

Cancer treatment vaccine: DNA vaccines in treating prostate cancer and colorectal cancer

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Abstract. Therapeutic DNA cancer vaccines are now widely regarded as a potential technique for stimulating the immune system's response to cancer. Several clinical experiments involving plasmid DNA vaccines have already the induction of a wide and targeted immune response. Unfortunately, due to the tumor's immunosuppressive mechanisms, these vaccinations often showed very modest therapeutic results in clinical trials. This paper looks at how DNA vaccines for prostate cancer are progressing, as well as colorectal cancer clinical trials. This paper also examines the rationale for various treatment combinations and antigen selection strategies being developed to circumvent the limits of the cancer DNA vaccine. This paper focuses on the most promising discoveries as well as major concerns that must be addressed before therapeutic cancer DNA vaccines may be approved as part of standard cancer care for prostate cancer and colorectal cancer.

Keywords: Cancer Vaccine, DNA Vaccine, Prostate Cancer, Colorectal Cancer.

1. Introduction

Surgery, chemotherapy, and radiotherapy were the three recognized cancer treatment procedures in the past, however, cancer immunotherapy is now considered the new cancer treatment approach. Tumor immunotherapy has become a hot issue in recent years. According to relevant statistics, immunotherapy helps roughly 20% of cancer patients and offers long-term benefits. Vaccines for cancer treatment are a type of immunotherapy that boosts the body's natural defenses against cancer. Cancer treatment vaccines, unlike cancer-preventive vaccinations, are designed to combat cancer cells in people who have already been diagnosed with cancer. Cancer treatment vaccines are based on the fact that cancer cells carry tumor-associated antigens. It can teach the immune system to recognize and react to these antigens, leading to the death of cancer cells.

DNA vaccines are made up of antigen-encoding genes that are introduced into a bacterial plasmid and controlled by a eukaryotic. DNA plasmids are internalized by the host cell which are subsequently transcribed in the nucleus and then translated into the cytoplasm by the host cell. The peptides are then displayed on the membrane of host antigen-presenting cells (APC) alongside major histocompatibility complex (MHC) molecules. This can happen either through direct transfection of DNA by APC or via cross-presentation from non-APC to APC. Antigen-specific T cells identify the peptide-MHC complex, triggering a cellular host immunological response. DNA vaccination has become a popular immunotherapeutic technique for cancer treatment because of its simplicity, durability, and safety [1]. DNA vaccinations have been shown in numerous clinical trials to be well tolerated by patients and to have no notable negative effects [1].

Nucleic acid vaccines based on DNA are a novel immunogenicity method in development. Employing DNA vaccines as a straightforward antigen delivery method for prostate cancer treatment has proven to be a particularly appealing strategy. There are now many clinical studies exploring DNA vaccines as prostate cancer treatments, all of which have shown to be safe and immunogenic [2]. In addition, DNA vaccines are being evaluated as therapeutic gene vaccines for Colorectal cancer

because of their ability to stimulate CD8 T-cells growth [3]. Several studies have shown that nucleic acid vaccines can induce an immune response against a variety of diseases. The CEA66 DNA vaccine was well tolerated, with no signs of an autoimmune reaction [3]. Plasmid DNA vaccines outperform existing anti-tumor vaccine techniques in terms of simplicity, production, as well as no potentially infectious components. This paper focused on the most recent advancements in DNA therapy vaccines for prostate cancer and colorectal cancer, as well as an update on the status of DNA vaccines in human clinical studies.

2. The present status of DNA vaccines

DNA vaccines are made up of plasmid DNA encoding the desired antigen under the direction of a mammalian promoter and are easily generated in bacteria. The optimal gene sequence of interest is administered by one of many delivery techniques to the skin (intradermally), subcutaneously, or to the muscle. The diverse medical trials aimed to establish the candidate vaccines' safety and tolerability, as well as to investigate the efficacy of DNA vaccines in patients. Injection of the plasmid DNA construct is generally well tolerated by patients and seldom results in systemic toxicities. Currently studied DNA vaccines demonstrate insignificant amounts of incorporation into host cellular DNA. Additionally, preclinical investigations in nonhuman primates and early human trials failed to discover increases in antinuclear or anti-DNA antibodies. Participants in human trials using DNA vaccines are monitored for indications and symptoms of autoimmunity triggered by the vaccine, but there are no concrete indications that autoimmunity develops in combination with the DNA vaccine [4]. DNA vaccines can be delivered through gene guns to the epidermis, intramuscular injection, or intradermal injection. At present, the methods used in human clinical trials are prostate cancer and colorectal cancer [5].

