# Successful vaccine immunotherapy. An exciting novel approach to cancer treatment

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**Summary**

Global cancer spread to pandemic proportions, has reinforced the importance of disease surveillance and prevention programs, and has provided the stimulus for greater resources in vaccine development. Many reports from phase I or II trials indicate that both partial and complete responses have been observed, with little or no toxicity, in a small proportion of vaccine recipients. Future prospects will be to increase basic knowledge of immunogenic tumor antigens to vaccine administration, which will make the cancer patient develop an immune response able to induce tumor regression. Clinical trials already under way in patients with malignant diseases may yield more definitive conclusions.

*Key words:* Cancer; Vaccine; Immunotherapy.

## Introduction

It has been recognised for centuries that individuals who recover from certain diseases are protected from recurrences. Immunization results in the production of antibodies which are directed against the infecting agent or its toxic products. It may also initiate cellular responses mediated by lymphocytes and macrophages.

The first still effective empirical-immunization was performed by Edward Jenner, an English physician, who observed that persons who got well after infection with cowpox were protected against smallpox. He introduced vaccination with cowpox as a means of protection against smallpox (1796). A century later, Louis Paster and his collaborators investigated the possibility of protection against infection by vaccinations with attenuated strains of microorganisms. More than 100 years ago, William Coley thought about cancer vaccination and published the first report of tumor regression induced by the immune system activation.

## Cancer vaccination

The recent advances in molecular biology and immunology have offered a greater insight into the pathogenesis of various types of malignant neoplasms and the way humans respond to them. This has led to a virtual explosion in the number and types of vaccines, globulins, monoclonal antibodies and immunomodulating agents. A large amount of preclinical and clinical data over the past 30 years has demonstrated that the development of cancer vaccines depends on the identification and administration of cancer antigens to the patients (in the form of tumor vaccine) that give rise to immune reactions able to destroy malignant cells.

Vaccination has a role to play in cancer treatment when radiation treatment and anticancer drugs have overwhelmed the immune system rendering the patient susceptible to lethal infections. Many adjuvants, immunostimulants and drugs with specific or non-specific effects are now being intensively investigated. Although most are still restricted to a few research centers, others are already widely used in the therapy of cancer and other disorders. The development and delivery of safe and effective vaccines for cancer control rank among the greatest achievements in the history of biomedical research. The central hypothesis behind active specific immunotherapy for cancer is that tumor cells express unique antigens that tell the immune system that something about these cells is foreign. A vaccine is a way of delivering an antigen to the immune system so that the immune cells recognize the antigen as foreign and destroy any cells bearing it.

Despite recent advances that have led to significant enthusiasm for development of anticancer vaccines, several major obstacles must be overcome. Vaccination for cancer can be given as adjuvant treatment after cytoreductive treatment of measurable cancer in order either to destroy micrometastases or for macrometastatic or disseminated cancer as well as in addition to postoperative chemotherapy to decrease the incidence of relapses or kill the residual tumor cells. In general, all of these vaccine agents are more effective when the antigenic mass is small.

Major advances in cellular and molecular immunology, and our increased understanding that the immune system is capable of recognizing immunogenic tumor-associated antigens (TAAs) have led to the development of several cancer vaccine approaches [1-4]. The critical importance of both cytotoxic ($CD_8^+$) and helper ($CD_4^+$) T-cells in achieving tumor rejection has already been demonstrated by several studies [5, 6]. Knowledge of the critical role of the cellular immune response has led to the realization that tumor-specific antigens are not only immunogenic but may be expressed on normal tissues.

Since the 1970s, when melanoma tumor antigens were initially studied [7], technology has improved and several antigens have been recognized by the immune system. Anti-tumor immunization strategies have taken many forms, such as, antigen-specific vaccines (vaccines targeting defined antigens) as well as peptide-vaccines, protein vaccines, DNA vaccines, recombinant viral vaccines and dentritic cell vaccines, including the use of whole tumor cells as vaccines.

Melanoma is the cancer prototype in the cancer immunology field, to which many forms of immunotherapy have been applied extensively because both many tumor antigens were discovered and cellular immune responses were characterized in patients with this type of malignancy [8].

Cervical cancer is the third most common cancer among women, with approximately 450,000 newly diagnosed cases each year and another 200,000 women who die from the disease annually [9-11]. Although women with early stage disease have an excellent prognosis with either surgery or radiation therapy, patients with more advanced disease have poor hope for prolonged survival.

Human papillomavirus (HPV)-induced carcinogenesis has been extensively studied and over 90% of cervical cancers (and cervical dysplasias) are associated with HPV infection, particularly types 16 and 18. Two early viral genes, HPV $E_6$ and $E_7$ play an important role in carcinogenesis by transformation and immortalisation of the infected cells [12] and binding to or inactivating the tumor suppressor gene products p53 and pRb, respectively [13]. $E_6$ and $E_7$ oncoproteins are expressed in cervical carcinoma cells and their expression is required for the maintenance of the cells in their transformed state. Therefore, $E_6$ and $E_7$ are ideal targets for specific T-cell mediated therapy of HPV-associated disease. The effectiveness of HPV vaccination as a strategy for cervical cancer prevention and control has been tested in experimental and clinical trials. The approach to immunization with HPV virus-like particles (VLPs) in order to generate virus-neutralizing antibodies has led to the development of an HPV preventive vaccine. VLPs are non-oncogenic, non-infectious and do not contain any viral DNA, making them suitable for prophylactic vaccines against genital HPV infection [14, 15]. Also, strong humoral immune responses have been achieved when VLP vaccines were given intramuscularly and at various doses [16-19] (phase I and phase II clinical trials). Future trials with HPV VLPs are needed to detect if the antibody response is able to prevent subsequent viral infection. The importance of the cellular immune system in the pathogenesis of HPV-associated cervical lesions is well supported and forms the critical step for therapeutic HPV vaccines [20].

