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Immunizing against breast cancer: A new swing for an old sword

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SUMMARY

Therapeutic potential of vaccination has been explored in many clinical trials involving patients with breast cancer. A large variety of cancer immunogens have been tested. The majority of clinical vaccination studies have been carried out in patients with metastatic breast cancer, characterized by extremely aggressive malignant tumors, resistant to all standard cytotoxic treatments and with longest-lasting disease. With active specific immunotherapy, tumor-associated antigens coupled to appropriate adjuvant can elicit a powerful antitumor responses. The potential advantages of therapeutic cancer vaccines are that they can augment an established immunogenic response to the tumor (which is generally weak in breast cancer), they target specific tumor antigens (although there are few), they are potentially non-toxic, they can be combined with conventional therapies and/or other immunotherapies, and they elicit immunologic memory to prevent recurrence of the tumor. It is unclear whether therapeutic vaccines for cancer prolong survival. Data of clinical activity have been observed by using vaccines targeting HER-2/neu protein, human telomerase reverse transcriptase, carcinoembryonic antigen (CEA), and carbohydrate antigen given after stem cell rescue. A better understanding of the relation between innate and adaptive immune responses, and of the immune escape mechanisms employed by tumor cells, the discovery of mechanisms underlying immunological tolerance, and acknowledgment of the importance of both cell-mediated and humoral adaptive immunity for the control of tumour growth are necessary for leading to a more comprehensive immunotherapeutic approach in breast cancer.

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Introduction

Genetic and epigenetic events can generate a large number of new antigens that are constantly expressed in tumors as they progress. The immune system can identify these antigens and generate humoral and cellular responses thus inhibiting tumor development.[1,2] An active immunization has the potential advantages of a non-toxic therapeutic modality capable of inducing antitumor immune responses in patients with tumors.[2] Induction of strong immunity by cancer vaccines is expected to lead to the establishment of immunological memory, thereby preventing tumor recurrence. Vaccination in patients with breast cancer could induce an expansion of CD8+ cytotoxic T lymphocytes (CTLs) capable of rejecting tumor cells via recognition of tumor-associated antigenic (TAA) epitopes presented on the surface of cancer cells in association with human leukocyte antigen (HLA) class I molecules. The antigens used in breast cancer vaccination strategies can be represented by whole tumor cells/dendritic cells (either allogeneic or autologous) or of specific TAAs, which are delivered as DNA (naked or comprised in recombinant viruses),

RNA, protein or HLA class I/II restricted peptide epitopes.[3] An ideal and successful vaccine should have: a target antigen on tumor cells to direct the immune response; a platform to present the vaccine-derived antigen to immune system; an adjuvant to enhance immune stimulation, and appropriate monitoring techniques.[2]

Results on vaccination trials are not exciting. These negative results can be related to the selection of a population of metastatic patients that is characterized by large tumor burden; as a consequence we observe the ability of large tumors to escape the immune system and the difficulty to break immune tolerance.[4] Therapeutic cancer vaccines will probably be more active in patients with minimal residual tumor burden, but most of the trials so far have been conducted on metastatic patients and limit the success of phase I/II trials.[5] Another issue is related to the difficulty in comparing the various approaches because monitoring assays haven't been standardized yet. Identification of univocal surrogate immunological markers of vaccine activity should be a challenge for the future.[6,7]

Many tumor antigens used in breast-cancer immunotherapy are expressed on normal tissues but are overexpressed or mutated on tumor cells: MUC1, HER-2, CEA, hTERT, p53 and carbohydrate antigens.

