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Review

Research Progress of Tumor Neoantigen Nano-Vaccines

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Abstract

A personalized vaccine based on neoantigen is a new immunotherapy method showing great potential in solid tumors. Neoantigen vaccines can induce specific immune responses and antitumor effects. It can significantly prolong the survival time of patients after surgery and inhibit cancer metastasis. The nano-vaccines make cancer vaccines more stable and effective. Currently, the technology of neoantigen based nano-vaccines has developed rapidly. This review will describe the research progress of tumor neoantigen nano-vaccines on three aspects, recognition, processing, presentation and identification of neoantigen, current research progress of nano-vaccines and their adjuvants and the clinical trials of neoantigen nano-vaccines.

Keywords: Neoantigen; Nano-Vaccine; Tumor Vaccine; Adjuvant; Identification of Neoantigens; Personalized Neoantigen Vaccine.

Introduction

Cancer kills millions of people, making it one of the leading health killer worldwide. From 2018 to 2020, cancer cases increased from 18.1 to 19.3 million, and deaths increased from 9.6 to 10 million (1). These data show that malignant tumor are globe healthy threaten. Some studies have predicted that cancer cases will reach 28.4 million by 2040 (2). Therefore, it is necessary and urgent to prevent and

treat cancers. Scientists have been committed to finding novel therapeutic strategies to conquer malignant tumors for years. Surgery is the most common treatment for early diagnosed patients, while radiotherapy, chemotherapy and targeted therapy also play an irreplaceable role. But the recurrences are common within 5 years. Scientists are trying to find effective methods to eliminate cancer incidence. Neoantigen vaccines as novel immunotherapy methods have shown some successful outcomes (3).

Based on the successful development of vaccinology, the utilization of cancer-related vaccines was boldly accepted. Vaccines can induce robust immune responses and produce long-term protective effects (4). Cancer vaccines can be divided into preventive and therapeutic cancer vaccines. The preventative vaccine aims to prevent cancers in high-risk populations infected with carcinogenic viruses. Therapeutic cancer vaccines, as we know, can treat patients who have pre-existing cancers (5). For example, one of the therapeutic cancer vaccines was based on the $CD8^+$ cytotoxic T lymphocyte (CTL) and $CD4^+$ T helper (Th) cells to stimulate the immune response via MHC class I and CLASS II presentation (6-8). Due to the rapid development of genomic sequence, the development of personalized cancer vaccines (PCV) can target tumor-specific mutated neoantigens (neoAg).

However, the road to cancer neoantigen vaccine is tortuous, but the outcomes are hopeful. The ideal cancer vaccine should contain powerful antigens and a strong and effective delivery vehicle. This review will describe the progression of neoantigen from three different aspects. The extraction and processing of neoantigen, the current research progress of nano-adjuvant and the clinical research of nano-antigen vaccine.

1. Neoantigen

1.1 Neoantigen generation, processing and presentation

In the development of cancers, genetic alterations and instability includes point mutations (95%), inset-deletion and frame-shift mutations (13, 14). Normally, the mutations occur in non-coding or coding regions, which lead to amino acid sequence changes, and produce short peptides (2-20 amino acid residues) not found in normal cells. These short peptides are called neoantigens. Neoantigens were first identified in melanoma in 1995 (9). Since then, many neoantigens have been found in non-small cell carcinoma (11) and other cancers (12). The neoantigens are transported to the endoplasmic reticulum and loaded onto the major histocompatibility complex (MHC) (15). These major histocompatibility complexes then enter the membrane through the Golgi apparatus, causing antigen presentation and recognition by receptors on cytotoxic T cells, which are activated to exert their tumor-killing effects (16, 17). At the same time, it can cause anti-tumor immune memory and resist tumor recurrence. The second mechanism involves gene mutations that alter the peptide residues on T cells interacting with the T cell receptor (TCR) (18). These mutated peptides activate T cells with TCR libraries, unlike those that recognize non-mutated peptides. However, current technologies have limited potential to predict T cell recognition or therapeutic potential and research has focused on the first generation mechanism.

Neoantigens are expressed only in tumor tissues, not in normal tissues, which determines their specificity and ability to overcome central and peripheral immune tolerance and minimize the risk of autoimmunity. These visible advantages lay a solid foundation for the realization of personalized cancer therapy vaccines.

