The Current Status of Human Anti-Cancer Vaccines

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Abstract— Therapeutic cancer vaccines (or active immunotherapy) aim to guide the patient’s personal immune system to eradicate cancer cells. In contrast to chemotherapy, the theoretical benefits arising from this approach are represented by a greater capacity to selectively eradicate the modified cells, thus triggering lower toxicity, as well as the capacity to identify and invade multiple target molecules, including the newly emerging antigens on rapidly mutating tumor cells (as a result of epitope spreading). The success of the frequent pursuit to use the immune system in order to fight cancer in a systematic approach was restricted, eliciting a one hundred year-long dispute on the pertinence of the immune system in cancer observation and therapy.

Keywords— cancer vaccine, peptides, monoclonal antibodies, dendritic cells,

I. INTRODUCTION

The main purpose of cancer immunotherapy is that of inducing directed immune responses against cancer cells. [1-3] Immunotherapies like interferon-alpha (IFN-α) and interleukin-2 (IL-2) can trigger immunologic responses with possible antitumor effects in an outnumbered group of patients. [2] There have been significantly more extensive studies on the use of tumor vaccines and immunotherapies as attractive alternatives or additions to conventional cancer treatments throughout the last two decades (2–3). These perspectives are mainly based on instructing the patient’s immune system to identify and eradicate cancer cells. The fundamental benefits of such approaches lie in their capacity to: 1) generate specific eradication of tumor cells, with the slightest damage to normal cells, 2) systemically trigger anti-tumor immune responses able to direct primary and secondary metastases, and 3) produce immunological memory that would determine long-term protection against potential further tumor recurrence. [2-4]

This work was funded by the Romanian Ministry of Education and Research (PN-II-PT-PCCA-2011-3.1-1586, PN-II-RU-TE 2011-3-0251; PN-II-RU-PD-2011-3-0287 PN-II-PT-PCCA-2011 -3.1-1551; PN-II-PT-PCCA-2011-3.2-1289).

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II. TARGETED THERAPY BY MEANS OF PEPTIDE VACCINES

The exquisiteness of therapeutic cancer vaccines lies in their ability to trigger a directed antitumor immune response by targeting specific tumor-associated antigens (TAAs) as a result of T-cell stimulation. Human cytotoxic T cells can identify 9- to 14-mer antigenic peptides expressed within the major histocompatibility complex (MHC) on the surface of all cells. These peptides originate from endogenously expressed proteins, along with TAAs converted by proteases inside cells. The successful stimulation of T cells results in the detection of specific TAAs with the MHC and initiates directed immune-mediated cell death.[5, 6] Optimal TAAs are specific to or overexpressed on cancer cell surface. Even if vaccines usually aim specific TAAs, the resulting immune response may not be limited to the aimed TAA. Clinical data note that vaccine-activated immune cells prone to lysed cancer cells are likely to identify supplementary TAAs that the vaccine does not consist of, and finally target tumor cells by means of these secondary antigens. This phenomenon is called antigen cascade or antigen spreading. The focus of clinical immunotherapeutic studies in cancer has been on three classes of tumor antigens: tumor-specific and shared antigens (MAGE-3, NY-ESO-1, TRA-3); mutated unique onco genes (p53, α-actinin-4 and malic enzymes); oncofetal antigens (carncoembryonic antigen); self-antigens overexpressed in tumors (HER2, MUC1, survivin); antigens expressed in some normal tissues (WT-1, PRAME, Survivin-2b). Even though about 20 antigens have reached phase III clinical trials, cancer immunotherapy is still restricted by a lack of antigens that are universally correlated with disorders, strongly immunogenic, continuously expressed and cancer specific.[7] Nowadays, about 50 antigens are reported to be in progress, encompassing both membrane-bound and intracellular targets. The use of an attrition factor to this number, considering the acknowledged high lack of success rate of potential candidates, to develop into late stage progress and regulatory submission, makes it clear that it is not possible to ensure a future acceptable number of successful products. Thus, there is an immediate requirement for future tumor antigen findings and for a thorough immunological target confirmation.