High levels of $CD_8^+$ cytotoxic T cells (CTLs) in cellular infiltrates have been demonstrated on cervical malignancies [21]. HPV viral proteins expressed in infected cells are able to be recognized and targeted by therapeutic vaccines through stimulation of the immune system. Therefore, vaccines act both by eliminating already existing lesions (patients who are already infected with HPV) and by preventing the development of lesions (patients at high risk of developing cervical cancer or patients already with HPV-lesions).

Expression of the key oncoproteins $E_6$ and $E_7$ in cervical cancer cells and precancerous lesions makes them potential tumor specific antigens that can be targeted by the immune system [22, 23]. Peptide vaccines using $E_6$ and $E_7$ proteins, have been used in phase I/II clinical trials. Immunostimulatory peptides obtained from

the $E_7$ protein that bind to the human HLA-A*0201 MHC class I allele have been tested in patients with advanced-stage vaginal and cervical cancers showing specific cellular immune responses. Unfortunately clinical improvement was not detected in those clinical trials [24, 25].

Protein-based vaccines, able to induce antibody and helper T cell immune response, and also cytoxic T cell responses, are another type of vaccines under investigation for HPV-induced lesions. A few protein vaccines have already entered phase I and phase II clinical trials [20].

Chimeric papilloma virus-like particle vaccines (HPV VLPs) which include the proteins $E_6$ and $E_7$ or peptide epitodes originating from such proteins offer an exciting strategy for a combined therapeutic and prophylactic vaccine against HPV-induced lesions [26]. They are able both to achieve a protective humoral immune response against infection from HPV, and offer antigens to the immune system for priming of T-cell responses. Chimeric HPV VLP vaccines have been tested so far only in experimental animal studies [20].

DNA vaccination is another alternative in therapeutic vaccine development for HPV-associated disease. DNA vaccines containing $E_6$ and/or $E_7$ genes that have been altered by mutation, insertions or deletions, have already been studied in many murine models with marked elevation in the $E_7$-specific CTL response [27, 28]. In order to increase the immunity of DNA-based vaccines, genes that encode for proteins able to enhance immunogenicity are fused with HPV genes (heat shock proteins) with activation of $CD_8^+$ T-cells [29-33].

Viral vector-based vaccines, including recombinant vaccinia virus and adenovirus is another approach for tumor vaccination [20]. Recombinant vaccinia virus vaccines that express $E_6$ and $E_7$ proteins of HPV 16 and 18 have been administered to patients with advanced-stage and recurrent cervical carcinoma [34]. Also, protective antitumor immunity has been noticed by using recombinant adenoviruses that encode tumor rejection antigens as vaccines [20].

Recent vaccine developments for preventing HPV infection and treating HPV-associated diseases are highly promising in being able to induce HPV specific CTL responses in patients with cervical cancer and preinvasive disease. Recently Merck and Company Inc. announced that the company's investigational vaccine against HPV 16 eliminated the risk of infection with HPV 16 in 100% of patients who were not previously infected [35] (2 years into a planned 4-year proof-of-principle study).

Currently, only prophylactic vaccines have entered and progressed to large human trials including a phase III NCI trial that started in 2003 in Costa Rica which will target HPV 16 and 18.

Breast cancer remains the most common cancer in women, and is second only to lung cancer as the leading cause of cancer-related death. Breast cancer mortality is decreasing in most of the industrialized countries due to the early detection of the disease, the wide use of mammographic screening and the availability of improved therapies. The development of breast cancer vaccine approaches depends on the identification of appropriate target antigens, methods of adoptive immunization strategies and finally methods to avoid the tumor's immunological escape mechanisms.

Potential target molecules for antigen-specific breast cancer vaccines include several categories, such as differentiation antigens (carcinoembryonic antigen, NY-BR-1), cancer-testis antigens (NY-ESO-1, MAGE-1, MAGE-3, BAGE, GAGE, SCP-1, SSX-1, SSX-2, SSX-4, CT-T), amplified/overexpressed gene products (Her-2/neu, NY-BR-62, NY-BR-85, tumor protein D52) and mutational antigens [36]. Over-expression of the HER-2/neu protein has been associated with more aggressive tumors, resistance to chemotherapeutic agents, poor prognosis and high risk of relapse. Monoclonal antibodies against the HER-2/neu oncoprotein have been developed and added to first-line chemotherapy for patients with metastatic breast cancer who over-express this specific tumor antigen. Prolonged disease-free survival, overall survival and increased response rates have been demonstrated in clinical randomized trials [37]. Understanding the biology of breast cancer may lead to joining molecular genetics to standard treatment strategies and development of therapeutic breast cancer vaccines.

## Conclusions

Cancer vaccine therapy has made extremely great progress and remains an active and important area of investigation and research. Prospective randomized trials will show us how to promote T-cell activation and proliferation while preventing T-cell death (T-cell apoptosis). Identification of additional tumor-associated and tumor-specific antigens, and a better understanding of the ability of tumor to escape immune surveillance will lead us to a new generation of vaccine trials. Therefore, it will increase the number of individuals that can be treated with tumor vaccines, thus replacing conventional therapies.

The major problems that immunologists face are which immune effectors are needed for the optimal antitumor response, and which immunological assays are reliable in monitoring the patient's immune response in an optimal fashion after immunization. The precise monitoring of immune parameters and immune response before and during clinical trials will help in optimizing treatment procedures that may be available to oncologists and beneficial for cancer patients. Prospective trials to confirm the efficacy of vaccination in preventing and treating malignancies would be warranted.

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