

NSCLC Vaccines: Mechanism, Efficacy and Side Effects

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Abstract. Lung cancer is the most prevalent cancer, with more than 2.2 million lung cancer diagnosed cases and more than 1.79 million deaths worldwide in 2020, accounting for approximately 18% of the total cancer deaths, of which non-small cell lung cancer accounts for the majority. Patients with stage I-II NSCLC can be treated by surgery, but most patients have missed the period of radical surgery when they are diagnosed. Cancer vaccines as a type of immunotherapy have low side effects and strong specificity against tumor cells. Cancer vaccines can be broadly classified into three categories: peptide vaccines, cell vaccines, and nucleic acid-based vaccines. Protein/peptide vaccines continue to make up a large percentage of all vaccination types. The main target at the moment is a tumor-associated antigen. The amount of research being done on cell vaccines, particularly DC vaccines, is expanding. NSCLC nucleic acid vaccines, particularly DNA vaccines, are in short supply.

Keywords: NSCLC, Cancer Vaccine, Immunotherapy.

1. Introduction

Cancer is the top cause of death for those under the age of 70 in most countries [1]. Lung cancer remains the most common cancer and the major cause of cancer death. More than 2.2 million newly diagnosed lung cancer cases and more than 1.79 million fatalities have been reported worldwide in 2020, accounting for around 18% of total cancer mortality, with NSCLC accounting for 80% to 85% of lung cancer cases [1].

Patients with stage I-II NSCLC can now be treated surgically, having a 5-year survival rate of greater than 50%. When most patients were detected, their lung cancer had progressed to the point that drastic surgery was no longer an option. Chest radiation and dual agent chemotherapy with cisplatin or carboplatin are routinely utilized for patients with locally advanced NSCLC (stage IIIa-b). Cisplatin or carboplatin will attack all cells indiscriminately, which leads to many negative effects, including vomiting, anemia, nephrotoxicity, and the recurrence rate of this treatment is high [2]. Many mutant tyrosine kinases, such as EGFR, ALK, ROS1, are surface markers in NSCLC and can be used as targets for detection and treatment. However, the targeted therapy of these targets has the situation that the target fails after further mutation [3]. Due to the obvious side effects or strong drug resistance of the above treatment methods, patients urgently need an innovative and effective treatment method. As a kind of immunotherapy, the cancer vaccine has low side effects and strong specificity for tumor cells.

Since Liam B. Coley found that erysipelas secreted by *Streptococcus pyogenes* can lead to tumor regression in patients with advanced sarcoma, there have been more and more studies related to tumor vaccine [4]. TAAs were largely discovered in the 1990s. In 1991, the first tumor-associated antigens (TAAs) - MAGE1 - were discovered, followed by the discovery of additional tumor antigens such as MUC1, MAGEA3, and HER2 by researchers. Since then, it has entered the rapid development period of tumor vaccine development and clinical trial.

The first new therapeutic tumor vaccine was approved by the FDA in 2010: sipuleucel-t (Provenge, Plavix), a prostate cancer vaccination based on autologous DC cells [5]. The mechanism, efficacy, and side effects of some NSCLC cancer vaccines will be summarized in this article, hoping to provide help for the development of NSCLC vaccines.

2. Protein/peptide vaccine

Protein/peptide vaccines still account for a high proportion of all vaccine types. At present, the tumor-associated antigen is still the main target of vaccines. Many TAAs are overexpressed in cancer cells and participate in tissue differentiation. Cancer testicular antigens (CTAs) are TAAs considered to have higher tumor specificity. Except for germ cells and trophoblast cells, these antigens are rarely expressed in normal adult tissues, but they are abundant in cancer cells [6]. Common CTAs include the MAGE family, of which NY-ESO-1 has received special attention and has been clinically tested in patients with melanoma, high-risk myelodysplastic syndrome, and metastatic synovial cell sarcoma. When the high-level expression of TAA reaches the threshold of T cell recognition, on the one hand, TAA can induce an anti-tumor immune response, on the other hand, it may also induce the immune system to attack the corresponding normal tissues [4].