The effectiveness of a therapeutic cancer vaccine is given by its ability to achieve two goals. It must first activate specific immune responses against the relevant target. Secondly, the
immune responses must be enough to defeat immunosuppressive mechanisms that can be used by tumors. [3] A few immunologic strategies may attain these purposes, two of which are displayed in this study. Autologous vaccines are produced from a patient's individual cells or tumor. These patient- specific vaccines are produced ex vivo in a labor-intensive method of production. Another possibility is patient-nonspecific, where a peptide or vector-based vaccine is produced to convey a TAA to the immune system in vivo, enabling immune stimulation inside the patient. [2] Approaches allowing cancer vaccines to beat immunosuppressive obstacles in order to trigger an immune response are also debated in this study. No matter the specific method employed, cancer vaccines are not as toxic as chemotherapy or targeted molecular inhibitors.

Developing antitumor vaccines is more demanding, due to the fact that causative agents of most infectious diseases are identified by the immune system as “non-self”. On the contrary, with their development in host tissues, tumors usually express “self” antigens, to which the immune system has previously been induced immunological tolerance, thus surrendering the development of tumor vaccines. The recent identification of several non-tolerogenic, tumor- associated antigens (TAAs), has emphasized their usefulness for the development of antigen- specific anticancer vaccines, including antigens stemming from mutations in oncogenes or oncopressor genes (e.g., KIT, BCR/ABL, RAS, BRCA1, BRCA2, HER2 and TP53), developmental antigens (e.g., MAGE, tyrosinase, melan-A, gp100); cancer/testis antigen (NY-ESO-1), antigens upregulated during malignant transformation (e.g., oncofetal antigens, carcinoembryonic antigen, α- fetoprotein), and viral antigens correlated with oncogenesis.[3, 8, 8-11] Some of the most common TAs are CTL (cytotoxic T lymphocyte, also known as CD8+ T- cells or killer T cell) epitopes. [12, 13] Peptide antigens are normally 8–10 amino acid long with 2-3 primary anchor residues that communicate with MHC class 1-molecules and 2-3 residues which attach to T-cell receptors. [12] CTLs targeted against peptides produced by MHC class I molecules represent strong effectors of the immune system against tumor cells. The T-cell antigen receptor (TCR) on T cells identifies the complex of a small peptide situated in the antigen-binding groove of an MHC molecule. MHC molecules (also called human leukocyte antigens (HLAs) in humans) are sub-grouped into class I molecules, which are found on all nucleated cells and class II molecules, appearing on specialized antigen-presenting cells (APCs) such as dendritic cells, macrophages, B cells and selected activated endothelial or epithelial cells. CD4+ T cells identify antigens attached to MHC class II molecules, and, as indicated, class II molecules are expressed on APCs that are able to capture antigen through phagocytosis and to bind to surface antibodies.[14, 15] After being subjected to phase I and II clinical experiments, a few peptide vaccines have shown encouraging results in terms of immunological and clinical responses. Several noticeable peptide vaccines have been subjected to phase I, II and III clinical experiments, such as HER-2/neu immunodominant peptide (lung, breast or ovarian cancer)[15, 16]; [17], Mucin-1 (MUC-1, Stimuvax) peptide (breast or colon cancer)[18], carcinoembryonic antigen (colorectal [19], gastric, breast, pancreatic and non-small-cell lung cancers) [20-22], prostate-specific membrane antigen (prostate cancer) [20, 23, 24], [25], HPV-16 E7 peptide (cervical cancer)[25], Ras oncoprotein peptide (colorectal and pancreatic carcinomas) [26-28, 28] and melanoma antigens (Melanoma) [29-31]. Some of the characteristics of peptide vaccines are their low prices, easy manufacturing and manipulation, defined structure, synthetic nature, batch-to-batch variation ability. One of the drawbacks of peptide vaccines lies in their poor immunogenicity.