3. DNA vaccine for prostate cancer

The prostate is a part of the male reproductive system. It is located in front of the rectum and below the bladder. The prostate gland wraps around the urethra, which is the tube through which urine is expelled. A healthy prostate is about the size of a walnut and it is a gland that produces the fluid that forms part of the semen. Prostate cancer is an epithelial malignant tumor that occurs in the prostate gland. Prostate cancer is the second most frequent malignancy, behind lung cancer, according to epidemiological data. The most common non-cutaneous cancer in men worldwide, and the sixth leading cause of death from malignancy in men worldwide. There were an estimated 1,276,000 new cases and 359,000 deaths in 2018. The highest incidence of prostate cancer is in highly developed countries, mainly in the United States, Canada, Australia, and the EU countries. It is estimated that by 2040, the incidence of prostate cancer will increase to nearly 2.3 million cases and 740,000 deaths [6].

3.1. Etiology for prostate cancer

The relative risk of prostate cancer in a family with no prostate cancer is 1 and the absolute risk is 8. Therefore, prostate cancer is a disease that runs in families [7]. Those who are more sexually active have an increased risk of prostate cancer. Smoking and endogenous hormones are also factors that affect prostate cancer [8]. A high-fat diet is also associated with the development of the disease. Some have previously mentioned a possible association with BMI [9], but data from research studies show little evidence that hereditary height or BMI has a substantial effect on prostate cancer risk [10]. Sexually transmitted infections and vasectomies have also been linked to the development of prostate cancer in studies. Some of the foods (such as processed meat products) and nutrients (saturated and trans-fatty acids) that we eat on a daily basis can also contribute to prostate problems, which can lead to oxidative stress and inflammation, as well as changes in lipid metabolism and growth factor signaling, all of which can lead to prostate cancer [6].

3.2. Prostate cancer drugs that have been authorized

Several medications for the treatment of prostate cancer have been approved in the last decade. Doxorubicin and cabazitaxel are chemotherapy medications; abiraterone and enzalutamide are androgen receptor-targeting drugs; radium-223-targeted radiation treatment; and sipuleucel-T is an autologous cellular vaccination. Given that enhanced patient survival is the primary requirement for new medicine approval, all these medications have been tested and approved for late-stage MCRPC (Metastatic Castration-Resistant Prostate Cancer) patients. But the bad news is that the median survival benefit of each therapy is only a few weeks to a few months [11].

3.3. The improvement of DNA vaccine for prostate cancer

The discovery of novel and effective treatments for prostate cancer is required due to the limits of existing medications. Immunotherapy has received a great deal of attention in recent decades as one of the cancer treatment techniques. Sipuleucel-T is an FDA-approved vaccine for the treatment of metastatic ulcer-resistant prostate cancer [12]. The vaccination must be able to elicit a tumor-specific T-cell response to a weakly immunogenic "autoantigen" in order to be effective. And vaccination had to overcome the immune evasion tactics deployed by cancer cells [11].

In phase III clinical research targeting persons with terminal mCRPC in a variety of clinical trials, three potential vaccine approaches have been investigated. GVAX, a whole-tumor cell vaccine, and antigen-specific vaccines, such as a poxvirus vaccine against PSA, are two examples (Prostvac-VF). The FDA approved sipuleucel-T, an autologous cell vaccine targeting the prostate-specific protein prostatic acid phosphatase (PAP), and patient survival compared to placebo, with improved mCRPC treated with sipuleucel-T. In conclusion, these findings suggest that treatment of the prostate by the vaccine is feasible. In addition, it continues to have a significant impact on patients early in the disease, following depot treatment, in conjunction with some certain immune-targeted treatments, or in combination with other conventional treatments. One particularly attractive approach is the use of the vaccines as a simple method of antigen delivery.

Six trials are now being conducted to determine the efficacy of DNA vaccines for the cure of prostate cancer. Three experiments employing pTVG-HP are the most promising. The goal of the first experiment was to have no metastases at 2 years, with patients having the option of receiving either a vaccine with GM-CSF protein or a vaccine without GM-CSF protein which started about ten years ago. It was a stochastic phase II trial in some non-metastatic prostate cancer patients, where PSA became doubled in time very quickly. The evaluation of the use of this vaccine with the sipuleucel-T vaccine is from a second trial that began in 2012, the sipuleucel-T vaccine, as previously indicated, is an FDA-allowed vaccination that also targets the hPAP antigen. In a third trial, this DNA vaccine is being tested in patients with metastatic disease, anti-ulcerative prostate cancer in a mixture, or sequentially with pembrolizumab, a PD-1 blocking antibody. Table 1 [11] also shows the results of clinical studies using DNA vaccines (as combination therapy and monotherapy) in prostate cancer to date.