1.2 Neoantigen recognition and identification

It is well known that antigen recognition is at the heart of an effective immune response, and a common way of recognizing neoantigens is through

T cells. Two studies by Wolfel *et al.* (19) and Coulie *et al* (9) provide the first evidence that T cells recognize human cancer mutant peptides. The study showed that cDNA libraries prepared from tumor cell lines were used to identify tumor-associated RNA transcripts that enhance the sensitivity of target cells so that they are recognized by tumor-specific T cells. Similar techniques were used to identify neoantigens in other patients in the following years. To identify neoantigens, people have attempted to develop research strategies with high throughput strategies (20) to detect somatic mutations. These strategies ideally include differential gene expression analysis of tumors and corresponding normal tissues combined with HLA elution and mass spectrometry. Because a large amount of tumor tissue is required, it is not suitable for routine operation. The advent of cancer genome sequencing, beloved by researchers for its rapid and systematic identification of unique tumor antigens, has broken this dilemma. Subsequently, genome sequencing has played an irreplaceable role in identifying cancer neoantigens (21). Massively parallel DNA sequencing (next generation) technology reduces the cost and time required to sequence the human genome. Further, it reduces the time needed to sequence the cancer genome by expanding it.

Currently, the standard strategy for identifying neoantigen candidates is to screen for somatic mutations by comparing the genomes of tumors and normal tissues based on analysis of whole-exome sequencing (WES) data (22). In addition, gene expression analysis by RNA sequencing (RNA-SEQ) or microarray has been used to predict candidate neoantigens from somatic mutations detected by WES. However, RNA-seq or microarray expression analysis does not necessarily mean that mutated mRNAs exist in cancer cells because the gene expression level is determined independently of the mutated location (23). Therefore, target fragments containing each mutation should be amplified from tumor cDNA and sequenced to verify the mRNA sequence of the mutation in the cancer cell itself. The next step is to detect the binding affinity between the peptide and human leukocyte antigen to predict the neoantigen. At the same time, the possible neoantigen is sorted. The immune response induced by the strong binding affinity is bound to be stronger than the immune response induced by the weak binding affinity. One study showed that not all predicted neoantigens are immunogenic. Only a small number of mutated and processed peptides can be presented on MHC-I and recognized by T cells to be effective (24). Predicting the binding affinity between peptides and human leukocyte antigen (HLA) is crucial in developing potent tumor vaccines. Therefore, predicting the binding affinity between mutated peptides and MHC- molecules to predict neoantigens is the focus of current research. Mutated peptides are presented on the surface of tumor cells by MHC-I-like molecules and recognized by MHC-I-like molecules to generate neoantigen epitopes that drive effective $CD8^+$T cell responses (25). Predicting the recognition of neoantigen epitopes by T cells can also yield neoantigens.

Although neoantigens have strong immunogenicity, different neoantigens in the same tissue have different immunogenicity and different abilities to be recognized by T cells. Therefore, it is necessary to prioritize them through certain calculations to develop the most effective personalized vaccine. Identifying and prioritizing neoantigens include mass spectrometry in vitro T cell analysis and silicon predictive peptide-MHC binding (26). At present, the accuracy of the MHC-I class combination prediction algorithm is relatively high, up to 99% (27, 28). However, the MHC-II class combination prediction algorithm has low accuracy, and further research is needed to improve its prediction accuracy. Current studies have shown that the combination of MHC-I and MHC-II classes shows promising results (29-31). It has been confirmed by experiments (32) that next-generation

sequencing, and epitope prediction strategies can identify and prioritise candidate neoantigens for immune targeting in breast cancer.

1.3 The positive rate of neoantigen in different tissues and related clinical studies

In recent years, oncology clinical trials have demonstrated the feasibility, safety and immunogenicity of personalized neoantigen vaccines for cancer patients. They all made use of next-generation sequencing technology and the ability of computing pipelines to recognize neoantigens in real-time (33-38). The following categories describe the clinical trials and progress of neoantigens studied.

Melanoma： Melanoma is a malignant tumour with a high degree of malignancy and a high mortality rate. There are also many clinical studies on neoantigen therapy for melanoma, and some progress has been made in neoantigen dendritic cell vaccines. Spontaneous immunization in patients with advanced melanoma has expanded the antigen breadth and clonal diversity of anti-tumor immunity. However, there is still a lack of systematic evaluation on whether neoantigens can be effective targets of anti-tumor immunity (33). The body's response to neoantigen epitopes is low. Ugur Sahin *et al*. injected melanoma patients with a vaccine made of mutated RNA-based new epitopes. The results showed that patients had higher T cell response to multiple vaccines and new epitopes, reduced tumor metastasis rate and prolonged progress-free survival. The experiment opens the way for personalized immunotherapy for cancer patients (37). Ott *et al*. demonstrated for the first time the feasibility, safety and immunogenicity of the combination of personalized neoantigen vaccines and PD-1 inhibition in treating advanced solid tumors. Vaccines-induced T cells will last for some time, show cytotoxicity and migrate to tumors (38). A phase I/II trial of Adoptive T-cell therapy(ACT) in 27 patients with melanoma showed that patients' clinical benefit was associated with a higher predicted neoantigen load, and higher mutations and predicted neoantigen load were significantly associated with improved progression-free and overall survival. Clinical benefits were related to the expression of immune-activating markers, including higher MHC-I antigen treatment and performance scores (39). The same neoantigen long peptide vaccine trial demonstrated that the T-cell response induced by the individual neoantigen peptide vaccine could last for many years and amplify tumor-specific cytotoxicity in melanoma patients (40). It can be concluded from the above clinical studies that the immunotherapy of neoantigen has shown great potential in melanoma, which will show good clinical effects without major adverse events when combined with other treatment methods to treat melanoma.