The examination of approaches including epitope augmentation, employment of various T-cell epitopes, adjuvants, incorporation of co-stimulatory molecules, ex vivo carrying into antigen presenting cells aims to improve the immunogenicity and potency of these vaccines. [31-34] There are well known mechanisms enabling tumors to elude immune responses. These mechanisms ought to be counteracted in order to enhance the action of therapeutic vaccines. Suppression of T-cell stimulation can be achieved via inhibitory receptors such as cytotoxic T- lymphocyte associated antigen-4 (CTLA-4) and programmed death I (PD1), by means of CD4+CD25+FOXP3+ regulatory T cells (Tregs) or IL-2-mediated activation-induced cell death (AICD). [4] Immunosuppressive cytokines are also released by tumors and tumor-associated immune cells, such as IL-10 and transforming growth factor β (TGFβ). [35-37] Some of today’s immunotherapeutic interventions aim at the processes engaged in T-cell survival, stimulation, migration and tumor eradication. A comprehensive method adjusting T cell activity is CTLA-4 blockade, an important molecule restricting T-cell activation. [38] The antibody-mediated CTLA-4 blockade attached to B7 (CTLA-4 binding partner) exercises a strong supplementary impact on T cells. [38-40] The fully human monoclonal antibody, ipilimumab, obstructs the inhibitory signals transmitted by CTLA-4, enhancing T-cell stimulation and the infiltration of effector T cells into tumors, eventually inducing tumor-cell eradication.[40,41] Therapies based on ipilimumab, both standalone interventions and associated therapies, show an enhanced antitumor potency of certain therapeutic vaccines. [42] There have been early-phase clinical trial ipilimumab tests in patients with metastatic melanoma and CRPC. Their potential results led to FDA approval to use ipilimumab in metastatic melanoma individuals. [43] The employment of ipilimumab is now examined in Phase III clinical trials for the treatment of advanced castrate resistant prostate cancer (CRPC). Nevertheless, the association between CTLA-4 blockade and autoimmune adverse effects confirms the major impact of this receptor in controlling normal immune homeostasis.[44, 45] There is ongoing examination of PD-1 negative immunoregulatory receptor as a target for anticancer immunotherapy. Like CTLA-4, PD-1 is an inhibitory receptor expressed on activated T cells, B cells and monocytes. [51] Programmed cell death protein 1 (PD-1) can attach two ligands,
PD-L1 and PD-L2. [46-48] PD-L1 is expressed on both immune and non-immune cells, whereas PD-L2 is generally expressed on APCs. [46] There are elevated PD-1/PD-L1 levels in many types of cancer, due to bad prognosis in skin, lung, renal, pancreatic and ovarian tumors. [49-51] PD-1/PD-L1 (PD-L2) interactions are reported to appear more preferentially within the tumor microenvironment, thus playing a major role in the inhibition of the effector performances of tumor-infiltrating activated T cells. As a therapeutic approach, PD-1 inhibition has been anticipated to generate fewer side effects than CTLA-4 inhibition, mostly as a result of a more rigorous selectivity for immunosuppressive signals, which are straightly transferred by the tumor microenvironment in the case of PD-1, which predominantly takes part in the effector phase of T-cell responses. Current clinical experiments investigating anti-PD-1 (by Topalian et al.) [51] and anti-PD-L1 (by Brahmer et al.) [52] antibodies have shown surprisingly greater rates of long lasting responses in patients with advanced tumors, thus being able to supply a novel reference point for antitumor activity. Moreover, the interactions between PD-1 and PD-L1 blockade have been related to long-lasting tumor responses in patients with lung cancer, until now antagonistic to all immunotherapeutic approaches. Many preclinical debates and current clinical debates note the importance of immunosuppressive cells, for instance CD4+CD25+FOXP3+ Tregs, in establishing anticancer vaccine efficacy. [53, 54] Tregs convey T-cell tolerance to self-antigens and inhibit immune reactions. They concentrate in patients with cancer and lead to tumor evolution. Treg subset depletion is examined by different current approaches. Ontak is a recombinant cytotoxic protein consisting of the diphtheria toxin and full-length IL-2. It attaches to CD25-expressing cells by means of its IL-2 moiety and, after endocytosis, causes cell death as a result of diphtheria toxin action. Preclinical and clinical trials have indicated that the use of Ontak can determine CD25+ Treg depletion and can enhance vaccine efficiency by boosting T-cell responses and enabling higher concentrations of tumor-specific T effector cells on the sides and inside tumor lesions. [55] The interaction between Ontak and CD25 may also lead to activated T-effector cell depletion, limiting its antitumor efficiency. Recent investigations regarding additional Treg-targeted therapies include the employment of agonistic anti-GITR and antiox-40 antibodies as well as the blockade of CCR4/CCL22 synergies. [56] Several clinical studies have proven that cyclophosphamide (CTX), an immunomodulatory agent, enhances anticancer vaccine potency. The infusion of low-dose CTX as a “metronomic” regimen in patients with advanced cancers has been shown to generate an intense and selective reduction of circulating Tregs, impeding their inhibitory function. [56-58] Anticancer vaccines can also be improved by developing agents that restrict immunosuppressive factors such as TGFβ and IL-10. Expression of TGFβ receptors on immune cell types indicates that this cytokine is likely to have a wide immunosuppressive activity, disturbing the reaction of cytotoxic CD8+ effector T cells, CD4+ effector helper T cells, Tregs, natural killer (NK) cells and APCs. [59] Antigen-specific CD8+ T-cell effector functions in individuals with melanoma are restricted in vitro by adding TGFβ. TGFβ deletion highly amplifies antitumor immunity in animal models [60-62], promoting it as an applicable approach in humans, mainly as an addition to current therapies (chemotherapy and antitumor vaccines). A fully humanized monoclonal antibody aiming multiple TGFβ1 ligands (fresolimumab, Cambridge Antibody Technology/Genzyme/Sanofi) has advanced to multiple phases of preclinical studies, being now under clinical investigation for both oncological and non-oncological manifestations. The TGFβRII-blocking antibody, IMC-TR1, developed by Eli Lilly has just been introduced in clinical experiments for the treatment of breast and colon cancer.

