roche has started a clinical trial combining a personalized neoantigen vaccine with PD-L1 therapy to treat melanoma and non-small cell lung cancer [81]. Neoantigens predicting algorithms like HLA-thena, have improved based on mass spectrometry data and can better predict HLA-binding preferences for various types of patients [82].

Although current research mainly focuses on HLA class-I molecule to

Table 2
Ongoing clinical trials investigating peptide vaccines in cancer.

Clinical Trial Identifier Code	Investigation Plan	Vaccines, Drug/s	Clinical Setting Lines of therapy	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT01789099	18 participants, Single Group Assignment, Open Label	UV1 synthetic peptide vaccine and GM-CSF	Second or later lines	Safety and tolerability, Immunological response	1/2	Active, not recruiting
NCT01784913	22 participants, Single Group Assignment, Open Label	UV1/hTERT2012P	First line	Safety and tolerability	1/2	Active, not recruiting
NCT03012100	280 participants, Randomized, Parallel Assignment, Triple masking, Double Blind	Cyclophosphamide, Multi-epitope Folate Receptor Alpha Peptide Vaccine, Sargramostim	Second or later lines	DFS	2	Recruiting
NCT01720836	30 participants, Non-Randomized, Parallel Assignment, Open Label	Vaccine + PolyICLC	Second or later lines	Immunologic response	1/2	Recruiting
NCT00194714	26 participants, Single Group Assignment, Open Label	HER-2/neu Peptide Vaccine	Second or later lines	Immunologic response, AE	1/2	Active, not recruiting
NCT03606967	70 participants, Randomized, Parallel Assignment, Open Label	Carboplatin, Durvalumab, Gemcitabine Hydrochloride, Nab-paclitaxel, Personalized Synthetic Long Peptide Vaccine, Poly ICLC, Tremelimumab	First line	Clinical response	2	Recruiting
NCT04998474	15 participants, Single Group Assignment, Open Label	FRAME-001 personalized vaccine	Fourth or later lines	FRAME-001-specific immune responses	2	Not yet recruiting
NCT02795988	36 participants, Randomized, Parallel Assignment, Partially blinded (outcomes assessor)	IMU-131, Cisplatin and either Fluorouracil (5-FU) or Capecitabine or Oxaliplatin and capecitabine.	First line	Safety and tolerability, Recommended Phase 2 dose and clinical efficacy of IMU-131	1/2	Active, not recruiting
NCT04747002	100 participants, Randomized, Parallel Assignment, Opel label	DSP-7888	Second line	RFS	2	Recruiting
NCT03761914	90 participants, Non-Randomized, Parallel Assignment, Opel label	galinpepimut-S, Pembrolizumab	Second or later lines	TRAEs, ORR, CR	1/2	Active, not recruiting
NCT02396134	133 participants, Randomized, Parallel Assignment, Triple masking	CMVpp65-A*0201 peptide vaccine	First line	CMV reactivation and CD8+ T cells binding	2	Active, not recruiting
NCT02636582	13 participants, Randomized, Parallel Assignment, Single masking (Participant)	Nelipepimut-S Plus GM-CSF Vaccine, Sargramostim	First line	Evaluate CTL	2	Active, not recruiting
NCT05127824	42 participants, Randomized, Factorial Assignment, Open label	Autologous alpha-DC1/TBVA vaccine, Cabozantinib	First line	Immune response, Safety	2	Not yet recruiting
NCT04197687	480 participants, Randomized, Parallel Assignment, Double blinded (participant, care provider)	Multi-epitope HER2 Peptide Vaccine TPIV100, Pertuzumab, Sargramostim, Trastuzumab, Trastuzumab Emtansine	Second or later lines	iDFS	2	Recruiting
NCT05243862	28 participants, Single Group Assignment, Open label	PolyPEPI1018, Atezolizumab	Third or later lines	AEs, Safety	2	Not yet recruiting
NCT02126579	62 participants, Randomized, Parallel Assignment, Open label	Peptide Vaccine (LPV7) + Tetanus peptide, PolyICLC	Second or later lines	Safety and toxicity, T cell response	1/2	Active, not recruiting
NCT00703105		DC vaccination	Second or later lines	Immune response	2	Recruiting

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Table 2 (*continued*)