Another antigen is often produced by the variation of normal protein, and most of it only exists in cancer cells [6]. It can be called mutant antigen, which is often directly related to the development of cancer (cell proliferation, anti-apoptosis, and other functions). The mutant antigen is also often called "tumor-specific antigen" (TSA), but this specificity is relative, because this antibody may exist in all cells where the gene is mutated. There are many common mutant antigens, such as p53, RAS, and B-Raf, which are produced by point mutation and gene fusion [6].

In multiple types of cancer, the human telomerase reverse transcriptase (TERT) is overexpressed. It may be a target of therapeutic drugs for many types of cancer, and it is also expressed in cancer stem cells [7,8]. Vx-001 (vaxon biotech) is a vaccine against human telomerase reverse transcriptase (TERT). This vaccine is composed of HLA-A * 0201 related optimized cryptopeptide TERT 572y and its natural counterpart TERT 572, which can stimulate the immune response of patients to tumors expressing hTERT [8]. In a clinical trial, Vx-001 successfully induced a particular immunological response in 66 percent of patients, however, 52 percent of patients experienced an early adverse reaction (EAE). Most patients presenting with EAE have symptoms of a local skin reaction. A few patients had weakness, anemia, and nausea. One patient had elevated grade 3 aminotransferase, but no symptoms of late toxicity or autoimmune syndrome were found [8]. In a separate clinical experiment, vx-001 only managed to elicit a particular immunological response in 29% of patients. The OS of the treatment group was 14.3 months, which was longer than that of the placebo group for 11.3 months [7].

Uv1 is another hTERT targeted vaccine, which consists of multiple synthetic long peptides, including many epitopes determined from previous hTERT vaccination trials. Uv1 has good safety, and the main side effect is the reaction at the injection site. In patients with immune response, the median OS was 38 months [9].

WT1 encodes a protein with a complex pattern of alternative splicing that regulates the expression of genes that favour cancer cell survival and has been implicated in carcinogenesis. Dsp-7888 is a vaccine containing two synthetic peptides derived from WT1, which can promote the corresponding specific immune response [10]. In a clinical trial, the median OS of patients was 180.0 days, and more than 50% of patients who received intradermal administration successfully induced specific CTL, which was better than that of subcutaneous administration. In this study, dsp-7888 did not show dose-limiting toxicity, but many patients who received the drug had injection site reactions. A few patients had serious adverse reactions such as hyperbilirubinemia, atrial fibrillation and anemia, and cardiopulmonary arrest, but the researchers of the clinical trial believed that these adverse reactions were not associated with dsp-7888[10]. In another study on the myelodysplastic syndrome, the most common side effect caused by dsp-7888 was injection site reaction. A few patients had supraventricular tachycardia, cellulitis, pyoderma gangrenosum, and febrile neutropenia [11].

IDO is an enzyme that can catalyze tryptophan degradation in cells. The elevated expression of IDO in tumor cells is linked to the suppression of T cell activity and survival. In 2014, a vaccine prepared with IDO5 peptide, sequence, and Montanide was reported. A vaccine containing the ido5 peptide sequence and Montanide was published in 2014. Six individuals had long-term stable disease and one patient had partial remission among 15 patients. In this clinical trial, the patient's median

overall survival (OS) was 25.9 months and no significant toxicity was observed [12]. Subsequent long-term follow-up showed that 3 of the 15 patients survived after 6 years, and two long-term responders had an IDO-specific T cell response produced by the vaccination [13].

Vaccines are often used as an adjuvant treatment after chemotherapy, and the combination of peptide vaccines and immunosuppressants is worth trying. It has been reported that a treatment mode of combining NEO-PV-01 phase vaccine with PD-L1 through a personalized new antigen vaccine has achieved an average one-year overall survival (OS) of 84% in 18 NSCLC patients. All patients have a specific immune response induced by the vaccine, and no obvious side effects of the vaccine on patients have been observed in the trial [14].