Lung cancer is first cancer threatening human health, with high morbidity and mortality, while non-small cell lung cancer is the most malignant and studied tumor. Karsaki *et al.* created a shared list of missense mutations for lung cancer patients and used this list to infer ready-made, personalized neoantigens for vaccine use. However, the results were not satisfactory. Only 20% of the 15 lung cancer patients had an overlap with the existing neoantigen, and the remaining 80% had no overlap with the existing neoantigen. This indicates that even though the cost of multi-targeted sequencing analysis is lower than that of whole-genome sequencing, the prediction efficiency of individual neoantigens is far less than that of whole-genome sequencing (41). Whole-genome sequencing has shown an irreplaceable role in the development of tumor neoantigens. Neoantigen-based dendritic cell vaccine in lung cancer can control the development of lung cancer, prolong progression-free survival and overall survival with high safety and no more than grade 2 adverse events (42).

Hepatocellular carcinoma: Zhixiong Cai *et al.* obtained tumor tissue and precancerous tissue of patients through surgery, conducted a series of

sequencing, identification, processing and extraction to prepare a new antigen vaccine, and then inoculated patients. The results showed that the neoantigen vaccine effectively resisted the recurrence of liver cancer and had a high safety (43).

Spongioblastoma: A phase I/Ib, neoantigen vaccine trial in patients with glioblastoma showed that the neoantigen vaccine-induced intratumor invasion of the T-cell tumor with high safety (35), and the progression-free survival of the patients was effectively prolonged. However, more research is needed to prepare the glioblastoma neoantigen vaccine.

Breast cancer: Analysis of fresh tumor samples from breast cancer patients showed no positive correlation between neoantigen load and immune-related gene expression, but synthetic neoantigen peptide-pulsed dendritic cells induced CTL from peripheral blood lymphocytes. Endogenous neoantigens in the breast cancer microenvironment are insufficient to trigger an immune response in the tumor microenvironment (44). Thus, neoantigen analysis may help develop strategies to induce T-cell responses.

Leiomyosarcoma of uterus: The identification of neoantigen vaccines can also be used to study the mechanisms of tumor immunotherapy sensitivity and drug resistance. A study of metastatic leiomyosarcoma of the uterus showed diffuse staining for PD-L2 and sparse staining for PD-L1 in both tumors, and PD-1 $^{+}$ cell infiltration was significantly reduced in the drug-resistant group (p=0.02). On the genome, the treatment-resistant tumor-specific double-allele PTEN gene was lost and reduced the expression of two highly immunoreactive neoantigens with the patient's T cells (45). Loss of PTEN is associated with resistance to anti-PD-1 checkpoint blocking therapy in metastatic uterine leiomyosarcoma.

Pancreatic cancer: A study of 23 patients with surgically resected pancreatic adenocarcinoma vaccinated with a vaccine consisting of long mutated peptides found that 85% of twenty evaluable patients developed an immune response, and three patients developed a memory response nine years after vaccination. The median and 10-year survival was increased in all patients so that k-RAS vaccination could consolidate the outcome of surgery in patients with pancreatic adenocarcinoma (46). A 62-year-old woman who underwent surgery for pancreatic cancer with Surviving 2B peptides (SVN-2B) vaccines, including IFA and INF- was well controlled after isolated lung metastases and survived for more than 10 years. It demonstrates the therapeutic potential of a personalized neoantigen vaccine (47). The pancreatic cancer long peptide vaccine into vac-P01 prolongates patients' mean overall survival (OS) and progression-free survival (PFS), stimulates long-term immune function of memory T cells and has the potential to kill cancer cells. It was one of the first studies to address the issue of a personalized vaccine for pancreatic cancer (48). As a good treatment for patients with advanced pancreatic cancer, the timing of administration is important because of the difference in the therapeutic efficacy of patients.

Ovarian cancer: By analyzing new epitope recognition in patients with epithelial ovarian cancer, a tumor type with relatively low mutation volume, we found that new epitope recognition in tumor-infiltrating lymphocytes was easier to find than in circulating lymphocytes and showed higher functional affinity and higher predictive affinity. This opens the possibility of personalized immunotherapy based on mutations. However, this experiment was limited to $CD8^{+}$ T cells, ignoring a potentially critical $CD4^{+}$T cell response and failing to identify neoantigen-reactive T cells that recognize autologous tumors due to the inability to generate autologous tumor cell lines (49).