III. DENDRITIC CELL-BASED THERAPEUTIC CANCER VACCINES

Dendritic cells (DCs) are potent antigen-presenting cells (APCs) widely employed in the design of anti-cancer vaccines. [63-66] There has been comprehensive study of the approach based on the vaccination with DCs pulsed with specific tumor-associated antigen (TAA)-derived peptides, [67, 68] Nevertheless, one of the main disadvantages of these strategies lies in the defined number of known TAAs available for multiple HLA molecules. Moreover, the efficient downregulation of a specific TAA is in contrast with cancer cells evading TAA-targeting immune responses. Therefore, there has been focus on the development of various strategies delivering as many whole TAAs as possible to DCs by means of cancer cell-derived RNA, whole cancer-cell lysates or apoptotic malignant cells. [68-70] The combination of DCs and whole neoplastic cells and use of the resulting DC/cancer cell chimera as an anticancer vaccine have also generated antitumor immune responses. Therefore, all TAAs (including familiar and unfamiliar molecules) are delivered to DCs, processed and presented to T cells in correlation with MHC Class I and II molecules and in the background of co-stimulatory signals. [71, 72] The chemical, physical or biological association of a DC with a cancer cell generates a heterokaryon expressing DC-derived co-stimulatory molecules, an efficient antigen-processing and antigen-presentation complex, as well as TAAs. [72] Therefore, at least from a theoretical perspective, this method enables DCs exposure to the entire collection of TAAs, primarily expressed by the malignant cell, processing them endogenously and presenting TAA epitopes thought the MHC Class I and II pathways, activating both CD8+ and CD4+ T cells. [73] Following exposure to polyethylene glycol (PEG), DCs and cancer cells become hybrid cells with united cytoplasm but maintaining separate nuclei identity. [74] This structure enables TAA and DC-derived MHC molecule co-expression, generating TAA processing and presentation on the cell surface, alongside DC-derived co-stimulatory molecule expression. Endogenous DCs in patients with cancer are a target of tumor-correlated suppressive factors, resulting in their abnormal performances and defective progression of effector
functions in tumor-specific lymphocytes.[75, 76] IL-10, TGF-β, VEGF, IL-6 and prostanooids, such as PGE2 – 6[77] are some of the mediators of this tumor-induced DC impairment, resulting in the defective maturation of DCs developing in their presence, increasing the expression of co-stimulatory molecules required for T-cell stimulation and producing cytokines required for survival and effector performances in tumor-specific T cells. Dendritic cell dysfunction has been observed not only in individuals with ovarian, breast, melanoma, renal and prostate cancer[78-80], but also in the blood of those with head and neck and lung cancer.[53, 81] STAT-3 activity is modulated by tumor-derived factors which disrupt key intracellular signaling pathways needed for DC stimulation and final growth, including NF-kB stimulation.[82-84] The lack of suitable co-stimulation and cytokine production by DCs results in the anergy of naive, memory and effector T cells, and theirtransformation into regulatory T(reg) cells, supporting tumor escape. Besides inducing abnormally tumors of tumor-infiltrating DCs, tumor-derived factors also trigger DC apoptosis [85, 86], able to promote T-cell lack of responsiveness.[87] These DC apoptosis-inducing factors are represented by ceramides, gangliosides, nitric oxide and IL-10, able to produce DNA fragmentation in DCs and to put an end to anti-tumor immunity.[88]

