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IFN-conditioned dendritic cells for the therapy of melanoma: what is missing?

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Viewing melanoma as one of the most immunogenic tumors has led to the development of a number of recent strategies for therapeutic manipulation of immune responses. One of the most interesting approaches in this field includes the utilization of specific vaccines. IFNα-conditioned dendritic cells (IFNα-DCs) are seen by many as promising candidates for the immunotherapy of melanoma. *Farkas and Kemény* [1] in their research suggest that IFNα-DCs are capable of producing Th1 cytokines, including interleukin-12 (IL-12), which in turn leads to T-cell derived IFNγ production, thus boosting the immune response. In addition, the combination of this approach with chemotherapy takes advantage of a presumptive "preconditioning phenomenon", which occurs when cytotoxic agents are administered prior to the autologous IFNα-DC inoculation. However, despite the theoretical advantages foreseen with this approach, potential limitations of the IFNα therapy are to be acknowledged, as the formerly mentioned authors believe that only selected patients are likely to benefit from this therapeutic modality.

IFNα-activated human monocytes have been shown to achieve a near-total eradication of select tumors *in vitro* [2]. These cells are able to differentiate into DC in the presence of IFNα *in vivo*, which can further lead to $CD4^+$ activation and consequent anti-melanoma activity. New research has identified very potent mechanisms by which IFN-α influences

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antigen processing and cross-presentation ability of monocyte-derived DCs, with potentially important implications for the development of DC vaccines. As such, IFN-DCs were shown to exert not only an enhanced expression of all the immunoproteasome subunits, as well as of TAP-1, TAP-2 and tapasin, but also to induce a higher proteasome proteolytic activity as compared to non-stimulated immature or mature DCs [3]. Particularly, IFN-DCs loaded with apoptotic LCL (what are LCL??) have been shown to be more potent than fully mature DCs in triggering the cytotoxicity of CD8+ T lymphocytes when recognizing a subdominant HLA-A*0201-restricted epitope derived from EBV latent membrane protein 2. Moreover, IFN-DCs loaded with apoptotic human melanoma cells are highly effective in cross-presenting the MART-1(27–35) epitope to a specific CD8(+) cytotoxic T cell clone, a proteasome-dependent function. As a result, IFN-α influences antigen processing and cross-presentation ability of monocyte-derived DCs, with potentially important implications for the development of DC-based therapeutic vaccines [3]. An interesting but yet unexplored question is the ability of IFN- α treatment of DC to alter costimulatory molecule expression on shed exosomes. Conversely treatment of DC with the anti-inflammatory cytokine IL-10 resulted in generation of exosomes that are pro-tolerogenic. Indeed, previous studies have shown that IFN-gamma treatment stimulated DC exosome immunogenicity by augmenting expression of CD40, CD80 and CD86 [4].

Isolated positive results with IFN-conditioned dendritic cells have sparked interest for this approach, but did confirmatory results occur? In a study of Ribas *et al.* involving monocyte-derived dendritic DC matured with IFN-gamma plus GM-CSF and IL-13, administered to patients with metastatic melanoma, a promising growth control rate of 27% was achieved with statistical significance, along with a high frequency of immune activation (90% of patients had detectable TAA-specific CD8+ T cells in peripheral blood). Interestingly, 66% of patients experienced boosted or induced immune responses to single or multiple pools of TAA-derived peptides [5].

On the contrary, an accumulated negative experience with DC vaccines compared to standard chemotherapy has evidently diminished the enthusiasm for this immunotherapeutical approach over the past decade. This occurred at least as a result of a small study involving a head-to head comparison of autologous peptide-pulsed dendritic cells (DC) with single-agent chemotherapy (dacarbazine, DTIC) [6], but also due to the lack of clinical activity in many clinical instances where its use was attempted [7]. In a pool of recent studies recently summarized in our work, antitumor responses with DC immunotherapy average 16.3% [7], compared to only 8.1% cumulative for the interferon-conditioned DC vaccines studies of Banchereau *et al*, Trakatelli *et al*, and Di Puchio *et al*, which were cited by *Farkas and Kemeny* [1].

In clinical practice, treatment with IFN-α alone results in disease remission in circa 15% of metastatic malignant melanoma patients. In addition, it appears that an additional one third of patients attain short-lived remissions. The short-lived immune response in these patients and the incomplete tumor eradication could be due to either selection of non-immunogenic tumor cells or down-regulation of the immune reactivity of melanoma cells. The findings of Håkansson *et al.* [8] suggest that IFN-α therapy results in immune-mediated tumor cell destruction early in the treatment, followed by immunosuppression within a few weeks. This could therefore explain the relatively inefficiency of IFN-α use in advanced melanoma. The lack of significant clinical activity of IFN-conditioned DC vaccines may be also explained in several additional ways. One possibility is that the IFN exerts a negative action on immune activation of DCs, either directly, or through modifications of the quality of the antigenic material pulsed *per se*, or its interaction with the receiving DC [9].