Clear cell carcinoma of the kidney: Personalized mRNA vaccine based on neoantigen is a novel strategy for treating renal clear cell

carcinoma (KIRC). Hang Xu *et al.* identified potential candidate genes for the development of mRNA vaccines in KIRC through gene sequencing in tumor tissues, combined with the expression and mutation data of KIRC, and finally selected four genes positively correlated with APCs (TOP2A, FCN4, FMNL1 and DOK3), which eventually trigger a robust immune response against the tumor (50). They also identified two subtypes of renal cell carcinoma, which benefited differently from the mRNA vaccine.

Gastroenteric tumor: Based on neoantigens mRNA vaccine safety and immunogenicity of antigen research has obtained the inevitable result in the gastrointestinal tract tumor, the vaccine encoding of patients with metastatic GI cancer antigen of defining new driver mutations and HLA-I predict table, but because of the number of cases is less and less tumor tissue, there is no analysis within the tumor cell bank after vaccination. It is still significant for combination therapy of epithelial tumors (51). There are also many ongoing studies on neoantigen therapy for tumors, and the positive rate of neoantigen in each tissue and its clinical trials have been summarized in **Table 1**.

2 Research status of the nano-vaccine platform

We report a kind of biomimetic nanoparticle anticancer vaccine that can transport tumor antigen materials from autologous cells and adjuvants for immune stimulation, promote the enhancement of antigen presentation, and activate tumor-specific cell response. In addition, when combined with checkpoint blocking therapy to help break tumor immunosuppression, nanovaccine formulations can significantly inhibit tumor growth in a therapeutic setting (52).

2.1 Research status of nano adjuvants used in vaccines.

Vaccine adjuvants have been developed to enhance the immunogenicity of vaccines. According to their primary mechanism of action, adjuvants can be divided into two categories, vaccine delivery systems and 'immune-stimulating adjuvants'. Vaccine delivery systems are usually granular, such as emulsions, particles, iscom and liposomes. Immune-stimulating adjuvants are primarily derived from pathogens and usually represent pathogen-associated molecular patterns (PAMP), such as LPS, MPL, and CpG DNA, that activate cells of the innate immune system (53). In a phase II adjuvant study compared with IFN- 2b, CSF-470 vaccine (BCG and GM-CSF as adjuvants) significantly extended distant metastasis-free survival (DMFS) in stage II-II-III melanoma patients, and the vaccine-induced an immune response to vaccine cells (54). With the wide application of adjuvants, more and more adjuvants have been developed, and nano adjuvants have attracted wide attention. There are known types of nano adjuvants liposomes and lipid NPs, polymeric NPs, protein conjugate, inorganic particles and microneedles. Next, the research progress, advantages and disadvantages of various nano adjuvants will be described.

2.1.1 Liposomes and lipid NPs

Due to their biocompatibility and biodegradability, liposomes are a critical platform for drug delivery and formulation. They are typically made of synthetic lipid bilayer membranes, biofilms that mimic cell membranes and use to trap drugs in a water core. Under the protection of the lipid membrane, drugs inside can be transported to the target tissue or cells. Lipid membranes also protect the drug from hydrolysis or oxidative degradation, which reduces toxicity and increases the duration of drug circulation in the body, thereby increasing drug bioavailability and providing sufficient time for drug molecules to reach disease targets. The ability of liposomes to carry lipophilic and hydrophilic compounds makes liposomes one of the favorite research topics of drug carriers for scientists of various disciplines (55). It is these

Table 1. The positive rate of neoantigen in different tissues and related clinical studies.

Cancer (ref)	Neoantigen (%)	Vaccine type	Date	Result	Patient	Trial	Advantage	Disadvantages	Nanoparticle	Adjuvant
Hepatocellular carcinoma (43)	33.5	Long peptide vaccine	2021	RFS prolonged, no obvious adverse reactions occurred, effectively resisting the recurrence.	10	ChiCTR1900020990	High specificity, low side effects, easy preparation	Small sample sizes	NO	0.5 mg poly: IC
Melanoma (37)	52.0	RNA vaccine	2017	Enhance T cell immune response, inhibit tumor metastasis and recurrence, and prolong progression-free survival.	13	NCT02035956	High specificity, safe and feasible	Sensitivity needs to be improved	YES	NO
Spongioblastoma (35)	50.9	Long peptide vaccine	2019	T cell immune responses and neoantigen-targeted vaccines can potentially alter the immune environment of glioblastoma beneficially.	10	NCT02287428	Low toxicity, high specificity, feasibility	Small sample size, tumor recurrence, and death from malleable disease	NO	poly-ICLC, Hiltonol, Oncovir
Breast cancer (44)	72.5-78	DC vaccine	2021	There was no positive correlation between neoantigen load and immune-related gene expression, and endogenous neoantigens in the breast cancer microenvironment were insufficient to trigger an immune response in the tumor	31	FGCC-EC001	High specificity	Small sample sizes, it was not examined whether the predicted neoantigen peptides were expressed on the tumor surface with MHC class I molecules.	NO	NO

