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Generation of more effective cancer vaccines

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Cancer vaccines represent a promising therapeutic approach for which prime time is imminent. However, clinical efficacy must be improved in order for cancer vaccines to become a valid alternative or complement to traditional cancer treatments. Considerable efforts have been undertaken so far to better understand the fundamental requirements for clinically-effective cancer vaccines. Recent data emphasize that important requirements, among others, are (1) the use of multi-epitope immunogens, possibly deriving from different tumor antigens; (2) the selection of effective adjuvants; (3) the association of cancer vaccines with agents able to counteract the regulatory milieu present in the tumor microenvironment; and (4) the need to choose the definitive formulation and regimen of a vaccine after accurate preliminary tests comparing different antigen formulations. The first requirement deals with issues related to HLA restriction of tumor antigen presentation, as well as usefulness of tumor antigen spreading and counteraction of immune escape phenomena, linked to tumor antigen down-modulation, for an effective anti-cancer immune response. The second point underscores the necessity of optimal activation of innate immunity to achieve an efficient adaptive anti-cancer immune response. The third point focuses on the importance to inhibit subsets of regulatory cells. The last requirement stresses the concept that the regimen and formulation of the vaccine impacts profoundly on cancer vaccine efficacy. A new generation of cancer vaccines, provided with both immunological and clinical efficacy, will hopefully soon address these requirements.

Keywords: cancer vaccines, tumor antigen, adjuvants, telomerase, tumor immune escape, combinatorial therapy

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The approval by the Food and Drug Administration of Sipuleucel-T (Provenge®) for the treatment of advanced prostate cancer provided a boost to the development of cancer vaccines.[1] For the first time the therapeutic potential of cancer vaccines has been officially recognized. However, those working in the field recognize that substantial improvements are required to make cancer vaccines a viable alternative or complement to traditional anti-cancer therapies. Although the rate of vaccine-specific immunological responses is often elevated, the rate of clinical responses is generally low.[2–4] One of the reasons for these unsatisfactory results could be the inappropriate use of follow-up criteria adopted for conventional cancer therapy. Indeed, RECIST criteria may not be suitable for immunotherapy since they are mainly based on the evaluation of the treatment's eradication potential applied, for instance, to cytolytic therapies.[5,6] Effective cancer vaccines usually are not directly cytotoxic, leading to inflammation of the tumor rather than immediate necrosis. Hence, an immunotherapy-sensitive lesion could show stable or even increased size for long time, thus failing to show amelioration when RECIST criteria are used to assess disease progression. This could cause premature withdrawal from the immunotherapy protocol, thus precluding potentially positive responses to treatment from being discovered. For this reasons modified RECIST criteria have been proposed for immunotherapies, and

future analyses will enable an understanding of whether vaccination can improve the rate of successful treatments.[7]

Apart from these considerations, we must be aware that optimal schedules for cancer vaccine protocols must be identified, which is the real challenge. In fact, several aspects must be taken into consideration in the setting of an optimal vaccine regimen. Indeed, the first point of discussion is the choice of the immunizing antigen. A plethora of tumor antigens has been identified—but how to choose among them? An attempt to clarify the issue by the National Cancer Institute categorizes each tumor antigen on the basis of its capacity to fulfill criteria such as therapeutic function, immunogenicity, oncogenicity, specificity, expression level, stem cell expression, number of patients with antigen-positive cancers, number of epitopes, and cellular location of expression.[8] What emerges from this proposal is that the ideal tumor antigen does not exist and hardly one will be identified with such characteristics. Therefore, before selecting a tumor antigen, an answer must be provided to these questions: (1) Is the vaccine antigen specific to a single tumor type, or is it common to many types of cancer? (2) Is it a surface antigen? (3) Is the candidate tumor antigen necessary for tumor growth and survival, or not? (4) Can several tumor antigens be associated with each other? In addition, tumor-specific antigens[9,10] need to selectively induce immune responses against tumors while sparing normal tissues.