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Table 1

Main trials with breast cancer vaccines in metastatic setting

Reference	Vaccine	No. of patients (breast)	Research phase
Disis '98[12]	HER-2 peptides + GM-CSF	17 patients with HER-2-overexpressing breast and ovarian cancer	Phase I
Disis '04[13]	HER-2 HLA-A2 (if HLA-A2+) or ECD or ICD (if HLA-A2−)	64 patients with HER-2-overexpressing breast and ovarian cancer	Phase I
Knutson '01[14]	HER-2 peptides with HLA-A2 epitopes	19 with advanced HER-2-overexpressing breast or ovarian cancer	Phase I
Goydos '96[15]	Five MUC1 epitopes plus BCG	63 with adenocarcinoma (9)	Phase I
Marshall '04[16]	rF-CEA-Tricom +/− rV-CEA-Tricom	58 with CEA-expressing adenocarcinoma (3)	Phase I
MacLean '96[17]	Theratope with KLH and DETOX-B: CTX versus no-CTX	18 breast cancer	Randomized Phase II
Mayordomo '04[18]	Theratope vs KHL with concomitant HT following first-line therapy	1028 breast cancer	Randomized Phase III
Melisko '03[19]	APC8024 (HER2-GMCSF)	17 advanced HER2 positive breast cancer	Phase II
Kylstra '03[20]	APC8024 (HER2-GMCSF)	16 advanced HER2 positive breast cancer	Phase I/II

HER2 = HER-2/neu; CEA = Carcinoembryonic antigen; IDC = intracellular domain; ECD = extracellular domain; GM-CSF = granulocyte-monocyte colony stimulating factor; CTX = cyclophosphamide; HT = hormonal treatment; rF = recombinant nonreplicating avipox virus; rV = recombinant virus vaccines; BCG = Bacillus Calmette-Guerin

Table 2

Trials with breast cancer vaccines in adjuvant setting

Reference	Vaccine	No. of patients	Research phase
Disis '04[13]	HER-2 ICD peptide	29 with HER-2-overexpressing breast and ovarian cancer	Phase I
Limentani '05[21]	HER2-ECD/ICD peptide	15 with stage II/III breast cancer	Phase I
Gilewski '00[22]	MUC-1 keyhole limpet hemocyanin conjugate plus QS-21	9 high risk breast cancer	Phase I
Holmberg '00[23]	Theratope following HDCHT	33 high risk or advanced breast cancer	Phase I
Chui '03[24]	Her2E75 (4); CEAmRNA (3); HER2ICD (1); CEAmRNA-HER2ICD (1)	9 high risk or advanced breast cancer treated with HDCHT/ASCT	Phase I
Wiseman '95[25]	allogeneic tumor cell/BCG immunotherapy	13 with inflammatory breast cancer	Phase I

HER2 = HER-2/neu; CEAmRNA = Carcinoembryonic antigen messenger ribonucleic acid; IDC = intracellular domain; ECD = extracellular domain; HDCHT = high dose chemotherapy; ASCT = autologous stem cell transplantation; BCG = Bacillus Calmette-Guerin

Therapeutic cancer vaccines

Passive immunotherapy is based on the adoptive transfer of ex-vivo activated immune cells, immunomodulators (including cytokines) or tumor-specific antibodies. Although not generally associated with the generation of immunological memory, up to now antibody-based therapy has proven to be more beneficial than other immunotherapy approaches, leading to complete regression of tumors in a good proportion of treated individuals, when combined with chemotherapy.[8] Some antibodies can act either directly by blocking signal transduction pathways (i.e. when targeted to growth factor receptors) or indirectly via the activation of NK-mediated killing (ADCC: antibody-dependent cellular cytotoxicity). Alternatively, antibodies can be conjugated to radioactive molecules, chemotherapy drugs or toxins and act as carriers to deliver in a highly specific manner the active compound. Other passive immunotherapies based on the selection of patient's specific anti-tumor T lymphocytes and the reinfusion in the same patient after in vitro expansion with cytokine cocktails and/or transduction with high affinity tumor-specific TCRs also hold promises.[9] Nevertheless although extremely promising, only episodically beneficial effects on highly selected cohorts of patients were observed so far, suggesting that further improvements are still awaited.