				microenvironment.						
Lung cancer (42)	43.1	DC vaccine (Neo-DCVac)	2021	Control the development of lung cancer, prolong the progression-free survival and overall survival, and has high safety, without the occurrence of grade 2 adverse events.	12	ChiCTR-ONC-16009100, NCT02956551	Low toxicity, high specificity, tolerable.	Antigen-specific $CD4^+$ T cell and $CD8^+$ T cell responses were analyzed in only six patients.	NO	Poly (I: C)
Melanoma (39)	33.6	Adoptive T-cell therapy	2017	Predicted neoantigen load were significantly associated with improved progression-free and overall survival.	27	NCT00937625	-	The sample size was small, and the threshold of mutation load was not refined.	NO	NO
Lung cancer (41)	41.5	Immunotherapy	2015	Using off-the-shelf tubes is not a good thing for most patients with NSCLC. Comprehensive genome sequencing is a satisfactory method of identifying mutations.	15	G3545	-	-	NO	NO
Pancreatic cancer (48)	16.4	Long peptide vaccines (NeoVac-P01)	2021	In-vac-p01 may improve the limited clinical efficacy of pancreatic cancer.	7	03645148	Low toxicity, no grade 3 or 4 adverse events, good specific immunogenicity	With the small number of cases, the retrospective study did not unify the dosing time.	NO	GM-CSF
Melanoma (36)	-	Long peptide vaccine	2017	Personal neoantigen vaccines greatly expand the pool of neoantigen-specific	10	CLIA22D205565 2	Safety, feasibility		YES （Poly-ICLC）	Hiltonol

				T cells, addressing tumor heterogeneity and reducing the chance of tumor escape.						
Clear cell carcinoma of the kidney (50)	-	mRNA vaccine	2021	TOP2A, FCN4, FMNL1 and DOK3 were identified as candidate genes for specific neoantigens, and renal cell carcinoma immune subtype 1(RIS1)H and RIS2 were identified, and RIS2 may benefit more from mRNA vaccine.			Feasibility		Unknown	Unknown
Melanoma (40)	-	Long peptide vaccine (NeoVax)	2021	The T-cell response induced by the individual neoantigen peptide vaccine can persist for many years and magnify tumor-specific cytotoxicity in melanoma patients.	8	NCT01970358	Safety, feasibility, specificity.	Small sample size, difficult for a single neoantigen vaccine to induce a memory T-cell response, requiring a combination with other therapies.	NO	poly-ICLC
Melanoma (33)	60.2	Dendritic cell	2015	The antigen breadth and clonal diversity of antitumor immunity were broadened.	3	NCT00683670 BB-IND13590	Safe, feasible and immunogenic	Small sample size	NO	NO
Gastroenteric tumor (51)	-	mRNA	2021	Induction of $CD8^+$ and $CD4^+$ neoantigen-specific T cells.	4	NCT03480152	Safe	Small sample size, less tumor tissue.	YES	NO

characteristics that have led scientists to use liposomes and liposome nanoparticles in the preparation of cancer vaccines. Liposomes and lipid nanoparticles encapsulate various antigens and enhance immune responses by using ligands and binding immunomodulators such as TLR agonists or other PRR agonists.

At present, liposomes and lipid nanoparticles are widely used as carriers of polypeptide vaccines. Varypataki *et al*. encapsulated a synthetic growth peptide (SLP) containing a model OVA polypeptide in a cationic liposome consisting of DOTAP and 1, 2-diolyl-Sn- glycerol-3-phosphocholine (DOPC). The results showed that liposomes effectively delivered SLP to DCs in vitro and induced functional CD8+ T cells to respond to CTL epitopes in SLP in vivo at least 25 times as much as poly I: C adjuvant soluble SLP (56). To improve cellular uptake of soluble protein antigens, Neda Kordalivand *et al*. covalently bounds long peptides (SLPs) containing CTL and $CD4^+$ T Help to a polymerization network of cationic glucan nanogels via disulfide bonds. The results showed that covalent SLP-loaded poly (I: C) nanogels exhibited higher $CD8^+$ T cell responses in vivo and in vitro than soluble and physically loaded nanogels (57). Jeroen heuts *et al.* also confirmed that cationic liposomes could accommodate various SLPs, making them a potential delivery platform for personalized cancer vaccines (58).

