Mini-Review

Multi-peptide immunotherapeutic vaccine for renal cell carcinoma: getting the troops all worked up

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Submitted Sep 10, 2012. Accepted for publication Oct 11, 2012.
doi: 10.3978/j.issn.2223-4683.2012.10.03
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These are exciting times in the development of new immunotherapeutic strategies for cancer therapy. In particular, synthetic peptide-based vaccines, aiming at the induction of T-cell mediated anti-tumor immunity, hold immense promise for prevention and therapy of malignant diseases. Synthetic peptides are easily produced, are chemically stable, easily deliverable, free of infectious contaminants and devoid of oncogenic potential. Importantly, the simplicity of producing clinical grade peptides allows for rapid changes in the design of peptide vaccines and, therefore, rapid translation into phase I/II trials in humans patients. Some clinical trials with therapeutic peptide vaccines so far are promising; a number of cancer patients exhibit immune responses against their tumor antigens and, to some extent, tumor regression (1,2). However, in many advanced cancer patients, peptide-based experimental therapy has shown limited benefit. Most encouragement for the immunotherapy community to pursue similar lines of therapeutic approach has come from cases where T cell responses against a tumor associated antigen (TAA) are induced by immunization. Unfortunately, while tumors expressing the immunized antigen may be destroyed, tumors that have lost expression of the antigen (or MHC) will remain untouched. The reason for such tumor escape is probably because tumor cells undergo antigenic variation and thereby avoid recognition and elimination by the immune system. Furthermore, antigen negative tumor variants will be positively selected upon the pressure of tumor destroying T cells. Thus, current immunotherapeutic strategies have evolved to include immunizations not only with one or two but with a number of different antigens simultaneously in order to circumvent the issue of tumor escape.

Walter *et al*. have used a similar approach to develop a multi-epitope cancer vaccine-IMA901. Their study, published in *Nature Medicine* (3), describes a systematic, multicenter Phase I/II clinical trial set up to test the therapeutic potential of a multi-epitope vaccine-IMA901 - in advanced renal cell cancer (RCC) patients.

The overarching goal of their study was to develop an effective immunotherapeutic peptide vaccine consisting of multiple tumor-derived antigens in order to induce a broad and specific T cell-mediated immune response against various cancer cells. For a tumor-antigen derived peptide vaccine to be successful as a cancer immunotherapeutic, it is essential for the vaccine to prime cytotoxic $CD8^+$ T cells *in vivo*, especially in the case of those cancers for which only a few tumor antigens have been defined so far. The authors address several key challenges involved in designing such a T cell response inducing peptide vaccine.

Two important criteria were considered when selecting peptides to be included in their vaccine formulation - The first is the frequency of expression, in RCC patients, of the parent tumor antigen from which the peptide is derived, and the second is the frequency of expression of the HLA alleles to which the peptides are naturally restricted. The tumor-derived epitopes that are formulated into their vaccine are restricted by HLA-A*-02 molecules, the more frequent and globally present HLA serotype. Thus, each of these tumor-derived antigens in their vaccination regimen may have a broad application in a majority of RCC patients. The authors use a unique and valuable

antigen discovery platform - given the vaguely political acronym 'XPRESIDENT' -to identify, select, and validate large numbers of HLA class I - associated peptides derived from tumor-associated antigens (TUMAPs) (4,5). Their approach combines methods from genomics, proteomics, bioinformatics, and T-cell immunology to identify clinically relevant TUMAPs from primary RCC tissues. Step 1 in this process involves the identification of naturally presented HLA-associated peptides directly from primary RCC cells. Step 2 is selection of tumor-associated peptides from step 1 by differential gene expression analysis; i.e., comparing the mRNA expression profiles between RCC and different healthy tissues. Step 3 involves validating the immunogenicity of selected candidate peptides by monitoring *in vitro* antigen-specific T-cell responses in peripheral blood cells obtained from healthy donors. Through such a process the authors then selected nine HLA-A*-02-restricted TUMAPs and one HLA-DR (MHC Class II) restricted TUMAP. This peptide pool of 10 antigens was designated the IMA901 vaccine. An HLA-A*-02-restricted Hepatitis B viral peptide was added to the vaccine formulation as a non-specific marker peptide.