Clinical Trial Identifier Code	Investigation Plan	Vaccines, Drug/s	Clinical Setting Lines of therapy	Primary Endpoint	Stage of Development	Clinical Trials Status
	36 participants, Single Group Assignment, Open label					
NCT02818426	54 participants, Single Group Assignment, Open label	UCPVax	Third or later lines	DLT	1/2	Recruiting
NCT03047928	50 participants, Single Group Assignment, Open label	Nivolumab, PD-L1/IDO peptide vaccine	Second or later lines	AEs	1/2	Recruiting
NCT02960230	49 participants, Non-randomized, Parallel Assignment, Open label	K27M peptide, Nivolumab	Second or later lines	AEs, OS	1/2	Recruiting
NCT04206254	80 participants, Randomized, Parallel Assignment, Open label	gp96	Second or later lines	2-years RFS	2/3	Not yet recruiting
NCT04364230	44 participants, Single Group Assignment, Open label	6MHP, NeoAg-mBRAF, PolyICLC, CDX-1140	First line	Safety of CDX-1140 + melanoma peptide vaccine, Immunogenicity	1/2	Recruiting
NCT04280848	28 participants, Single Group Assignment, Open label	UCPVax	Second or later lines	Immunogenicity	1/2	Active, not recruiting
NCT03946358	47 participants, Single Group Assignment, Open label	Atezolizumab, UCPVax	Second or later lines	ORR	2	Recruiting
NCT03560752	36 participants, Single Group Assignment, Open label	Multi-peptide CMV-Modified Vaccinia Ankara Vaccine	Second or later lines	AEs	2	Recruiting
NCT04051307	48 participants, Single Group Assignment, Open label	PD-L1 peptide: PD-L1 Long(19–27), Arginase1 peptide: ArgLong2(169–206)	First line	Immune response	1/2	Recruiting
NCT03617328	30 participants, Randomized, Parallel Assignment, Open label	6MHP, Montanide ISA-51, polyICLC, CDX-1127	First or second line	Safety and immunogenicity	1/2	Recruiting
NCT01885702	25 participants, Non-Randomized, Parallel Assignment, Open label	DC vaccination	First or second line	Safety and feasibility	1/2	Active, not recruiting
NCT02802943	56 participants, Non-Randomized, Parallel Assignment, Open label	Peptide Vaccine, Imiquimod	First or second line	Induction of peptide-specific T cell responses	2	Recruiting
NCT03715985	12 participants, Sequential Assignment, Open label	EVAX-01-CAF09b	Second or later line	Safety and tolerability	1/2	Active, not recruiting
NCT05096481	120 participants, Single Group Assignment, Open label	PEP-CMV, Temozolomide, Tetanus Diphtheria Vaccine	Second line	PFS, OS	2	Not yet recruiting
NCT04580771	35 participants, Single Group Assignment, Open label	Cisplatin, Liposomal HPV-16 E6/E7 Multipeptide Vaccine PDS0101, Radiation Therapy	First line	Toxicity	2	Recruiting
NCT02455557	66 participants, Single Group Assignment, Open label	Montanide ISA 51 VG, Sargramostim, SVN53-67/M57-KLH Peptide Vaccine, Temozolomide	Second line	PFS	2	Active, not recruiting
NCT03821272	20 participants, Randomized, Parallel Assignment, Open label	PepCan	Second line	AEs	1/2	Recruiting
NCT02358187	25 participants, Randomized, Single Group Assignment, Open label	HLA-A2 Restricted Glioma Antigen-Peptides with Poly-ICLC	Third line	Tumor shrinkage or stable disease	2	Recruiting
NCT04114825	180 participants, Randomized, Parallel Assignment, Quadruple masking	RV001V	Second or later line	Time to PSA progression	2	Active, not recruiting

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Table 2 (*continued*)

Clinical Trial Identifier Code	Investigation Plan	Vaccines, Drug/s	Clinical Setting Lines of therapy	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT05232851	24 participants, Randomized, Parallel Assignment, Open label	Liposomal HPV-16 E6/E7 Multipeptide Vaccine PDS0101, Pembrolizumab	First line	ctHPVDNA response	1/2	Recruiting
NCT01697527	6 participants, Single Group Assignment, Open label	Aldesleukin, fludarabine phosphate, cyclophosphamide, NY-ESO-1 reactive TCR retroviral vector transduced autologous PBL, DC therapy, fludeoxyglucose F 18, PET	Second or later line	Clinical response	2	Active, not recruiting
NCT03384914	110 participants, Randomized Parallel Assignment, Open label	DC1 Vaccine, WOKVAC Vaccine	Second or later line	Immunogenicity	2	Recruiting
NCT05163080	265 participants, Randomized Parallel Assignment, Double-Blind	SurVaxM	Second or later line	OS	2	Recruiting
NCT01814813	90 participants, Randomized, Parallel Assignment, Open label	HSPPC-96, bevacizumab	First line	OS	2	Active, not recruiting
NCT04912765	60 participants, Single Group Assignment, Open label	Neoantigen DC Vaccine, Nivolumab	Second or later line	24-months Relapse Free Survival	2	Recruiting
NCT03633110	24 participants, Non-Randomized, Single Group Assignment, Open label	GEN-009 Adjuvanted Vaccine, Nivolumab, Pembrolizumab	First line	AEs, T-cell responses	1/2	Active, not recruiting
NCT02506933	102 participants, Randomized, Parallel Assignment, Double blinded (Participant and Investigator)	Multi-peptide CMV-Modified Vaccinia Ankara Vaccine	First line	CMV events, Severe AEs	2	Active, not recruiting
NCT02134925	110 participants, Randomized, Parallel Assignment, Double blinded (Participant and Investigator)	MUC1 Peptide-Poly-ICLC Vaccine	First or second line	Change in Anti-MUC1 Immunoglobulin G (IgG) Levels	2	Active, not recruiting
NCT04060277	128 participants, Randomized, Parallel Assignment, Double blinded (Participant and Care Provider)	Letermovir, Multi-peptide CMV-Modified Vaccinia Ankara Vaccine	First line	Clinically significant cytomegalovirus	2	Recruiting
NCT03284866	536 participants, Randomized, Parallel Assignment, Double blinded (Participant and Investigator)	Recombinant Human Papillomavirus Nonavalent Vaccine	Second or later line	Lesions occurrences	3	Recruiting
NCT02543749	30 participants, Single Group Assignment, Open Label	DC vaccine	First line	DC toxicity Parameters using CTC	1/2	Recruiting
NCT02334735	36 participants, Randomized, Parallel Assignment, Open Label	DC Vaccine, Montanide Vaccine, Poly-ICLC	First line	Humoral immune response	2	Active, not recruiting
NCT04445064	11 participants, Randomized, Parallel Assignment, Open Label	IO102	First line	Number of participants with a T-cell peptide-specific response to the vaccine	2	Recruiting

generate T cells that kill cancer cells, research on class II HLA to induce memory T cells, had been conducted to induce a long-lasting response.