Lipid nanoparticles may contain many drugs and other lipid-binding molecules in addition to the bilayer membrane of liposomes, resulting in thermodynamically stable lipid nanoparticles (55). Xu *et al.* developed lipid calcium phosphate nanoparticles as a Trp2 peptide vaccine delivery system for melanoma. To improve the co-precipitation of the p-TrP2 peptide with calcium phosphate, two serine phosphorylated residues were added to the N-terminal of the peptide. CpG ODN was also encapsulated in the Mannitol-modified LCPs. The results showed that the LCP vaccine coated with p-TrP2 and CpG had a better inhibitory effect on tumor growth in both subcutaneous and lung metastasis models of B16F10 compared with free Trp2 peptide/CpG. This study suggests that wrapping phosphopeptide antigens in liposomes may be a promising method to enhance the immunogenicity of autoantigens with poor immunogenicity (59). Chunhui Lai *et al.* assembled DC-targeted mannan and immune adjuvant CPG-ODN onto liposome surface and loaded melanoma-specific TRP2180-188 peptide as liposome vaccine. In conclusion, the tumor-specific antigen polypeptide vaccine M/ CPG-ODN-TRp2-Lipo can effectively inhibit the growth of B16 melanoma, improve the survival rate of mice, and enhance the anti-tumor response (60). A DC-targeted mannose-modified liposome Lip E7/CpG was used to prepare the HPV16 E7 peptide and CpG ODN vaccine. Its antitumor effects and effects on systemic immune response and tumor microenvironment (TME) were measured in a rat tC-1 transplanted tumor model. These results suggest that Lip E7/CpG induces antitumor effects by enhancing cellular immunity and improving tumor-associated immunosuppression. Mannose-modified liposomes are a promising vaccine delivery strategy for cancer immunotherapy (61). These experiments confirm that cancer antigen vaccines using liposomes and lipid nanoparticles are promising.

2.1.2 Polymeric NPs

Polymers include natural sources and synthetic. Polymers used commonly include -hydroxy, acid polyanhydride and natural sugars, while natural sources of polymers include chitosan, Y-polyglutamic acid (Y-PGA) and hyaluronic acid. Synthetic polymers include polyethyleneimine, polylactic acid, polypropylene sulfide, acrylic polymer and PLGA (62). Synthetic biodegradable polymers are preferred for tissue engineering or drug delivery applications as they have less variability and immunogenicity than biodegradable polymers from natural sources (63). The success of

the first microparticle developed for clinical use validates the concept of polymer-controlled release and lays the foundation for the era of polymer-controlled release NPs (64). The development of antibody and biological coupling technology has facilitated the generation of disease-specific NPs (65, 66). Polymer NPs that have been shown to enhance anti-tumor immune responses include chitosan, γ-PGA, PLGA, and acrylic-based polymers (67-70). A simple physical mixture of antigen and PC7A NP forms the simplest nonvaccine. PC 7A NP prepares synthesized methacrylate monomer and PEG-B-PR block copolymer into synthetic polymer nanoparticles (PC7A NP) by solvent evaporation. The nano-vaccine has been demonstrated by Luo *et al*. to enhance antigen delivery and cross-delivery and stimulate the STING pathway to enhance anti-tumor immunity (71). Synergistic activation of STING by PC7A nano vaccine and ionizing radiation can improve tumor immunotherapy (72).

2.1.3 Inorganic particles

Using inorganic particles to target TAAs in solid tumors has been interesting. At present, gold nanoparticles and aluminum nanoparticles are the most studied. Advantages of gold nanoparticles as vaccine carriers are more, such as non-toxic, immunity inertia and good control properties (such as size and shape) synthesis. They have been the object of research (73-75). Kang *et al*. showed that GNPs may be related to the diameter of OVA in terms of LNs transfer efficiency and inducing $CD8^+$ T cell response (76). The FDA has used aluminum-containing adjuvants in all aspects of human life. Aluminum-containing adjuvants can enhance the immune response by directly or indirectly stimulating dendritic cells (DC) (77); activated complement (78); induced chemokine release (78, 79). Conventional aluminum-containing adjuvants have weak or moderately enhanced antigen-specific antibody responses (80). Li *et al*. experiments showed antigen adsorption on aluminum hydroxide NPs (diameter 112 nm) induced a robust antigen-antibody response that generated a relatively mild inflammatory response compared to the inflammatory response induced by microparticles. Moreover, the solid auxiliary activity of aluminum hydroxide nanoparticles can promote the absorption of antigens by antigen-presenting cells (81). A recent study showed that the size and crystallization of AH NPS are essential in mediating the activation of enhanced antigen-presenting cells (APCs) and improving antigen-specific solid immune response. They are critical parameters for the rational design of aluminum-based Th1-type adjuvants to induce a more balanced antigen-specific immune response (82).