Initially, Walter *et al*. tested the safety and clinical benefit of IAM901 vaccine in an open-label, single-arm phase I study in HLA-A*-02$^+$ metastatic RCC patients. Patients (*n=28*) received intradermally (i.d.) at least 8 IMA901 vaccinations in combination with an immunomodulator - granulocyte macrophage-colony stimulating factor (GM-CSF). Immunostimulatory cytokines such as GM-CSF have been reported to induce potent and specific anti-tumor T cell immunity (6-8). The initial results were encouraging; 8 of the patients exhibited vaccine-induced T cell responses to multiple TUMAPs and the other 20 immune-evaluable patients responded positively to at least one TUMAP. Further analysis revealed a positive correlation between patients' immune response to multiple TUMAPs and disease control when compared to subjects who responded to only one TUMAP. No adverse events or toxicity was observed during the entire study period.

Based on these observations, Walter *et al*. set up a randomized, multicenter, phase II clinical trial. Metastatic RCC remains a disease with dismal prognosis. Several immunosuppressive circuits operate in advanced disease stage, making immunotherapeutic intervention a bigger challenge. Patients have been shown to harbor increased numbers of circulating and tumor infiltrating $CD4^+CD25^+Foxp3^+$ T regulatory cells (T_{regs}) that exert functional inhibition on tumor-specific T cells (9). This increase in T_{regs} in RCC patients is associated with worse prognosis (9). Therefore, to improve the therapeutic efficacy of IAM901 vaccine, the authors included a second immunomodulator - a single dose of cyclophosphamide (CTX; 300 $mg.m^{-2}$) into their vaccination regimen. Recent studies show that low dose CTX selectively depletes T_{reg} cells and hence can enhance the vaccine - induced anti-tumor effects (10,11).

A total of 68 HLA-A*-02$^+$, metastatic RCC patients with documented disease progression during or after systemic therapy (cytokines or tyrosine kinase inhibitors) were recruited into the study. Patients were randomly divided into two subgroups - one group (+CTX; n=33) was given 17 intradermal IAM901/GM-CSF vaccinations after pretreatment with CTX while the second group received the vaccinations alone without CTX pretreatment (–CTX; n=35). The authors ensured that baseline characteristics and risk factors of the patients recruited into their trial were well balanced between the two groups, an important factor to consider when assessing the clinical benefit of their combinatorial vaccine regimen.

Results obtained from this second trial showed that the vaccine was well tolerated, corroborating their phase I study. Although no shrinkage of established tumors was observed with vaccinations and the progression free survival rates were similar in the two study arms (+CTX and –CTX), their data indicated an increase in survival rates in patients only in the CTX pretreatment group (23.5 months for+CTX arm when compared to 14.8 months for the –CTX group). T cell-mediated immune response analysis showed that 26% of all subjects responded to multiple TUMAPs and CTX pretreatment had no effect on the induction of T cell responses in these patients. The authors next compared survival rates between immune responders and non-responders. Data from such an analysis indicated that: (I) Among immune responders, survival was significantly prolonged in patients pretreated with CTX and no such increase was observed in immune responders from the –CTX group, (II) Significant increase in survival rates was observed in +CTX patients who responded to multiple TUMAPs when compared to +CTX subjects showing immune response to single TUMAP (III) No difference in survival rates was observed among non-immune responders from either group. Based on these results, the authors concluded that IMA901/GM-CSF induced T cell-mediated immune responses which positively correlate with better

clinical outcome in RCC patients. Importantly, immune responses were associated with increased survival only in subjects who were pre-treated with CTX.