3. Cell vaccine

Dendritic cell vaccination and cancer cell vaccine are the two most often utilized cell vaccines. Some antigen-presenting cells (APCs) called dendritic cells (DCs) can help immature T cells grow and memory T cell responses. DCs can capture antigens and internalize them into peptides [15]. The peptide is delivered to the DC surface after binding to MHC I and MHC II. Then the MHC peptide complexes are recognized by TCRs on CD8 + and CD4 + T lymphocytes. There are many antigen sources of DC vaccine, including various types of antigen-related peptides and proteins, autologous tumor cell lysates, and dead allogeneic tumor cells. DCs transfected with DNA and RNA or transduced with a viral vector can also activate an adaptive immune response. Some reports have shown that DC tumor hybrids can be used for T cell activation [15].

The main component of another cell vaccine can be a tumor cell lysate or irradiated intact tumor cells. These tumor cells are derived from autologous tumor tissue or established heterologous tumor cell lines and are mostly genetically engineered to secrete cytokines such as GM-CSF [6]. Irradiated tumor cells will be administered to patients together with adjuvants in clinical trials. Vaccines using specific tumor cells as the main component can induce specific immune responses to antigens expressed on such cancer cells. For example, Canvaxin has been studied in melanoma. Some patients have expressed immune response and have some improvement [16].

Survivin and MUC1 were highly expressed in NSCLC. Survivin can inhibit apoptosis and regulate cell fraction and is expressed in at least 80% of NSCLC patients. MUC1 is a highly glycosylated large glycoprotein, which can participate in tumor invasion by regulating cell adhesion [18]. A 2017 clinical trial took peripheral blood mononuclear cells from each patient to generate DCs and co-cultured the DCs with flagellin, survivin, and MUC1 peptides, in addition to downregulating SOCS1 on DCs using siRNA. Common side effects include fever, fatigue, elevated C-reactive protein (CRP), and grade 1 flu-like symptoms. The number of cells expressing CD14 was down-regulated in co-cultured cells, whereas cell populations expressing costimulatory markers CD80, CD86, and CD40 were rise, resulting in an evident immunological response in patients. It's worth noting that 11 of the 15 patients in this experiment had recurrence-free survival (RFs) in the long-term follow-up [19].

Personalized treatment has also been applied to DC vaccines. Wang et al reported a DC vaccine transfected with prefabricated common TAA mRNA. After the overexpression of TAA was identified in individual patient samples, the PBMC of the patient was taken to generate DC and transfected with corresponding TAA mRNA. The patient then received an intravenous injection of DC after 3-16 times of transfection. The median survival time of patients was 17 months. The main adverse reactions were local skin reactions and influenza-like low fever [20].

CCL21 interacts with the CCR7 and CXCR3 receptors to recruit effector T cells and DCs. Ad-CCL21-DC is an autologous dendritic cell vaccine transduced with adenovirus containing the CCL21 gene. In a clinical trial, 25% of patients were in stable condition (SD) after inoculation with Ad-CCL21-DC, with a median survival time of 3.9 months, and nausea was the most common vaccine-related side effect [21].

GM.CD40L is also a cellular vaccine. The major components of such vaccines are human bystander cell lines that can express GM-CSF and CD40L. In the vaccine site's microenvironment,

GM.CD40L can release GM-CSF, recruit antigen-presenting cells like DC, and activate DCs with CD40L. GM.CD40L had a median OS of 9.3 months. Injection site response, tiredness, and anorexia were the most prevalent TRAEs [22].

DCVAC / LuCa is a vaccine composed of isolated pulsed autologous DC and killed NSCLC h522 cell line. More than 30% of 44 patients obtained PR and more than 60% obtained SD. The most common trae is grade 1 constipation, anorexia, and fatigue. A few patients have anemia, leucopenia, and neutropenia [23].

Belagenpumatulcel-L is a cancer vaccine made up of four NSCLC cell lines that are activated by transfection with the (TGF)-2 antisense vector. In a phase III clinic in 2015, patients who received the vaccination had a median OS of 20.7 months, with 260 patients experiencing injection site reactions and one patient experiencing anaphylaxis [24].