Active immunotherapy is aimed to activate the patient's own immune system via the administration of a therapeutic vaccine. This strategy has the main advantage that, if successful, can elicit long-lasting immunological memory that can protect against minimal residual disease and tumor recurrence. Therapeutic cancer vaccines are usually applied in the metastatic setting of disease in order to reverse the lack of tumor control by the immune system. Several approaches have been tested so far, the most promising being based on the use of dendritic cells as nature's adjuvants.[10,11] Several phase II and III trials are actually ongoing in metastatic disease and in the adjuvant setting; the most significant ones are reported in Tables 1 and 2. Overall, the most frequent scenario of these therapies was that even when an immune response was effectively induced, the immunological outcome did not correlate with a significant clinical response. Using conventional criteria for clinical tumor response, objective response rate in cancer vaccines trials was only 3.3%.[26] One major problem is that assessment of therapeutic effect is based on criteria developed for cytotoxic agents, which have different clinical characteristics than heterogeneous biologics.

The Cancer Vaccine Clinical Trial Working Group together with pharmaceutical and biotechnology industries and the US Food and Drug Administration (FDA), have defined developmental guidelines to address the unique characteristics of therapeutic cancer vaccines.[27] They suggested that therapeutic cancer vaccines be investigated in two sequential steps: proof-of-principle trials and efficacy trials. Proof-of-principle trials should include a homogeneous population of nearly 20 patients in an adjuvant or in a metastatic setting without rapidly progressive disease to allow vaccines adequate time to induce biologic activity. Objectives should include initiation of a safety database, determination of dose and schedule, and demonstration of biologic activity (defined as any effect of the vaccine on the target disease or host immune system). Only if proof-of-principle trials show such immune response, or other biologic or clinical activity, efficacy trials may be initiated. Efficacy trials are encouraged to be randomized studies and should formally establish clinical benefit. Altogether this differs from single-arm phase II trials used for cytotoxic agents, which often use tumor response rate as the primary end point and historical controls

as a comparator. Efficacy trials can then expand from randomized phase II into phase III studies if well-defined trigger-point criteria are met.

The identification of specific tumor escape mechanisms gives the opportunity to envisage possible strategies for the rational design of tumor immunotherapies to translate into clinical practice. In these approaches the specificity of the antigen might still be extremely relevant, but it will have to be coupled to other devices to target immune-escape mechanisms. In this setting what is important is to identify the better population candidate to a vaccine trial, before than the better antigen. Large population analyses on specific subtypes of breast cancer are necessary in order to select patients who have higher probability to express that specific antigen. First select the patient, then drive the design of the clinical trial. In order to design a 'second generation' immunotherapy protocols we should highlight 3 issues: (1) the ability to initiate tumor-specific immunity, either directly by providing tumor associated antigens or indirectly, by favoring the cross-presentation of endogenous tumor antigens; (2) the capacity to recruit effector immune cells within the tumor site, by increasing tumor visibility; (3) the ability to preserve immune cell functionality within the tumor microenvironment through the subversion of immune-escape mechanisms. It is becoming clear that these three features cannot be provided for by a single modality and combined therapies should be proposed.

Prediction of clinical efficacy based on immunologic monitoring is crucial for the rational design of cancer vaccination studies. There are a number of new techniques permitting investigators to dissect T-cell responses ex vivo. It is now possible to determine molecular features of human T-cell responses in great detail, going much beyond what is usually done to assess T cells in animal models. It would be useful to identify some surrogate markers to improve vaccine activity and address questions about the best treatment schedules, and shift from laboratory surrogate markers to clinical practice end-points. A higher immune response should theoretically correspond to a higher survival rate. Doses, immunization schedules, methods of administration, timing of vaccinations and of following boosts to maintain a durable immune response need to be addressed in prospective clinical trials. Optimal combination vaccine therapy with a variety of novel approaches (e.g., monoclonal antibody as trastuzumab or tyrosine kinase inhibitors) is a great promise but it also requires evaluation in clinical trials to assess its benefit. More focused developmental guidelines are needed to address characteristics of therapeutic cancer vaccines. We should consider an adequate time to induce biologic activity and we should incorporate in our trial immune and molecular surrogate markers. A new area of investigation should be considered combination of chemotherapy with vaccine therapy. It is now clear that the way a chemotherapeutic drug kills a tumor cell determines how that dying cell interacts with the immune system and whether the interaction leads to an immune response. Chemotherapy depletes regulatory T cells, potentially enhancing immune responses. Furthermore, lymphodepletion triggers homeostatic T cell reconstitution, creating new populations of pre-T cells that need education in the thymic environment. An understanding of the underlying cellular and immunological events in both animal models and patients undergoing chemotherapy will guide decisions about which immunomodulatory approaches may be effective with different cytostatic drugs and hence to develop appropriate scheduling for integration of the treatment modalities.