2.1.4 Microneedles

Adequate vaccination is essential to ensure a robust immune response (83). Common routes of vaccine administration include subcutaneous injection (SC) and intramuscular injection (IM). The advantages of SC are low cost, rapid and direct. Its disadvantages include low patient compliance, potential contamination of safe needle handling, and the need for professional management (84). Compared with ordinary subcutaneous injection, needle-like microneedles can be used to deliver biological macromolecules through the cuticle barrier. Due to the dense distribution of different DC subsets in the dermis and epidermis, antigen delivery via microneedle array (MAs) has greater immunogenicity (85). Multifunctional particles containing antigens and/or adjuvants, such as liposomes and polymer particles, enable targeted drug delivery and controlled release (86). Zaric *et al*. found that antigen (OVA) -coated PLGA NPs could be successfully delivered to the skin layer by dissolving MAs by administering antigen-coated nanoparticle vaccines with microne, improving vaccination efficiency compliance and coverage (87). In 2018, Lin Niu *et al*. used hollow microneedle arrays to deliver polymeric nanoparticles intradermal to rats, achieving high

local lymphoid concentrations through the early bursts of draining lymph nodes and relatively limited systemic exposure. Using model antigen ovalbumin (OVA), TLR agonist imiquimod and monophonic lipid A-coated poly (d, L-lactide-glycolic acid)(PLGA) NPs as vaccine agents, they showed faster antibody affinity and maturation kinetics. Delivery of antigen-loaded NPS through hollow microneedle arrays significantly increased the IgG2a antibody response and the number of lymphocytes secreting interferon (IFN)-γ, both markers of the Th1 response. In conclusion, hollow microneedle-mediated intradermal delivery of poly NPs is a promising method for vaccine delivery (88).

2.2 Vaccine delivery of neoantigen and growth peptide

There are two ways to prepare vaccines based on peptide antigens using nanotechnology. One is to prepare peptide antigens and adjuvants with particle technology, but this is an empirical process in which the peptide load of each antigen and the properties of other agents may differ. Particles include polylactic acid glycolic acid (PLGA) (89), liposomes (90), lipid nanoparticles (91), polymers (92) and emulsions (93). Another approach is to use conjugate vaccines based on peptide antigens linked to hydrophobic carriers (such as lipids (94), fatty acids (95) and TLR (96-98)), which can induce particle assembly or binding of albumin for more efficient delivery to lymph nodes (94, 99). At present, many particles and the conjugate vaccine technologies have a major limitation which is that they do not consider the new antigen's extensive features, which may lead to the variability of preparations, including the hydrophobic peptide tend to micelles, which makes the production complicated, and form at the injection site storage, which may lead to suboptimal immune $CD8^+$T cells (100). To overcome the above limitations, a PCV platform based on charge modification of peptide-TLR-7/8 conjugates has been developed, which is capable of repeatable and precise loading of different peptide neoantigens and molecularly defined adjuvants in self-assembled nanoparticles (SNP-7/8) of specific sizes (20 nm in diameter) （such as TLR-7/8 , TLR-9 and STING ）. Compared with previous PCV platforms, it can increase the breadth and number of neoantigen-specific $CD8^+$T cells and improve tumor clearance. The main finding was that conjugate vaccines could be chemically programmed (via charge modification) to account for the physicochemical heterogeneity of peptide antigens to provide a consistent formulation optimized for T cell initiation (101).

An autonomous nanoparticle vaccine involving neoantigen peptides linked to TLR7/8 agonist (SNP-7/8) showed that a higher proportion of TCF1+PD-1+$CD8^+$T cells were induced by intravenous (SNP-IV) nanoparticle vaccine than by subcutaneous immunization (SNP-SC). In the treatment model, SNP-IV-generated stem cells proliferate and differentiate into effector cells upon checkpoint blockade, leading to a better antitumor response than SNP-SC. The quantity and quality of $CD8^+$T cells depend on the duration of antigen presentation by dendritic cells (102). Again, according to a study by intramuscular nanoparticles vaccine compared with subcutaneous injections of nanoparticles vaccine, subcutaneous injection vaccine significantly enhances the immune response, enhancing the rise and activation of dendritic cells in the lymph nodes, promoting the antigen to the draining lymph nodes, enhancing the secretion of cytokines, increasing the $CD4^+$T cells and $CD8^+$T cells, activating cytotoxic T lymphocyte reaction, inducing a strong cellular immune response (103).

Evidence shows that the loading efficiency of peptides in PC7A nanoparticles is not dependent on the preparation method but mainly depends on the properties of the peptide antigen. Mutations in a peptide that disrupt the helix structure's formation will lead to inefficient loading. This research could facilitate the widespread use of personalized nano

vaccines (104). The antitumor efficacy of nano-vaccines in combination with immune checkpoint blockade therapy depends on the sequence and timing of each treatment (105). Gold nano vaccines are also feasible (106).