Recently we have witnessed the attempt to develop personalized cancer vaccines, and some groups are applying genomic and proteomic approaches to the search for unique single-tumor-specific antigens.[11,12] Theoretically, the more tumor-restricted the antigen, the more specific the immune response will be, thereby creating the conditions for high specificity of the response and greater avoidance of side effects related to collateral damage of healthy tissues by the vaccine-induced immune response. While appealing, this approach would result in the proliferation of numbers of cancer vaccines that exceed the types of cancer. An extreme characterization of this new trend will be a personalized cancer vaccine unique for each patient. Is this an effort we can afford? If we think to cancer vaccines as a therapy of the future, possibly adopted in any country and at any latitude, is it realistic to imagine a widespread diffusion of such an onerous (technically and economically) approach? An alternative could be the choice as immunogens of universal tumor-associated antigens (TAA, e.g., telomerase, survivin),[13,14] expressed by the majority of cancers. In this case, the same vaccine could be applied to the treatment of several tumor types. Telomerase-based vaccines are examples demonstrating feasibility and efficacy of this approach.[15–21] But are the two strategies really alternatives to each other?

Evidence suggests that antigen spreading may occur in effective vaccine-induced immune responses against cancer.[22,23] Hence, a scenario can be envisaged in which an initial anti-tumor immune response (in any kind of tumor) could be induced by a cancer vaccine based on a universal TAA, and a subsequent tumor-specific boost could be provided by immunization against a tumor-specific antigen. The immunization against the universal TAA would induce an initial immune response leading to in situ inflammation and recruitment of lymphocytes specific to a broad spectrum of tumor antigen specificities; the immunization against the tumor-specific antigen(s) would allow the expansion and affinity selection of (more) strictly tumor-specific T-cell clones. This dual-faced strategy also would have the advantage of targeting multiple epitopes/antigens at the same time. This is important since one of the most effective mechanisms of tumor immune escape is down-modulation of tumor antigen.[24] Indeed, the greater the number (and type) of target antigens, the more difficult it will be for the tumor to escape immune surveillance through antigen modulation. Accordingly, vaccines have been developed including multiple epitopes of tumor antigens.[11,15,25–27] However, the inclusion of multiple epitopes in a vaccine does not protect against the risk of tumor immune escape via antigen down-modulation. We reason that the biological characteristics of the immunizing molecule may have an impact on the immunogenicity of the cancer vaccine. For instance, tumor antigens likely to offer greater immunogenicity could be (1) those strictly related to the

Table 1. Some options and requirements relevant for the setting of a cancer vaccine

Issue	Options	Suggested requirements
Tumor antigen(s)	(1) Tumor-specific (2) Universal (3) Both	(1) Involved in pathways of tumor growth or survival (2) Surface antigen
Immunizing epitope(s)	(1) Single (2) Multiple	(1) Restricted by both HLA class I and II molecules (2) Restricted by multiple HLA alleles
Activation of innate immunity	(1) One adjuvant (2) Multiple adjuvants	Activation of multiple pathways and functions
Inhibition of regulatory cells	(1) Biological agents (2) Chemotherapy	Inhibition of multiple cell types and functions
Regimen and formulation	(1) Routes of administration (2) Schedules (3) Antigen formulation (cell lysate, protein, peptide, DNA, RNA) (4) Co-administration of cytokines	Selection through preclinical testing

mechanisms of tumor growth/survival, and (2) those expressed at the cell surface. The former type of antigens would tend not to be down-modulated due to the importance in the economy of the tumor growth; the efficacy of immunotherapies targeting protein tyrosine phosphatases supports this consideration.[28] The second type of tumor antigen could allow the combined targeting of the same molecule by vaccine-induced T cells and by specific cytotoxic/neutralizing antibodies. This combined but little-explored approach[29] could prove beneficial against immune escape due to antigen downregulation and/or an impairment of the antigen presentation machinery.[24]