Cell death following chemotherapy may be either immunogenic or non-immunogenic, and the type of cell death may have a profound influence on the subsequent immune response. Immunogenic cell death induces dendritic cell (DC) maturation, allowing the DC to activate relevant T cells. In contrast, non-immunogenic cell death is bland and does not activate DC. Historically, apoptosis has been considered to be a tolerogenic or non-immunogenic event.[28] However, there is now evidence that death by apoptosis resulting from chemotherapy may not be a tolerising event and may in fact prime the immune system for an anti-tumor response in experimental models,[29] particularly in the setting of massive apoptosis which overwhelms normal clearance mechanisms.[30] This suggests an opportunity to exploit cell death from chemotherapy with immunotherapy or cancer vaccines. We have evidences that lymphodepleting chemotherapy can be used in conjunction with tumor vaccination. Immunomodulatory effects have been described for several cytotoxic agents: cyclophosphamide, gemcitabine, adriamycin, taxanes and 5-fluorouracil. The immunomodulatory effects of cyclophosphamide have been extensively studied. Cyclophosphamide is a DNA alkylating agent which has been used over decades to treat hematological and solid malignancies. It displays either immunosuppressive or immunopotentiating effects, depending on the dosage and the timing of drug administration. Cyclophosphamide potentiates delayed-type hypersensitivity (DTH) reactions in murine models, with the optimum timing of the dose being 1 to 3 days before antigen administration. The mechanism of DTH potentiation is thought to be through a reduction in regulatory T cell function.[31] Cyclophosphamide also augments antibody responses in animal models, with the optimum timing of administration again being 1–2 days prior to antigen.

Future perspectives

Several questions are raised by all remarkable data presented in this lecture; answer to these questions should be considered possible area of research in the following years. Should all cancer patients be treated with an active immunotherapy approach or only individuals potentially more "responding"? How can we predict that the individual will develop an immune response against a particular antigen used in the vaccine formulation? What are the risks associated with such a vaccination, i.e. the possibility to develop an autoimmune response? What is the durability of immune protection? Can we combine vaccine therapy with therapeutic monoclonal antibodies or small target oriented molecules? Continued basic research into the molecular mechanisms regulating carcinogenesis and immunosurveillance will identify new potential target introducing vaccine therapy in prevention trials for patients at high risk for developing cancer. Target antigens that are directly involved in promoting the neoplastic process can induce an optimal antibody response. During tumor progression several genetic hits might make specific signalling pathways redundant and relevant for activating other downstream pathways. As preventive vaccines operate during the early phases of carcinogenesis, effective inhibition of the specific targets will arrest the whole. As for all preventive medicine, an extremely low incidence of adverse effects will be a prerequisite of preventive cancer vaccines. Another important long-term concern for cancer preventive vaccination is the induction of autoimmunity, which depends on the kind of tumor antigen that is targeted and the response that is elicited. Induction of a specific immune response against the most common oncoantigens over expressed by pre-neoplastic lesions might constitute a new scenario in cancer prevention. The translation of preclinical data into preventive treatments requires more attention since the plan is to vaccine a healthy individual. Vaccination against selected oncoantigens of healthy people who have a specific genetic risk of cancer, who have been exposed to an exogenous carcinogen, or who bear multifocal pre-neoplastic lesions would provide the most appropriate scenario.

There is no doubt that the findings reported in cancer prevention vaccination trials open a new field at the interface of basic science, clinical medicine, public health, and public policy. It

is important to keep in mind that these new treatments raise many scientific, medical, economic, and sociological questions. To improve the efficacy of the breast cancer vaccines are needed a better understanding of the relation between innate and adaptive immune responses, and of the immune escape mechanisms employed by tumor cells, the discovery of mechanisms underlying immunological tolerance, and acknowledgment of the importance of both cell-mediated and humoral adaptive immunity for the control of tumor growth.

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