IV. VACCINES TARGETED TO THE TUMOR NEOVASCULATURE

Targeting tumor vasculature by means of vaccines or other immunotherapies may produce possible benefits over targeting tumor cells. Firstly, tumor endothelial cells are more handy to the immune system than tumor cells far away from the vessels. Secondly, tumor endothelial cells are normally more balanced genetically than tumor cells, thus lowering the possibility of resistance to immunotherapies.[14, 15] Nevertheless, chromosomal abnormalities have been detected in solid tumor endothelial cells[16, 17], and in glioblastomas, the tumor cells and the endothelium come from common cancer stem-like cells.[89-91] The third advantage is given by the down-regulation of MHC I in tumor cells, occurring more rarely in tumor endothelial cells, thus generating a stronger CD8+ mediated response. Another benefit offered by immunotherapies targeted towards tumor endothelial cells lies in the possible amplified inhibitory impact, considering that inhibition of one endothelial cell can inhibit almost 100 tumor cells.[92] These presumed benefits and individually expressed proteins in the tumor endothelium led to a series of immunotherapeutic approaches targeting angiogenesis, along with monoclonal antibodies, vaccinations and additional co-activating therapies.[93, 94] So far, passive immunotherapy employing monoclonal antibodies has been the most efficient approach. In 2004, bevacizumab, the monoclonal antibody targeting angiogenesis via VEGF, was approved for use in colorectal cancer treatment.[95, 96] Bevacizumab has also shown efficiency against lung, renal and breast cancers.[97, 98] The achievement and capacity of bevacizumab to selectively target tumor endothelial cells was an incentive to develop other types of angiogenic immunotherapies. A few encouraging preclinical trials of tumor endothelial vaccines have primarily led to Phase I clinical trials. As part of the blooming sphere of tumor immunotherapies, our focus will be on tumor vaccines with a significant anti-angiogenic constituent. Nowadays, the best known types of nucleic acid demonstrating an anti-angiogenic impact in mouse models are plasmids encoding angiogenic self-antigens. Additionally, the most frequently used delivery system for plasmid-based vaccines has been represented by bacteria.[99] The potential of orally infused bacteria-based vectors, with weakened, non-replicating strains of Listeria or Salmonella, in cancer prevention and treatment by inhibiting angiogenesis has been proved in several animal models.[100-102] Considering the safety concerns regarding these bacterial delivery systems, it is important to mention that Salmonella enterica strain has been certified by the FDA for vaccine use.[103] A few bacterial vaccines that had obvious anti-angiogenic and anti-tumor activity have also produced little or no autoimmune responses, at least in animal trials. Electroporation is another attractive method employed in DNA or RNA vaccines targeting tumor endothelium.[104] The great amount of antigen presenting cells turns skin into a typical delivery path for various delivery systems, including electroporation. Long-lasting immune protection against tumor angiogenesis and maturation is provided by intradermal DNA vaccination. Electroporation might be less common than direct inoculation of plasmid DNA, but it might be more efficient. For instance, intradermal electroporation of "naked DNA" generated a much more powerful anti-angiogenic and anti-tumor immune reaction to survivin than intramuscular DNA inoculation.[105, 106] The angiogenesis-associated receptor VEGFR2 (vascular endothelial growth factor receptor 2, also known as FLK-1 or KDR) is expressed on tumor endothelial cells. There is rare occurrence of angiogenesis in non-tumor vessels, allowing preferential targeting of VEGFR2-mediated tumor angiogenesis with minimum side effects. VEGF-mediated signaling via VEGFR-2 is the major rate-limiting stage in tumor angiogenesis, of great importance in tumor neovascularization, evolution and maturation. Moreover, VEGFR2 has a key role in both physiologic angiogenesis and vasculogenesis as showed by the missing vasculogenesis in VEGFR2 null mice.[107] VEGFR2 is part of the tyrosine kinase receptor family together with VEGFR1, VEGFR2, VEGFR3 and neuropilin 1. As VEGFR2 is a selective marker for neoplastic endothelium, many approaches have employed it as a target. Such examples are small molecule kinase inhibitors, synthetic receptor tyrosine kinase inhibitors, monoclonal antibodies and vaccines.[108] The number of vaccines and immunization approaches designed and tested in pre-clinical experiments against VEGF/VEGFR2 has been greater than with that of any other tumor angiogenic marker. Some DC features, along with their full-growth status, migratory potential and cytokine secretion, proved to be significant for the potential generation of numerous Th1-type CD4+ T cells and CD8+ CTLs by DC-based cancer vaccines. Effective generation of anti-tumor CTL reactions needs adult