An important issue in formulating a multi-epitope cancer vaccine is selection of the immunogenic epitopes. Two major rules must be taken into consideration: (1) the need to activate both $CD4^+$ and $CD8^+$ tumor-specific T lymphocytes in order to achieve efficient tumor rejection;[30–33] and (2) the opportunity to conform to the widest possible array of HLA haplotypes in order to make the vaccine suitable for patients with the greatest genetic assortment. Several vaccination procedures allow to fulfill such requirements, e.g., those using as immunogens either whole molecules (as in the case of DNA vaccines, RNA vaccines, or vaccines consisting of tumor lysate-pulsed dendritic cells [DC])[34] or multi-peptide vaccines containing promiscuous peptides (i.e., peptides binding to various HLA molecules).[26] As for peptide vaccines, recent data from this and other laboratories corroborate the concept that multi-epitope vaccines may be both immunologically and clinically effective.[11,15,35]

An essential requirement for achieving effective anti-cancer immune responses is the optimal activation of innate immunity. In fact, the immune system generates effector responses when it "senses" danger signals.[36] Pattern recognition receptors are devoted to this function; when stimulated they induce the activation of genomic pathways leading to complex integrated effector functions.[37] Adoptive immune responses are strictly dependent on adequate activation of innate immunity.[38] Hence, the selection of the appropriate adjuvant is as important as that of the immunizing tumor antigen. Again, a wide choice of adjuvants is now available.[39] Each of them has specific activities, but none has the capacity to mediate alone the complex network of activating signals that pathogens have. This provides the rationale for the use of multiple adjuvants, preferably those with complementary functions.[40] Accordingly, in the GX301 vaccine we associated Montanide ISA-51 and Imiquimod. The first adjuvant generates a water-in-oil emulsion, which protects vaccine peptides from tissue proteases and favors uptake by antigen presenting cells. Moreover, it induces IFNγ release by innate immunity cells, favoring the expression of HLA molecules by tumor cells.[41] The second adjuvant is a potent activator of Toll-like receptors 7 and 8, inducing strong activation of DCs.[42] Collectively, these two adjuvants exert complementary functions fostering simultaneous uptake and presentation of vaccine peptides. It is of interest that by associating the multi-epitope and the dual adjuvant strategies, we achieved 100% of cancer-specific immune responses in a series of highly advanced prostate or renal cancer patients.[15] Indeed, future studies are needed to explore the feasibility and efficacy of other adjuvant associations in order to select the most effective ones.

Several other aspects deserve mention, including the importance of the route, tools and schedule of vaccine administration, the selection of the appropriate antigen-presenting cell, the usefulness of cytokine co-administration, and the opportunity to associate cancer vaccines and chemotherapy drugs. However, all these points have been exhaustively reviewed recently,[10,43–46] and will not be analyzed here.

A final issue has fundamental relevance to effective cancer vaccines: the inhibition of tumor-dependent regulatory functions. The therapeutic efficacy of Ipilimumab supports this point.[47] We are aware that several regulatory cell subsets co-exist within tumor microenvironment, e.g., $CD4^+$ and $CD8^+$ T regulatory lymphocytes, myeloid derived suppressor cells, and tumor-associated macrophages.[48,49] Hence, a combination of inhibitors that counteract different regulatory pathways is likely to enhance the immunogenicity of cancer vaccines. Thus, in an experimental model of melanoma, we consistently achieved 100% protection from tumor growth when the vaccine was co-administered with a neutralizing anti-IL10 antibody,[50] given that IL10 secretion is a hallmark of different subtypes of cells with regulatory function.

In conclusion, an ideal cancer vaccine must integrate many options with respect to its composition and the several requirements discussed above (**Table 1**). Furthermore every possible vaccine combination should be tested at the preclinical level to select the combination that best fulfills the requirements desired for clinical efficacy. It is no surprise that the same tumor antigen may yield different clinical results depending on the immunization setting.[50]

Collectively, the multitude of data obtained so far indicate that the right approach for generating more effective cancer vaccines is combinatorial. In fact, only by combining different (biological and traditional) agents will it be possible to fulfill the numerous requirements enabling cancer vaccinology to move from empiricism to a well-structured science, and cancer vaccines to move from promise to reality.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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