Currently, not all neoantigens in a vaccine produce T cell response, as neoantigens must bind to HLA class I molecules (on the surface of cancer cells), or to class II (on the surface of APCs), as well as to T cell receptors (TCRs) [82]. There are still several opportunities to improve the selection of neoantigen to elicit the best antitumor response.

As NGS and predictive algorithms continue to advance, personalized cancer vaccines will continue to impact the field of immunotherapy. The rationale is to act against cancer cells by promoting immunity by vaccines and removing suppression immunity by inhibitor drugs, besides conventional chemotherapies.

Cancer vaccines targeting immune checkpoint proteins

Immunotherapy has significantly revolutionized cancer therapy by using monoclonal antibodies targeted to immune checkpoint molecules that are very active, even in advanced stages of the disease [84,85 86-

Table 3
Ongoing clinical trials investigating viral vaccines in cancer.

Clinical Trial Identifier Code	Investigation Plan	Viral vaccine/ Drug	Clinical Setting Line	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT04745377	300 participants, Observational, Case-Control	SARS-COV-2	First line	Rate of Covid19 Infection post vaccination	Case-Control	Recruiting
NCT04410874	45 participants, Sequential Assignment, Open Label	Imvamune	First line	MTD	2	Recruiting
NCT03315975	40 participants, Interventional, Single group Assignment, Open Label	Inactivated influenza vaccine	First line	Neutralizing antibody response	4	Active, not recruiting
NCT04521764	33 participants, Interventional, Single group Assignment, Open Label	modified measles virus (MV-s-NAP)	Second line or later line	MTD	1	Recruiting
NCT03848039	1220 participants, Interventional, Randomized Assignment, Open Label	Gardasil-9	First line	Evaluation of DRR	3	Not yet recruiting
NCT01376505	100 participants, Non-Randomized, Parallel Assignment, Open Label	HER-2 vaccine	First line	Safety and duration of immune response	1	Recruiting
NCT04410900	41 participants, Non-Randomized, Parallel Assignment, Open Label	Wistar Rabies Virus	First line	Positive vaccine response	1	Recruiting
NCT03113487	28 participants, Interventional, Single group Assignment, Open Label	Vaccinia Virus expressing p53, Pembrolizumab	Second line or later line	PFS	2	Recruiting
NCT02432963	19 participants, Interventional, Single group Assignment, Open Label	Vaccinia Virus expressing p53, Pembrolizumab	First line	Tolerability	1	Active, not recruiting
NCT02285816	56 participants, Non-Randomized, Parallel Assignment, Open Label	MG1MA3 AdMA3	Second line or later line	MFD	2	Active, not recruiting
NCT03439085	77 participants, Interventional, Single Group Assignment, Open Label	IL-12 DNA plasmids, MEDI0457, Durvalumab	First line	ORR	2	Active, not recruiting
NCT04836793	300 participants, observational, Cohort	Additional biological samples	First line	IgG levels after Covid19 vaccination		Recruiting
NCT02700230	30 participants, Interventional, Single Group Assignment, Open Label	Measles Virus Encoding Thyroidal Sodium Iodide Symporter	First line	Dose Response	1	Recruiting
NCT02865135	11 participants, Interventional, Single Group Assignment, Open Label	DPX-E7 vaccine	First line	SAE	2	Active, not recruiting
NCT04355806	160 participants, Observational, Prospective Assignment,	PD-1/PD-L1 inhibitors, Inactivated trivalent influenza vaccine	First line	IgG levels		Not yet recruiting
NCT04667702	330 participants, Observational, Prospective Assignment,	HPV	First line	Vaccine Hesitancy		Recruiting
NCT04774887	1200 participants, Interventional, Single group Assignment, Open Label	HPV	First line	Risk to HPV	Not Applicable	Not yet recruiting
NCT00092534	12,167 participants, Interventional, Randomized, Single Group Assignment, Double Masking	Gardasil, HPV	First line	Incidence of Endpoint of HPV	3	Active, not recruiting
NCT02977156	22 participants, Interventional, Single group Assignment, Open Label	Pexa-Vec, Ipilimumab	First line	DLTs, ORR	1	Active, not recruiting
NCT03618953	75 participants, Non-Randomized, Parallel Assignment, Open Label	Ad-E6E7 MG1-E6E7 Atezolizumab	First line	Safety	1	Active, not recruiting
NCT03560752	36 participants, Interventional, Single Group Assignment, Open Label	CMV-Modified Vaccinia Ankara Vaccine	First line	Safety	2	Recruiting

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Table 3 (*continued*)