Current research into APC populations capable of direct and cross-presentation of DNA-encoded antigens will lead to more effective DNA vaccine targeting strategies, potentially against several antigen-presenting cells types. Moreover, the immunogenicity of bacterial DNA will be investigated in order to develop new molecular adjuvants, and more and better tumor-specific T lymphocytes will be developed for innate signaling pathways. The most rational combination therapy will be discovered from ongoing studies, which evaluate and estimate the mechanisms by which the tumors keep away immunological detection, and whether any of these processes are beneficial in the treatment of prostate cancer. DNA vaccines in combination with medications that target certain resistance mechanisms. The most effective research, according to the results of mouse studies, DNA vaccines have been used with checkpoint inhibitors [11]. All in all, the mixture of DNA vaccines with PD-1 inhibition has lately displayed promising results, with advanced prostate cancer patients with realistic clinical responses [12].

Table 1. Clinical trials with DNA vaccines (both as monotherapy and in combination) in prostate cancer to date [12].

Vaccine	Phase (Number of patients)	Principle / Method	Main Conclusions
Rhesus PSA	1 (N=15)	Safety of nmCSPC patients, dose rise, alterations in PSA kinetics and determination of PSA-specific immunoreactivity.	Vx was not harmful. PSA kinetics have not changed. Due to vx or ADT, 14/15 individuals developed PSA-specific immune responses.
PSA	1 (N= 8)	Safety and detection of PSA-specific cellular immunity of CRPC with rising doses.	PSA-specific humoral and cellular immunity were identified at the highest dose (900 Ig).
PSA +PSMA (+IL-12 DNA plasmid (INO-9012))	1 (N= 62)	PSA kinetics and PSA doubling time, as well as safety and tolerability, immunological response to PSA and PSMA. Patients with PCa that is biochemically recurrent (nmCSPC).	Approximately 85% of patients were progression-free at 12months. Patients whose PSA doubling time was less than one year prior to treatment initiation had increased PSA doubling time and approximately 76% of patients had specific immunity to PSA or PSMA.
PSMA +PRAME	1 (N= 24)	Two different amounts of peptide boost and a fixed DNA plasmid (prime). Harmless, specific immunological response to PSA or PRAME, and clinical benefit (stable illness) in CRPC.	With 4/10 exhibiting a drop in PSA or stable antigen-specific T cells above standard line, illness above-baseline antigen-specific T lymphocytes and disease control (illness stayed steady for > 0.5 year).
NY-ESO1	1 (N= 16)	Patients with various cancers, including some patients with metastatic prostate cancer, were studied for harmless and immunological response.	All patients had CD4+ immunoreactivity and 20% of them had CD8+ immunoreactivity.
AR LBD	1 (N= 40)	1.5-year PSA Progression-Free survival in mCSPc Progressive patients are safe and immunoreactive.	Of the 30 patients evaluated, approximately 14 developed AR-specific cellular immunity. And the time to PSA progression was significantly longer in patients with T-cell immunity.
PAP	1 (N= 22)	Harmless, PAP-specific immune response, dose rising, time to PSA doubling in nmCSPC patients.	About 40% of patients showed PAP-specific CD4+ and/or CD8+ cell proliferation. the time to PSA doubling increased from six months before treatment to 0.7 years after treatment and 0.8 years after treatment to 1 year.
PAP	1/2 (N= 16)	6 vaccine injections every 2 weeks for 2 years, followed by 3 injections every 3 months for 2 years, compared with 6 injections every 14 days, in people with nmCRPC based on immunological monitoring.	Immunosurveillance does not result in a more advantageous schedule. Antigen-specific T cells that have been elicited continue to exist over time.

4. DNA vaccine for colorectal cancer

4.1. Overview of colorectal cancer

Colorectal cancer (CRC) is the second most common cause of cancer mortality in males and the third most common cause in females, including skin cancers, allowing it one of the primary causes of cancer-related deaths globally. The American Cancer Society projects that in 2022, the United

States will see over 100,000 new instances of colon cancer and over 40,000 new instances of rectal cancer. From a holistic perspective, the rate of occurrence has decreased significantly since 2013. However, this trend is concentrated in older adults; for those under the age of 50, this rate has been increasing for years. Around one in five patients are diagnosed with colorectal cancer would then develop metastasis from the colon or rectum to other organs, culminating in a 12-percent reduction in survival. Despite significant advances made in chemotherapy, surgery, and radiotherapy, CRC is still a lethal cause of death for patients, ranking as the fourth most common cause of cancer-related mortality. DNA cancer vaccines have been proved to be efficient against not only prostate cancer but also colorectal cancer. Fortunately, colorectal cancer has been shown to be one of the most treatable cancers when detected early; this facilitates the delivery of specific sequences into patients' bodies, that also express specific proteins that efficiently activate the immune system to eliminate cancer cells, consequently limiting the spread of CRC.