Nevertheless, previous incertitude generated by the contradictory results with the use of interferon-enhanced DC vaccines in melanoma should not darken the perspectives generated by the use of CTLA-4 antibodies, which appear to represent a different way of generating and amplifying immune responses. The recent trial of Hodi *et al*., comparing the anti-CTLA4 antibody ipilimumab to a gp100 vaccine in patients with previously treated metastatic melanoma, has brought genuine hope for the field of immunotherapy [10]. However, the presence of a similar survival in the ipilimumab and ipilimumab + gp100 vaccine arms, and the observation of a deterioration in the response rate with the combination therapy, suggest that a better understanding of the immune activation pathway in the presence of exogenous antigenic stimulation is needed [10]. From a mechanistic standpoint, CTLA-4 blockade is releasing a negative immune regulatory mechanism, which is a very dissimilar approach to the stimulation of a T cell response to cancer antigens through a vaccine. While the former may augment the targeted natural immune responses to melanoma, the latter may in fact boost responses which are actually irrelevant for anti-tumor responses, and may actually even be acting as a confusing stimulus for the already weakened immune system of patients with cancer. Combining the two approaches may nevertheless hold promise for the future. It is in fact very significant that a pilot phase I trial of anti-CTLA4 antibody tremelimumab plus a MART-1 peptide-pulsed DC vaccine, demonstrated durable anti-tumor responses, which were detected at increased levels compared to those usually seen with each individual treatment modality [11].

The lack of correlation between immune parameters and response may have multiple determinants. One of these may be the low sensitivity of the detection of immune responses to uncharacterized antigens in the tumor lysates used to pulse DCs. Another possibility is that analysis of peripheral blood T cell responses in peripheral blood may not fully reconstitute the cardinal events in tumors, as reported for the therapy with anti-CTLA4 antibodies [11]. In addition, detection of T cells stimulated by a vaccine may not correlate with anti-tumor responses, as some cancer cells may not be adequate targets for T cells even in the presence of a significant vaccine-induced cellular response. Other cause for the observed clinical-immunologic dissociation may consist of a low MHC surface expression, the influence of various interferences in processing and presenting molecules, or resistance to the pro-apoptotic signals from T cells [9].

Use of chemotherapy prior to immunotherapy to increase the antitumor immune response is currently viewed as an option worth exploring, yet we believe caution with its approach is warranted. The most commonly employed agents in this regard are dacarbazine and temozolomide. Some authors believe that these drugs are able to modulate the TCR repertoire in terms of diversity/antitumor response and possibly enhance tumor-reactive, long-lasting effector-memory $CD8^+$ T-cells [12]. Others believe that chemotherapy preconditioning may lead to depletion of the regulatory T-cells, which may be important for the immune anti-melanoma responses. It is known that these agents cause significant $CD4^+$ lymphopenia in a vast majority of patients, which translates into an increased incidence of serious opportunistic infections [13]. Furthermore, profound lymphopenia (grade 3–4 CTC) has been documented in the context of biochemotherapy using temozolomide and it remained persistent after immunotherapy [14]. The controversy seems even deeper considering that IFN-α use *per se* is associated with significant immunosuppression and prolonged lymphopenia. In this context, we believe the long-term outcomes of metastatic melanoma patients treated with biochemotherapy plus/minus dendritic cell vaccines remain uncertain, and there is a tremendous need for a better understanding of the mechanisms by which melanoma escapes immune surveillance. However, our previous work indicates that the immune effector parameter that correlates with survival in melanoma is neopterin, a macrophage activator [15]. Consequently, we would like to direct attention towards including this parameter into the fine tuning of interferon-conditioned DC therapies, along

with other time honored parameters, such as measuring the CD45RA-CCR7- effector memory cells, CD45RA+CCR7- terminally differentiated effectors, CD8+ tumor antigen-specific recall memory cells, CD14+CD16+ monocytes, etc.

Evidently, many questions remain regarding the long-term outcome of melanoma patients treated with IFNα-DC therapy. At present time, an array of genetic and molecular investigations is under way to explain the mechanisms of action of IFN-α underlying tumor response. Additionally, more light needs to be shed over the elusive triangle chemotherapy preconditioning - IFNα-DC vaccine - long-term outcomes in advanced melanoma. Alongside others, we believe that further research in the area of DC vaccination for advanced melanoma might offer additional insights to this intensely debated topic, which in turn may yield new avenues for melanoma immunotherapy.

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