Clinical Trial Identifier Code	Investigation Plan	Viral vaccine/ Drug	Clinical Setting Line	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT02653118	4453 participants, Observational, Cohort, Open Label	V503, GARDASIL	First line	Incidence of HPV		Active, not recruiting
NCT04847050	220 participants, Non-Randomized, Parallel Assignment, Open Label	mRNA-1273	First line	Safety	2	Recruiting
NCT04854980	55 participants, Observational, Prospective Assignment,	Blood	First line	Immune response to vaccine		Recruiting
NCT04580771	35 participants, Interventional, Single Group Assignment, Open label	Cisplatin Liposomal HPV-16 E6/E7 Multi-peptide Vaccine PDS0101	First line	Rate of grade	2	Recruiting
NCT03547999	78 participants, Parallel Assignment, Interventional, Randomized, Open Label	mFOLFOX6, MVA-BN-CV301, FPV-CV301, Nivolumab	First line	OS	2	Active, not recruiting
NCT02415387	180 participants, Crossover Assignment, Interventional, Randomized, Quadruple	typhoid vaccine	First line	Change in IL6 levels	Not Applicable	Recruiting
NCT04935528	430 participants, Single group Assignment, Interventional, Randomized, Open Label	ELISPOT, Serology	First line	seroprevalence of SARS-CoV-2	Not Applicable	Recruiting
NCT05237947	5000 participants, Parallel Assignment, Interventional, Randomized, Double	DTP, Questionnaire, HPV	First line	Incidence of persistent HPV infection	4	Enrolling by invitation
NCT02649439	97 participants, Parallel Assignment, Interventional, Randomized, Open Label	PROSTVAC –V, PROSTVAC-F	First line	Tumor growth rate	2	Active, not recruiting
NCT01867333	57 participants, Parallel Assignment, Interventional, Randomized, Open Label	PROSTVAC-F/TRICOM PROSTVAC-V/TRICOM, Enzalutamide (Xtandi)	First line	Increase in time to progression	2	Active, not recruiting
NCT05078866	45 participants, Single group Assignment, Interventional, Randomized, Open Label	GAd-209-FSP, MVA-209-FSP	First line	Adverse events	2	Not yet recruiting
NCT04041310	84 participants, Sequential Assignment, Interventional, Non-Randomized	GAd-209-FSP, MVA-209-FSP	First line	Toxicity	2	Recruiting
NCT04977024	240 participants, Parallel Assignment, Interventional, Triple masking	COVID-19 Vaccine	First line	biological activity	2	Recruiting
NCT02002182	15 participants, Non-randomized Parallel Assignment, Interventional, Open Label	ADXS11-001 (ADXS-HPV)	First line	HPV-Specific T Cell Response Rate	2	Active, not recruiting
NCT04442048	195 participants, Randomized Parallel Assignment, Interventional, Open Label	IMM-101	First line	rate of "flu-like illness"	3	Active, not recruiting
NCT03603808	80 participants, Single group Assignment, Interventional, Randomized, Open Label	HPV DNA Plasmids (VGX-3100)	First line	ORR	2	Recruiting
NCT05173324	8000 participants, Randomized, Parallel Assignment, Quadruple	HPV vaccine HAV vaccine	First line	HPV prevalent infections	3	Not yet recruiting
NCT03350698	100 participants, Randomized, Single group Assignment, Open label	Gardasil-9	First line	Prevention	4	Recruiting
NCT04635423	1050 participants, Randomized, Parallel Assignment, Triple	V503	First line	Combined incidence of HPV 6/11/16/18-related anogenital persistent infection	3	Active, not recruiting
NCT04274153	130 participants, Single group Assignment, Interventional, Open label	Gardasil9	First line	Immunogenicity of HPV vaccine	4	Recruiting
NCT02834637	930 participants, Parallel Assignment, Interventional, Randomized, Open Label	bivalent HPV vaccine, nonavalent HPV vaccine	First line	Proportion with HPV 16/18-specific seropositivity	3	Active, not recruiting
NCT03284866	536 participants, Parallel Assignment, Interventional, Randomized, Double	Gardasil 9	First line	Occurrence of cervical cancer	3	Recruiting
NCT02649855	74 participants, Parallel Assignment, Interventional, Randomized, Open Label	PROSTVAC-V, PROSTVAC-F, Docetaxel	First line	Response/efficacy	2	Active, not recruiting
NCT03315871			First line		2	Recruiting

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Table 3 (*continued*)