2.3 Delivery of RNA and DNA neoantigen vaccines (nucleic acid vaccine)

RNA vaccines are often used to design cancer neoantigen vaccines because they are relatively simple to design, manufacture, and cost-effective. DNA templates can produce fragments or linearized plasmid DNA by in vitro transcription (IVT) (107, 108). Because the translation of neoantigen peptides occurs in the cytoplasm, RNA vaccines are designed to enter the cytoplasm without entering the nucleus, dramatically reducing RNA integration into the host genome (109). The injection of naked DNA/mRNA leads to the rapid dissolution of endonucleases, resulting in a weak immune response. In cancer vaccines, we usually use non-viral vectors to deliver genes into cells, including lipids, polymers and inorganic molecules, peptides and nanoparticles synthesized from different materials of their combinations, *etc*. These vectors have higher safety, almost unlimited transgenic size and repeatable drug delivery ability. Lipid nanoparticles are the most widely used nanoparticle carriers at present.

Sahin *et al.* developed a kind of personalized RNA vaccine by neoantigens. All melanoma patients tested showed a high T cell response to multiple new epitopes of the vaccine, demonstrating the effectiveness of the customized antigen vaccine in treating cancer patients (37). A study has shown for the first time that precise DC targeting in lymphatic septa can be achieved using well-known lipid carriers (such as DOTMA, DOTPA, DOPE and cholesterol) without functionalization, simply by adjusting the negative net charge of nanoparticles(36).

2.4 Dendritic cells (whole-cell vaccines include dendritic cells and tumor cells)

Dendritic cells have been widely studied in vivo and in vitro as living carriers for transporting nanoparticles to lymph nodes. But few studies have been done on neoantigen dendritic cells. A recent neoantigen nano vaccines study for hepatocellular carcinoma has improved immunotherapy efficiency by reshaping associated neutrophils and enhancing the anticancer immune response. The nano-vaccine is constructed by SiPCCl2-hybridized mesoporous silica with coordination of Fe (III)-captopril and coating with the exfoliated membrane of matured DCs by H22-specific neoantigen stimulation. It was found that the vaccine directly induced the activation and proliferation of neoantigen-specific T cells in lymph nodes, thereby inhibiting the development of primary/distal tumors (110).

3. Advantages and disadvantages of nano-vaccines

Based on many experiments, we found that the nano vaccines have the following characteristics: no cytotoxicity was found in vitro; no measurable inflammation in vivo; no swelling at the injection site; no accumulation of inflammatory cells or cytokines; there was no allergic IgE production induced by aluminum adjuvant vaccines (111); safe and well-tolerated. Polyanhydrides-based nano vaccines can provide antigen delivery and DC activation while avoiding the apparent inflammatory response associated with conventional adjuvants (112). After the optimized CPG-DNA/peptide vaccine was injected into mice, compared with its parent compound, LN accumulation was significantly increased, the systemic dissemination was reduced, and T-cell initiation was increased by 30 times, which enhanced the anti-tumor effect and significantly reduced systemic toxicity (94). Gold nanoparticles have the advantage of non-toxic, immune inertia, and they can control the synthesis of properties, such as size and shape (73, 75). Aluminum hydroxide particles have a good safety profile, and

nano alumina causes less inflammation at the injection site (81). However, aluminum hydroxide only weakly or moderately enhances antigen-specific antibody responses.

4. Discussion

The development of tumor neoantigen vaccines is a tortuous process, and scientists have overcome obstacles to find multiple vaccine development paths. Firstly, scientists use WES to conduct comprehensive sequencing on the excised tumor tissues and peripheral blood mononuclear cells or tissues around the tumor and analyze all the somatic mutant genes. Secondly, they analyze the candidate neoantigens using RNA-sequencing (RNA-seq) or microarray gene expression analysis. Thirdly, they identify the new antigens by calculating the affinity with which the candidate antigens bind to the MHC. Finally, they inject the neoantigen vaccines into patients to enhance their T-cell response. Patients treated with conventional surgery and PD-1 therapy combined with vaccines may achieve better results, their progression-free survival will be prolonged, and their cancer recurrence rates will be reduced. High-mutated neoantigen vaccines for individual tumors have great potential and are the future direction of development. It is particularly important to find shared neoantigen vaccines for the same malignant tumors, and scientists are working on this aspect. This will benefit all humankind, and human treatment of cancer will stride to a new stage.

Declarations

1) ***Consent to publication***
   We declare that all authors agreed to publish the manuscript in this journal based on the signed Copyright Transfer Agreement and followed publication ethics.
2) ***Ethical approval and consent from participants***
   Not applicable.
3) ***Disclosure of conflict of interests***
   We declare that no conflict of interest exists.
4) ***Funding***
   None
5) ***Availability of data and material***
   We declare that the data supporting the results reported in the article are available in the published article.
6) ***Authors' Contributions***
   Authors contributed to this paper with the design (YYZ, HWX and YDY), literature search (YYZ, LJ, JPZ and YYW), drafting (YYZ), revision (YYW, HWX and YDY), and editing (YYZ, YYW, HWX and YDY) and final approval (YYZ, LJ, JPZ, HWX, YYW and YDY).
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8) ***Authors' biography***
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