Walter *et al*. performed immunophenotyping analysis in vaccinated individuals that indicated a significant (and expected) decrease in the number of FOXP3 expressing T_{reg} cells 3 days after CTX treatment when compared to T_{reg} numbers before treatment. To better define the immune-regulatory phenotype present in the RCC patients, the authors performed a detailed analysis of different cellular and serum biomarkers in subjects recruited to their trial. Samples were collected from these patients prior to CTX and IMA091/GM-CSF treatments and also from matched healthy controls. Among the cellular pretreatment markers, the authors analyzed the percentages of six subpopulations of myeloid derived suppressor cells (MDSC1-6) (12). In tumor-bearing hosts, including metastatic RCC patients, MDSCs have been shown to suppress tumor antigen-specific T cell-mediated immune responses (13). MDSCs induce T cell dysfunction by depletion of arginine, which induces T cell receptor downregulation (14) and also by generation of reactive oxygen species, marked by T cell tyrosine nitration (15,16). The authors report for the first time that MDSC4 and MDSC5 subpopulations are negatively associated with survival in RCC patients. Among the 300 serum pretreatment biomarkers that were analyzed, the authors identified apolipoprotein A-1 (APO-A1) and chemokine (C-C) ligand 17 (CCL17) as markers that might have prognostic significance in RCC patients. APO-A1 is a major component of the high-density lipoprotein and can suppress adaptive T cell responses via oxidative stress (17). CCL17 is a chemokine produced by dendritic cells and can influence anti-tumor T cell responses by altering antigen presenting functions of dendritic cells (18,19). Higher concentrations of APO-A1 and CCL17 were present in patients with significantly longer overall survival rates.

The current study reiterates the substantial role played by vaccine-induced T cell-mediated cellular responses and highlights the need for accurate evaluation of these responses in vaccine formulations for generation of effective anti-cancer therapies. Systematic, well-balanced, multi-center efforts such as the one described in this study can aid in formulation of specific guidelines to synchronize vaccine-directed immune response monitoring and patient based meta-analysis across several cancer clinical immunotherapy programs (20).

The approach of Walter *et al*. and their observations motivate further questions about the clinical benefit of multi-peptide vaccine as an immunotherapeutic strategy for other human cancers: (I) How long lasting are the multi-epitope T cell responses generated with IMA901 vaccine, especially in poorly immunogenic tumors that display a significant loss of tumor cell allelic class I expression? (II) Does the IMA901 multi-peptide vaccine stimulate the antibody-mediated and innate arms (NK mediated killing) of tumor immunity? (III) Why do some RCC patients within a HLA allelic group respond positively to multiple TUMAPs and others not? (IV) How feasible is it to include peptide sets in the vaccine formulation that fit to the less frequently expressed HLA class I alleles and also to multiple HLA class II molecules expressed in patients? (V) Is it possible to combine other co-stimulatory adjuvants with the IMA901 vaccine formulation that can bring about tumor shrinkage and also potentiate T cell responses in non-responders? (VI) Finally, is it possible that other TUMAPs to which patients *do not* naturally respond would be equally good or even better immunogens? Answers to these questions will greatly improve our understanding of the fundamental mechanisms that would enable TAA-derived vaccines to overcome the ever changing, tolerance inducing tumor microenvironment and ultimately might pave way for tailoring vaccine antigens for individual cancer patients. The more challenging part of this approach is the crucial and laborious task of identifying, selecting, and validating large numbers of naturally HLA-restricted tumor-associated peptides in different tumor types and for different HLA alleles (21). The new and efficient technology platforms now available such as those described by Walter *et al*. should allow for rapid determination of the tumor antigen profile of a particular patient. In an ideal scenario, from this profile, it should then be possible to design a suitable set of tumor antigenic peptides for vaccinating a particular patient.

The development of novel vaccine designs in conjunction with newer/more accurate immune response/biomarker monitoring methods are likely to facilitate ways in which to efficiently combine specific antigens in vaccine constructs and vaccination schedules, a major prerequisite for the optimization of vaccine-based treatment modalities. Consequently, such approaches can greatly increase the chances of attaining an objective response to first-line immunotherapy in advanced cancer patients. Metastatic RCC accounts for numerous deaths here in the US and world-wide. Chemotherapy is not very effective in controlling the disease progression. RCC has, however, demonstrated particular susceptibility to immune-based

therapy (22,23) and the encouraging results from the IMA901 vaccine trial lends credibility to the argument that immunotherapeutic vaccines can become a mainstay of treatment for advanced RCC.

Acknowledgements

Funding: NIH/NCRR P20RR018733 (KY), Kentucky Lung Cancer Research Program (KY). JWE is supported by the Commonwealth of Kentucky Research Challenge Trust Fund.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Cite this article as: Yaddanapudi K, Eaton JW. Multi-peptide immunotherapeutic vaccine for renal cell carcinoma: getting the troops all worked up. Transl Androl Urol 2012;1(4):229-233. doi: 10.3978/j.issn.2223-4683.2012.10.03