Clinical Trial Identifier Code	Investigation Plan	Viral vaccine/ Drug	Clinical Setting Line	Primary Endpoint	Stage of Development	Clinical Trials Status
	34 participants, Parallel Assignment, Interventional, Non-Randomized, Open Label	PROSTVAC-V, PROSTVAC-F, MSB0011359C		Response of combination immunotherapy		
NCT02396134	133 participants, Parallel Assignment, Interventional, Non-Randomized, Triple masking	CMVpp65-A*0201 peptide vaccine	First line	non-relapse mortality	2	Active, not recruiting
NCT05266898	150 participants, Single group Assignment, Interventional, Open Label	Human papillomavirus 9-valent vaccine	First line	change in serological response to Gardasil-9	4	Not yet recruiting
NCT01824537	1000 participants, Randomized, Factorial Assignment, Interventional, Quadruple	Gardasil 9, Hepatitis A vaccine	First line	Reduction in HPV type concordance	4	Recruiting
NCT04534205	285 participants, Randomized, Parallel Assignment, Interventional, Open Label	BNT113, Pembrolizumab	First line	TEAE and ORR	2	Recruiting
NCT04060277	128 participants, Randomized, Parallel Assignment, Interventional, Open Label	Letermovir, Multi-peptide CMV-Modified Vaccinia Ankara Vaccine	First line	Non-relapse mortality	2	Recruiting
NCT03702231	116 participants, Non-Randomized, Parallel Assignment, Interventional, Open Label	Zoster Vaccine Recombinant, Adjuvanted	First line	Safety and Tolerability	2	Active, not recruiting
NCT04484532	200 participants, Single group Assignment, Interventional, Open Label	Trivalent Influenza Vaccine	Second or later line	Antibody response	4	Recruiting
NCT03180034	25,000 participants, Randomized, Parallel Assignment, Interventional, Double	DTP adsorbed, HPV bivalent, HPV Nonavalent	Second or later line	Incidence of persistent human papillomavirus (HPV)-16 or 18 cervical infections	4	Active, not recruiting
NCT00834093	18 participants, Single group Assignment, Interventional, Open Label	Epstein-Barr Virus Specific Immunotherapy	First line	ORR	2	Active, not recruiting
NCT03728881	1240 participants, Non-Randomized, Parallel Assignment, Interventional, Open Label	Quadrivalent HPV virus, bivalent HPV vaccine	First line	Antibody levels of HPV16	3	Active, not recruiting
NCT02506933	102 participants, Randomized, Parallel Assignment, Double masking	Multi-peptide CMV-Modified Vaccinia Ankara Vaccine	First line	CMV events encompassing any CMV reactivation	2	Active, not recruiting
NCT04046445	96 participants, Non-Randomized, Parallel Assignment, Interventional, Open Label	ATP128, BI 754091, VSV-GP128	First line	safety and tolerability and SAEs	2	Recruiting
NCT02481414	125 participants, Randomized, Parallel Assignment, Interventional, Open Label	PepCan, Candin	Second or later line	Efficacy	2	Active, not recruiting
NCT03391921	170 participants, Randomized, Parallel Assignment, Interventional, Open Label	Vaccine	Second or later line	Rate of seroconversion in HPV antibodies against HPV	4	Active, not recruiting
NCT05262010	13,500 participants, Randomized, Parallel Assignment, Interventional, Open Label	11-valent recombinant human papilloma virus vaccine	First line	Person-years incidence of CIN2 + associated with HPV6/11/16/18	3	Not yet recruiting
NCT04436133	480 participants, Randomized, Parallel Assignment, Interventional, Double	11-valent recombinant human papilloma virus vaccine, Gardasil 9	First line	Anti-HPV neutralizing antibodies GMT	2	Active, not recruiting
NCT03943875	512 participants, Randomized, Parallel Assignment, Interventional, Open Label	9-valent HPV vaccine	First line	Efficacy	4	Recruiting
NCT04482933	30 participants, single group Assignment, Interventional, Open Label	Biological G207	Second line or later line	Efficacy	2	Not yet recruiting
NCT04199689	6000 participants, Randomized, Parallel Assignment, Interventional, Triple masking	9vHPV Vaccine	Second line or later line	Incidence of HPV	3	Active, not recruiting
NCT03903562	1990 participants, Randomized, Single group Assignment, Interventional, Open Label	V503	First line	Serum antibody titers for HPV	3	Active, not recruiting
NCT04953130	10,400 participants, Randomized, Parallel Assignment, Interventional, Open Label	Gardasil HPV vaccine	First line	Impact of HPV vaccination	4	Not yet recruiting
NCT04951323	anti-COVID19 mRNA-based vaccine	anti-COVID19 mRNA-based vaccine (BNT162b2)	First line	Quantification of anti-SARS-CoV-2 receptor binding domain specific IgG	3	Recruiting

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Table 3 (*continued*)

Clinical Trial Identifier Code	Investigation Plan	Viral vaccine/ Drug	Clinical Setting Line	Primary Endpoint	Stage of Development	Clinical Trials Status
NCT02750202	75 participants, Randomized, Parallel Assignment, Interventional, single masking	Quadrivalent HPV vaccine, Hepatitis B vaccine	First line	Change in the genital wart lesion	3	Recruiting
NCT05291845	75 participants, Randomized, Factorial Assignment, Interventional, open label	Candida antigen vaccine, Bivalent HPV vaccine	Second line or later line	complete response	2	Not yet recruiting
NCT05027776	1348 participants, Randomized, Parallel Assignment, Interventional, open label	HPV vaccine	First line	Primary immunogenicity	3	Recruiting
NCT04708041	700 participants, Randomized, Parallel Assignment, Interventional, open label	9vHPV vaccine	First line	GMT of HPV	3	Active, not recruiting
NCT04474821	300 participants, Randomized, Single group Assignment, Interventional, open label	Human Papillomavirus Infection	Second line or later line	Acceptance and completion rates of free HPV vaccination	4	Recruiting
NCT04895020	1200 participants, Single group Assignment, Interventional, open label	9-valent HPV vaccine	First line	primary immunogenicity	3	Recruiting
NCT04422366	8000 participants, Parallel group Assignment, Interventional, open label	9-valent Human Papillomavirus, GARDASIL	First line	person-year incidence of HPV	3	Recruiting
NCT03998254	6000 participants, Parallel group Assignment, Interventional, double label	V503, Gardasil	Second line or later line	Combined Incidence of HPV related 12-month Persistent Infection	3	Active, not recruiting
NCT05285826	8100 participants, Parallel group Assignment, Interventional, double label	9vHPV vaccine	First line	Combined Incidence of HPV 58-related External Genital and Intra-anal 12-month Persistent Infection	3	Recruiting
NCT05279248	300 participants, Parallel Assignment, Interventional, Open label	HPV + MMR,HPV	First line	GMT of anti-HPV 16 and 18 at 7 months	4	Active, not recruiting
NCT04870333	5000 participants, Parallel Assignment, Randomized, Interventional, Open label	Niclosamide, Ciclesonide, Sotrovimab	First line	Prevention	3	Recruiting
NCT05119855	400 participants, Parallel Assignment, Randomized, Interventional, Open label	9vHPV Vaccine, mRNA-1273 Vaccine	First line	GMT of HPV	3	Recruiting

90]. These results led to the study of peptide vaccines capable of generating antibodies against immune checkpoint proteins in the body.

PD-L1-based vaccine made from the fusion of extracellular domain of PD-L1 (PD-L1E) to C-terminal region of translocation domain of diphtheria toxin (DTT), showed to elicit CD4+ T cell response inducing Th1 antitumor immunity in mouse tumor models. In this study, PD-L1E was extracted from sera, blocked the binding of PD-L1 to PD-1 *in vitro*, which revealed a specific interaction. Moreover, PD-L1E vaccination induced an increase in TILs levels and a decrease in LAG3 + PD-1 + levels and CD8+ T cells. The data suggest that the PD-L1 vaccine reverses tumor suppression and could be a promising strategy for cancer therapy [83].