4.2. Causes of CRC

While the exact cause of colon cancer is unconfirmed, there are several plausible theories below. Colorectal cancer arises when normal cells develop genetic abnormalities. African-Americans have a higher rate of colorectal cancer as a result of racism issues. Chronic inflammatory diseases have been shown to perform a significant section in the development of colorectal cancer. Colorectal cancer has been linked to inflammatory bowel disease. Numerous inflammatory cells contribute to the establishment of a cancerogenic microenvironment, which may eventually lead to the development of cancer. As illustrated in Figure 1, alcohol and nicotine are also thought to have a detrimental impact on the microenvironment, thereby increasing the risk of colorectal cancer.

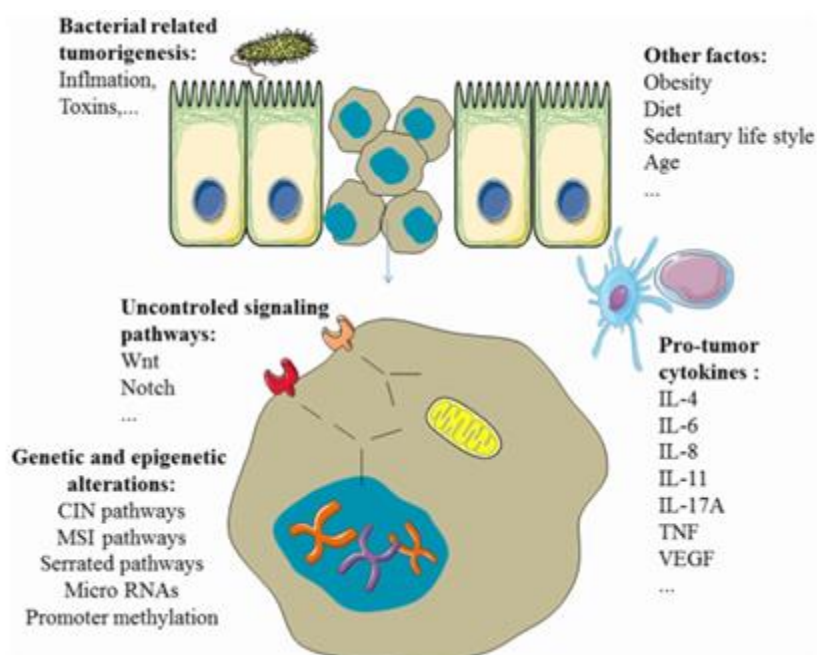


Figure 1. Mechanisms underlying the promotion of CRC [19].

In a nutshell, colorectal cancer is frequently caused by a series of genetic mutations occurring in germline and somatic cells. Previous research has revealed that APC, P53, RAS, BRAF, PIK3CA, WT1, and MYH are frequently mutated [13-16]. MYH is associated with APC mutations. APC and P53 are tumor suppressor genes; their mutations impair microtubule regulation, cell cycle regulation, and chromosome instability. RAS, BRAF, PIK3CA, and WT1 are oncogenes; their mutation results in an increase in the mutation of genes involved in cell growth and accelerates tumor progression. Genome analysis has revealed that a single CRC frequently contains more than 80 mutations [17,18]. The preceding examples are significant but not comprehensive. Tumor-associated antigens (TAAs) are significant molecules that are helpful in the immune response. TAA's components, small peptides,

can bind to human leukocyte antigen (HLA), triggering an immune response and killing cancer cells spontaneously. There are numerous specific molecular transformations in tumor cells; thus, analyzing specific transformations could play a significant role in more precisely connecting DNA vaccines [19].