4. Nucleic acid vaccine

A DNA vaccine is composed of an antigen coding gene and a bacterial plasmid containing a eukaryotic promoter. DNA vaccine can transmit the gene encoding antigen and trigger the systemic adaptive immune response to tumor cells containing the target antigen. Because DNA vaccine itself has a CpG sequence and double-stranded structure, DNA vaccine can also induce innate immune response [25]. Because the cancer DNA vaccine promotes systemic immune response, the vaccine is also effective for metastasis. DNA plasmids are introduced into the host and absorbed by the cell, where they are transcribed in the nucleus and translated into the cytoplasm. The proteins are then digested into peptides, which are then linked with MHC molecules and delivered to the surface of APCs in the host. Direct transfection of APC, such as DCS, with DNA or cross-presentation of DNA vaccine antigen from other cells, can be used to deliver DNA vaccination antigen [6,25].

An RNA vaccine is a messenger RNA (mRNA) that is produced in vitro utilizing phage RNA polymerase and target antigen template DNA. The mRNA transcript can be immediately translated into the cytoplasm once the vaccination has been swallowed by the host cell. Newly produced antigens in cells are delivered to APC to promote immune response, similar to DNA vaccines [25]. The proteasome digests the mRNA-encoded products and delivers them to CD8 + T cells via MHC I, but they never make it to the MHC II processing pathway, which is important for triggering CD4 + T helper cell responses. HLA class I and HLA class II presentation can be caused by tumor antigen mRNA combined with signal peptide and HLA class II sorting [25]. In vitro transcribed mRNA molecules are less durable because they are easily touched and destroyed by nucleases, and they are easily detected by TLRs and activated DCs, which stimulate innate immune responses. Some frequent mRNA nucleoside modifications, such as m6A, m5C, m7G, and 5' - terminal cap, might diminish the human innate immune response to foreign RNA.

Vaccinia virus, herpes simplex virus (HSV), and Coxsackie virus all induce immunogenic tumor cell death. The virus vector vaccine can produce mature progeny virus in only 6 hours and can accept large-scale exogenous DNA insertion. On the other hand, there are still genes with unknown functions in the virus, and there are certain safety risks and uncertainties [26]. Nowadays, people often enhance the immune response of vaccines to tumors by further modifying viral vectors and inserting genes encoding tumor-associated antigen (TAA) or new antigen. In addition, vaccinia virus antigen can further induce systemic antitumor immune response through STING and batf3-dependent dendritic cells [26].

Cv9201 is a vaccine composed of the mRNA of five antigens expressed on NSCLC. Cv9201 target contains three CTAs. These CTAs are only expressed in male germ cells or tumors including NSCLC and are potential targets of cancer vaccines. In 60 percent of patients, vaccine therapy led to a doubling of CD38 hi B cells expressing IgD, with a median survival time of 10.8 months. Injection site reactions accounted for the vast majority of vaccine-related adverse events, and 19 people may have autoimmunity [27]. CV9092 is a vaccine with one more MUC1 mRNA than CV9091, but there are few relevant research data.

MUC1 is overexpressed in several malignancies, and it can impact cancer cell proliferation via mediating growth factor synthesis, as well as tumor migration, invasion, angiogenesis, and anti-apoptosis. MUC1 is overexpressed in several malignancies, and it can influence cancer cell proliferation by regulating growth factors. It also plays a factor in tumor migration, invasion, angiogenesis, and anti-apoptosis. TG4010 is a vaccine against the MUC1 targeted recombinant vaccinia virus. Clinical experiments showed that the vaccine could increase interferon- γ secretion and increase CD8 T-cell numbers. TG4010 also can lead to a significant improvement in PFS. In a clinical trial, the median OS for HLA-A02 * 01 individuals treated with TG4010 was 15.5 months. The common adverse reaction was local injection reaction, and neutropenia occurred in a few patients [28].

5. Conclusions

At present, the vaccines of NSCLC in clinical trials are mostly used as a late adjuvant treatment of monoclonal antibody therapy and chemotherapy. At this stage, peptide vaccines still account for the majority of vaccine types, and some vaccines targeting IDO and personalized antigen peptides have shown good therapeutic effects. Cell vaccine is one of the most studied types of vaccine at present. At present, the DC vaccine is more studied. Cancer-related antigen peptides are commonly used or cancer cell pulsed DC is directly used to improve the vitality of DC against cancer cells. At present, there are few studies on nucleic acid vaccines, especially DNA vaccines. Among them, TG4010 is one of the more mature vaccines with good antitumor activity.

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