Another strategy consists of DCs loaded with an immunogenic PD-L1 (PD-L1-Vax), which has been shown to induce anti-PD-L1 immune responses and tumor inhibition in cancer cells expressing PD-L1 [84].

Kaumaya et al. recently developed a novel chimeric B-cell peptide epitope capable of targeting PD-1 linked to measles fusion protein (MVF, sequence 288–302) T-cell epitope, that could elicit polyclonal antibodies in the body, enabling blockage of PD-1 signaling, and mimicking the effects of nivolumab. The authors observed that their vaccine candidate with epitope sequence 92–110 (PD-1-Vax), significantly reduced tumor growth in the syngeneic BALB/c CT26 mouse model [85].

Neoantigen vaccines potentiating the immune response

Although neoantigens vaccines have been extensively studied for personalized immunotherapy, the vast majority of neoantigens have very minimal to no immunogenicity. An important role is played by adjuvants, because they can elicit a powerful immune response. Among other approaches that can potentiate immunogenicity of neoantigens to develop powerful and durable cancer response, there are: synergistic modulation of multiple immune signaling pathways, presence of multiepitope antigens that elicit a broad spectrum of immune responses, and cancer-specific antigens capable of inducing a specific adaptive immune response.

Currently, adjuvants such as polyinosinic-polycytidylic acid-poly-L-Lysine carboxy methyl cellulose (poly-ICLC) in combination with anti-CD40 have been used in neoantigen vaccines. However, not all adjuvants can induce a robust immune response, and some soluble vaccine formulations may also limit the immunogenicity of the vaccine itself [86,87]. To overcome these challenges, pathogen-imitating nanovaccines were created and seem to have a great potential in improving the immunogenicity of neoantigens for cancer immunotherapy. Due to their small size (5–100 nm), nanovaccines can be effectively delivered to secondary lymphoid tissues like lymph nodes and APCs, where they can be retained for a long time. Delivering neoantigens with multiple synergistic adjuvants into lymph nodes [106-109] or APCs [88-90] is an essential step for ideal immunotherapy.

The administration through encapsulation of adjuvants and neoantigens can improve the pharmacokinetic properties of drug payloads, further enhancing immunomodulation. Recently, bi-adjuvant neoantigen nanovaccines (banNVs) co-delivered a peptide neoantigen with two adjuvants, TLR7/8 agonist R848, and TLR9 agonist CpG oligos, were developed to enhance immunogenicity. The combination of banNVs together anti-PD-1-induced potent and durable cancer immunotherapy when combined with anti-PD-1 [91]. TLR7/8 agonists used for cancer treatment in clinics [91], especially imiquimod and resiquimod (R848) are US FDA-approved drugs to treat topical skin lesions. The combination of these immune adjuvants exhibited synergistic therapeutic efficacy through the TLR-Myd88 pathway [91]. However, these drugs' poor solubility and unfavorable pharmacokinetics have desisted them from being used together with immunotherapy [88-90]. The engineering of these drugs using nanocarriers could overcome this

Table 4
Neoantigen prediction softwares.

Software (references)	Principle	Year
NeoPredPipe [140]	Connects commonly used bioinformatics software using custom python scripts giving neoantigen burden, immune stimulation potential, tumor heterogeneity and HLA haplotype of patients.	2019
Strelka2 [141]	Estimates error or deletion parameters of each sample improved tumor liquid analysis	2018
MuPeXI [142]	Identifies tumor-specific peptides through the extraction and induction of mutant peptides, it can predict immunogenicity and evaluate the potential of novel peptides	2017
CloudNeo pipeline [143]	The docker container executes the tasks. After giving as an input mutant VCF file and bam FILE representing HLA typing, the software predicts HLA affinity all mutant peptides.	2017
pVAC-Seq [144]	Integrates tumor mutation and expression data to identify personalized mutagens through personalized sequencing.	2016
NetMHCpan [145]	The sequences are compared using artificial intelligence neural network and predict affinity of molecular peptide-MHC-I type	2016
VariantEffect Predictor Tool [146]	It uses automated annotations to manual review time and prioritize variants	2016
Somaticseq [147]	It uses a randomized enhancement algorithm, which has more than 70 individual genome sequence features based on candidate sites to accurately detect somatic mutations	2015
OptiType [148]	It uses an HLA type algorithm with a linear programming that gives sequencing databases comprising RNA, exome and whole genome sequencings.	2014
ATHLATES [149]	It assembles allele recognition, pair interface applied to short sequences and HLA genotyping at allele level achieved via exon sequencing	2013
VarScan2 [150]	It detects somatic and copy number mutations within tumor-normal exome data using a heuristic statistical algorithm.	2012
HLAminer [151]	Through a shotgun sequencing Illumina database platform, predicts HLA type through an orientation of the assembly of the shotgun sequence data to then compare it with databases of allele sequences used as references.	2012
Strelka [152]	It uses a Bayesian model that matches normal-tumor sample sequencing data to analyze and predict with high accuracy and sensitivity somatic cellular variations	2012
SMMPMBEC [153]	Through a Beyesian matrix based on optimal neural network they can predict peptide molecules with MHC-I	2009
UCSC browser [154]	The fusion of various databases can give fast and accurate access to any gene sequence.	2002

problem. Indeed, nanoparticles can be used to effectively encode more adjuvants and neoantigens and to enhance immune response. Adjuvants in neoantigenic vaccines promoted the response of cytotoxic T cells to specific neoantigens, leading to complete tumor destruction when combined with immune checkpoint blockade.