4.3. Clinical trials of the utilization of CRC

DNA or RNA-based nucleic acid vaccines, particularly non-coding RNA, is a novel immunogenicity strategy currently underway. Numerous researches have demonstrated the accessibility of nucleic acid vaccines against certain diseases through the induction of an immune response. While in vivo and in vitro studies provide a wealth of information, Clinical studies have not been conducted to determine the effectiveness of DNA vaccines in CRC. In prior work, we focused on a DNA vaccine that is now through phase I clinical trials, both Hepatitis B Surface Antigen and CEA tumor antigen-encoding plasmid were inoculated in 17 patients with CRC. Repeated DNA vaccine doses generated HBsAg antibody production in 6 of 8 patients and enhanced the level of protective antibodies in 4 patients. While 4 of 17 individuals exhibited a lymphoproliferative response to the CEA, no CEA-specific antibody response was observed following immunization. There were no distinct clinical responses to the DNA vaccine observed in this study's 17 patients with mCRC [20]. The previous research used an altered CEA antigen-encoding plasmid DNA vaccine (CEA66 DNA) in combination with a T helper cell-related epitope in another phase I study. Level 1 and 2 complications most frequently reported included symptoms like headaches, weariness, muscle discomfort, chest spasms, and joint pain that may occur at the injection site. As a consequence, the CEA66 DNA vaccination was fairly well tolerated and showed no evidence of an autoimmune reaction. [21]. The safety and feasibility of vaccination with autologous DCs transfected with CEA tumor antigen encoding mRNA were probed in CRC patients who have metastatic liver surgery in phase I and II clinical trials. DCs transfected with CEA mRNA were used in this study. The outcome indicated that the immunogenicity was well tolerated, with 1 out of every 24 assessed patients achieving a complete response, 2 achieving a limited response, 3 achieving stable disease, and 18 achieving progressive disease. 9 out of 13 individuals in Phase II of the trial reported a disease recurrence within 122 days. The induction of an immune response was demonstrated in samples taken from the DC injection site and peripheral blood. According to the acquired data, loading mRNA into DCs is not only safe but also possible in patients with mCRC [22, 23]. Seeing the limited efficacy of DNA vaccines, another study launched a new initiative that targets the MYB gene. The MYB gene regulates the development and function of the hematopoietic system, and in humans, aberrant MYB expression results in a spectrum of hematological and non-hematological malignancies. MYB leads to leukemia, adenoid cystic carcinoma breast cancer, and CRC. When patients with colorectal cancer have a high MYB level, their prognosis is poor. [24,25]. Earlier conducted research examined a feasible strategy for specifically targeting cells that overexpress peptides or other transcription factor peptides such as MYCN, as seen in neuroblastoma. This therapy does not necessitate long-term medication use and theoretically possesses the ability to eradicate remaining cancer cells over a lengthy period of time. DNA vaccines played a critical role in this situation.

To create this type of DNA vaccine, DNA sequences encoding two immunodominant, Tetanus peptides with MHC class II restriction were ligated to the amino and carboxyl termini of the full-length mouse MYB cDNA. Currently, the MYB vaccine has been utilized in a prophylactic context in pre-clinical studies, protecting mice in both transgenic and cell line-based models of CRC. Researchers progressed its therapeutic utilization of two preclinical types of aggressive colorectal cancer.

As a result, they evaluated vaccination techniques in a therapeutic context through injecting 5×10^{15} MC38 cells into the flanks of C57BL/6 mice on day 2 after tumor cell injection and commencing immunization with the DNA construct or saline [27], followed by boosting on days 7 and 12.

The vaccine offered considerable protection against tumor development in all animals in these settings. However, no survival benefit was observed when mice were vaccinated on day 5 and boosted

on days 10 and 15. These data indicate that the pVAXMYB DNA vaccine is efficacious only in the presence of a low tumor load and probably when the immune system is tumor naïve, with tumor-mediated immune system editing likely limiting its efficiency [27].

When the tumor burden is low, significant single-agent protection is evident. When the tumor burden is greater, protection can also be obtained by combining other immune modulators. The ability of vaccination approaches to harness the immune system in order to eradicate residual disease and provide protection for extended periods of time is an appealing quality. This study suggests that this may be possible in CRC [27]

5. Conclusions

For prostate cancer, using DNA vaccines as monotherapy and combination therapy has been conducted in several clinical studies. Although they have little efficiency and immunogenicity when compared to other immunotherapies, they have shown to be safe in triggering highly specific, effective, and widespread immune responses. Several studies have shown that DNA or RNA-based nucleic acid vaccinations can induce an immune response against a variety of diseases. It is safe and practical to use loaded mRNA in dendritic cells for patients with metastatic colorectal cancer. Recent preclinical and clinical trials of prostate cancer and colorectal cancer suggest that existing DNA vaccines are unlikely to have a significant impact on cancer outcomes as a single agent due to their low efficacy and immunogenicity. The effectiveness of DNA and RNA vaccines against colorectal cancer has yet to be thoroughly examined in clinical trials, despite a lot of evidence from research. In the future, combining DNA vaccines with other techniques may have a larger potential for enhancing clinical results than a single therapy. This could be the most effective way to improve the efficacy of cancer immunotherapy.

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