Ongoing clinical trials for neoantigens vaccines

The *in vitro* transcribed mRNAs have been administered differently and formulated as naked mRNA in buffer or LNP. These studies are based on the knowledge generated by Theilemans et al. showing that it is possible to generate powerful, clinical-grade IVT mRNA DC vaccines through electroporation [92-96]. Various studies have been conducted to find a way to directly deliver neo-antigen mRNA to APCs [97,98].

Shahin et al. identified somatic mutations in tumor biopsies of 13 patients with stage III/IV using whole genome/exome and RNA sequencing techniques compared to control. After ranking mutations, they predicted binding affinity to patients' HLA-I/II molecules. mRNA vaccines were generated against HLA-I and HLA-II, and mRNA doses of

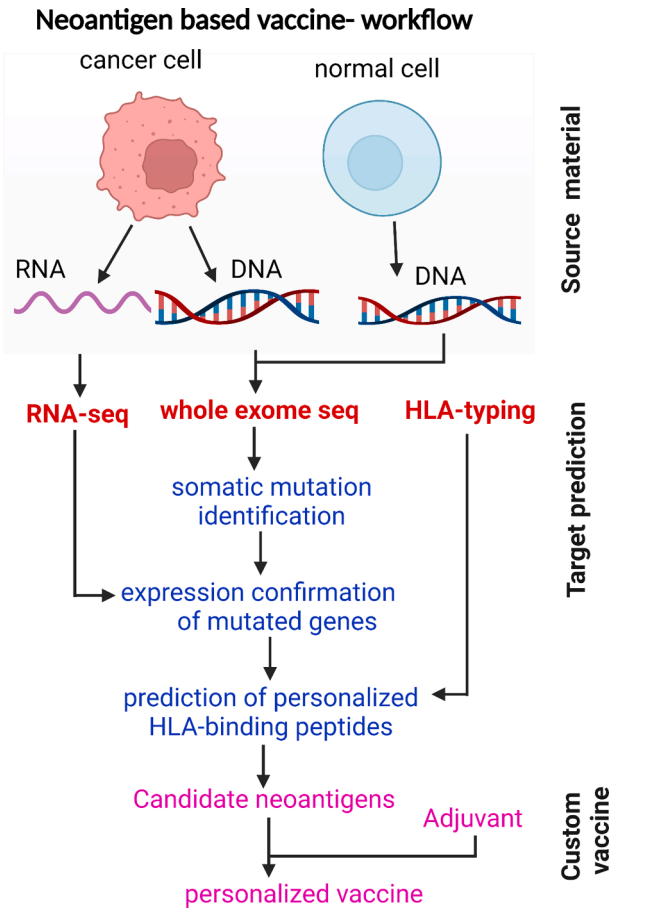

Fig. 2. Identification of neoantigens: Tumor-specific mutations are identified using whole-exome sequencing (WES), confirmed by RNA sequencing, then ranked by predicted affinity binding to HLA types; finally, neoantigens are synthesized based on mutated alleles followed by *ex vivo* T-cell reactivity analysis to confirm the immunogenicity.

0.5–1 μg per vaccination course and injected in the inguinal lymph nodes. Patients with tumors displaying TAAs such as NY-ESO-1 and tyrosinase received an mRNA-based vaccine targeting these TAAs [99]. The results were encouraging: eight patients had no radiologically detectable tumors when neoepitope vaccination started and remained without recurrence in 12–33 -months follow-up; five patients had the metastatic disease before vaccination, and two of them experienced objective responses, a third patient exhibited complete response for combined treatment of PD-1 blocking antibody. All patients showed Tcell responses against neoepitopes with 60% response. The same strategy was tested in a subsequent clinical trial with mRNA developed based on somatic mutations and LNP adjuvant delivered intravenously in patients with triple-negative breast cancer. Various drug administration methods could impact the efficacy of mRNA vaccination, as previously observed in animal studies using nanoparticle vaccines with neoantigen peptides linked to TLR7/8 agonist [100]. The authors showed that intravenous injection (i.v.) vaccination induced a higher proportion of CF1 + PD-1 + CD8+ T cells versus subcutaneous injection. Additionally, stem cells were induced by i.v injection, whereas effector genes were induced by subcutaneous injection [100]. Various clinical trials are currently investigating safety and efficacy of neo-antigens mRNA using different delivery methods and formulations either alone or in combination with other therapies. Ongoing clinical trials for neo-antigen vaccinations for cancer therapy are summarized in Table 5.

Table 5
Ongoing clinical trials investigating cancer neo-antigen vaccines.

Ongoing clinical trials investigating cancer neo-antigen vaccines Study	Cancer Type	Phase	Neo Antigen	Modality	Co-treatment	Status
NCT03639714	Solid tumors	I/II	GRT-C901/2	NN	Nivolumab/ipilimumab	Recruiting
NCT04864379	Solid tumors	I	iNeo-Vac-P01	NN	Anti-PD-1	Recruiting
NCT04072900	Melanoma	I	rhGM-CSF	NN	Anti-PD-1	Recruiting
NCT02287428	Glioblastoma	I	Personalized NeoAntigen Vaccine	NN	Pembrolizumab/ Temozolomide/Radiation Tehrapy	Recruiting
NCT03361852	Follicular Lymphoma	I	Neo Vax	s.c.	Rituximab	Not yet recruiting
NCT03219450	Lymphocytic Leukemia	I	NeoVax	s.c.	Pembrolimuzab/ Cyclophosphamide	Not yet recruiting
NCT02950766	Kidney Cancer	I	NeoVax	s.c.	Ipilimumab	Recruiting
NCT04810910	Resectable Pancreatic Cancer	I	iNeo-Vac-P01/ GM-CSF	NN	NA	Not yet recruiting
NCT04024878	Ovarian Cancer	I	NeoVax	s.c.	Nivolumab	Recruiting
NCT03953235	Solid tumors	I/II	GRT-C903/4	NN	Nivolumab/ipilimumab	Recruiting
NCT03807102	Lung Cancer	I/II	Neoantigen Tumor Vaccine	NN	NA	Recruiting
NCT04087252	Solid tumors	I	Tumor neoantigen	i.m.	NA	Recruiting
NCT03359239	Urothelial/Bladder Cancer, NOS	I	Multipeptide Personalized Neoantigen Vaccine (PGV001, ICLC)	NN	Atezolizumab	Recruiting
NCT04749641	Diffuse Intrinsic Pontine Glioma	I	Histone H3.3-K27M Neoantigen Vaccine Therapy	s.c.	NA	Recruiting
NCT04799431	mPCmCRC	I	Neoantigen Vaccine with Poly-ICLC adjuvant	s.c.	Retifanlimab	Not yet recruiting
NCT04912765	Solid Tumors	II	Neoantigen Dendritic Cell Vaccine	i.d..	Nivolumab	Recruiting
NCT04397926	NSCLC	I	Individualized neoantigen peptides vaccine	s.c.	NA	Recruiting
NCT04487093	NSCLC	I	neoantigen vaccine	s.c.	1) EGFR-TKI Anti-angiogenesis	Recruiting
NCT03122106	Pancreatic cancer	I	Personalized neoantigen DNA vaccine	NN	NA	Active, not recruiting
NCT03956056	Pancreatic cancer		Neoantigen Peptide Vaccine, Poly ICLC	s.c.	NA	Recruiting
NCT03199040	TNBC		Neoantigen DNA vaccine, TDS-IM system (Inchor Medical Systems)	NN	Durvalumab	Recruiting
NCT03655756	Melanoma Stage III/IV	I	IFx-Hu2.0	s.c.	NA	Active, not recruiting
NCT04397003	Extensive-stage SCLC	II	Neoantigen DNA vaccine	NN	Durvalumab	Not yet recruiting
NCT02129075	Cutaneous, Mucosal and Ocular Melanoma	II	DEC-205/NY-ESO-1 Fusion Protein CDX-1401, Neoantigen-based Melanoma-Poly-ICLC Vaccine	s.c.	NA	Active, not recruiting
NCT04266730	Squamous NSCLC SCC of Head and Neck	I	PANDA-VAC	s.c.	Pembrolizumab	Not yet recruiting
NCT03558945	Pancreatic Tumor	I	Personalized neoantigen vaccine	s.c.	NA	Recruiting
NCT03468244	Solid tumors, lymphoma	NN	Naked mRNA	s.c.	NA	Recruiting
NCT03815058	Metastatic melanoma	II	LNP	i.v.	Pembrolizumab	Recruiting
NCT03897881	High-risk melanoma	II	NN	NN	Pembrolizumab	Recruiting
NCT03908671	Esophageal cancer, NSCLC	NN	LNP	s.c.	NA	Not yet Recr4 times uiting
NCT04161755	Pancreas cancer	I	NN	NN	Atezolizumab, chemotherapy	Recruiting

Abbreviations: *i.d., intradermal; i.m., intramuscular; i.n., intranodal; i.v., intravenous; s.c., subcutaneous; NN, not known, NSCL, non-small cell lung cancer; SCC, squamous cell cancer; TAA, tumor-associated antigen; TNBC, triple negative breast cancer; LNP, lipid nanoparticle; NA, not applicable; mCRC, metastatic colorectal cancer; mPC, metastatic pancreatic cancer; TNBC, triple negative breast cancer, SCLC, small cell lung cancer.*

Conclusions

A new age of cancer vaccines has started with the first LNP mRNA vaccines being FDA approved as safe and effective in preventing infections by SARS-CoV-2 causing COVID-19. Such new methods could be important to other medical fields, especially therapeutic anticancer vaccines. Several clinical trials are testing LNP mRNA anti-cancer vaccines after encouraging *in vitro* results. Current methods for delivery of nucleic acid-based vaccines (RNA and DNA), like electroporation and intradermal needle-free system, are also advancing rapidly. Moreover, with the advent of immune therapies, stimulation of the immune system through checkpoint inhibition provides a valid rationale for the combination of therapeutic cancer vaccines together with immune-stimulating agents. Additional methods to further stabilize vaccines in the blood system should be considered in future research. DNA vaccines with more efficient delivery methods and combined with nanoparticles' adjuvants could become a valid and potentially superior alternative to current RNA vaccines. In fact, DNA vaccines are simpler to design. With the impetus to the vaccination field, significant improvements are also expected in cellular and peptide-based anticancer vaccines.

CRediT authorship contribution statement

Navid Sobhani: Conceptualization, Supervision, Data curation, Visualization, Writing – original draft, Reviewing and editing. **Bruna Scaggiante:** Conceptualization, Data curation, Formal analysis, Supervision, Writing and editing the draft. **Rachel Morris:** Data curation, Conceptualization, Software, Visualization, Writing – original draft.

Dafei Chai: Conceptualization, Investigation, Visualziation, Writing and editing. **Martina Catalano:** Data curation, Investigation, Resources, Software, Visualization, Writing – review & editing. **Dana Rae Tardiel-Cyril:** Data curation, Formal analysis, Methodology, Project administartion, Writing – review & editing. **Praveen Neeli:** Conceptualization, Software, Visualization, Writing and editing. **Giandomenico Roviello:** Conceptualization, Formal analysis, Supervision, Writing – original draft. **Giuseppina Mondani:** Conceptualization, Validation, Writing – review & editing. **Yong Li:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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