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Overcoming HLA restriction in clinical trials
Immune monitoring of mRNA-loaded DC therapy

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A decade of collective work by tumor immunologists has led to improved large scale generation, maturation, antigen loading and administration of dendritic cells (DCs) to cancer patients, promoting enhanced antitumor activity. We alleviated the HLA-restriction in DC therapy and demonstrated that it is meaningful to treat patients with DCs irrespective of their HLA type.

The incidence of melanoma doubles every ten to 20 years and becomes a major health problem in white-skinned Caucasians. Despite the high response rates seen with vemurafenib in selected patients with $BRAF^{V600}$ mutant melanoma, the duration of these responses is relatively short. Melanoma is an antigenic and immunogenic tumor and therefore immunotherapy is promising. Indeed, ipilimumab, an IgG1 antibody directed against cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), was one of the first treatments to improve the survival of advanced melanoma patients. The most professional stimulators of immune responses are dendritic cells (DCs), which exert multiple functions (including the regulation of tolerance) to bridge innate and adaptive immunity. Occasional observations of clinical responses after DC-based immunotherapy support their use in the clinic. Evidently, it would be desirable that all patients with eligible disease characteristics had access to such DC therapy. However, most clinical trials are restricted to patients with a defined HLA type, mainly for two reasons. First, loading of DCs often occurs with defined peptides that are HLA-restricted. Second, the golden standard tool to evaluate T-cell stimulation, i.e., peptide-HLA multimers, is also HLA-restricted. The use of DCs loaded with full-length tumor antigens (TAs) overcomes the first issue.[1,2] We addressed the second issue and we explored the immunologic potential of mRNA-modified DC therapy in melanoma patients.

Patients were treated with mRNA-modified DCs, the so-called 'TriMix-DCs' developed by our group. These are autologous DCs electroporated with mRNA encoding caTLR4, CD40L and CD70 (which render them highly potent), and a full-length TA linked to a Class II HLA sorting signal for presentation to both $CD8^+$ and $CD4^+$ T cells. TriMix-DCs are superior to golden standard cytokine-matured DCs in vitro and the combination of TLR4 and CD40 triggering holds great promises as for their resistance to regulatory T cells.[2–4]

The immunogenic epitopes derived from an antigen are patient-dependent as they rely on the individual's HLA type. Therefore, we developed a method to evaluate the treatment-specific $CD8^+$ and $CD4^+$ T-cell responses independently of the patient's HLA type or of the recognized epitope, de facto overcoming the second reason for HLA restriction as an inclusion criterion in DC therapy.[5] The T cells screened were skin infiltrating lymphocytes (SKILs) from a delayed-type hypersensitivity (DTH) response induced by a small amount of treatment-DCs. This was based on a report of De Vries et al. demonstrating a correlation between the presence of functional SKILs and clinical outcome.[6]

Using this method, the immune stimulatory potential of intradermally administered TriMix-DCs was demonstrated in a clinical trial. After immunotherapy, treatment-specific $CD8^+$ SKILs, which were undetectable before DC therapy, were observed. Moreover, the presence of such $CD8^+$ SKILs correlated with the secretion of IL-12p70 (after 24 h till 48 h) of the injected DC-product.[2] As DCs need 24 h to reach lymph nodes upon intradermal administration, this might reflect the need for DCs to be 'fit' when they encounter T cells.

Looking at the exact epitopes recognized by SKILs after DC treatment, we could demonstrate the stimulation of cytotoxic $CD8^+$ T lymphocytes (CTLs) and $CD4^+$ T helper cells in vivo recognizing previously unidentified epitopes that were presented in both common and less common HLA types. Moreover, different epitopes within the same antigen were recognized by T cells exerting different functionalities.[7] This indicates that DCs loaded with full-length TAs indeed stimulate a broader T-cell response than peptide-loaded DCs. In addition, $CD8^+$ T-cell responses were found to be directed to a xenogenic signal peptide linked to the TA for translocation to the endoplasmic reticulum and presentation by

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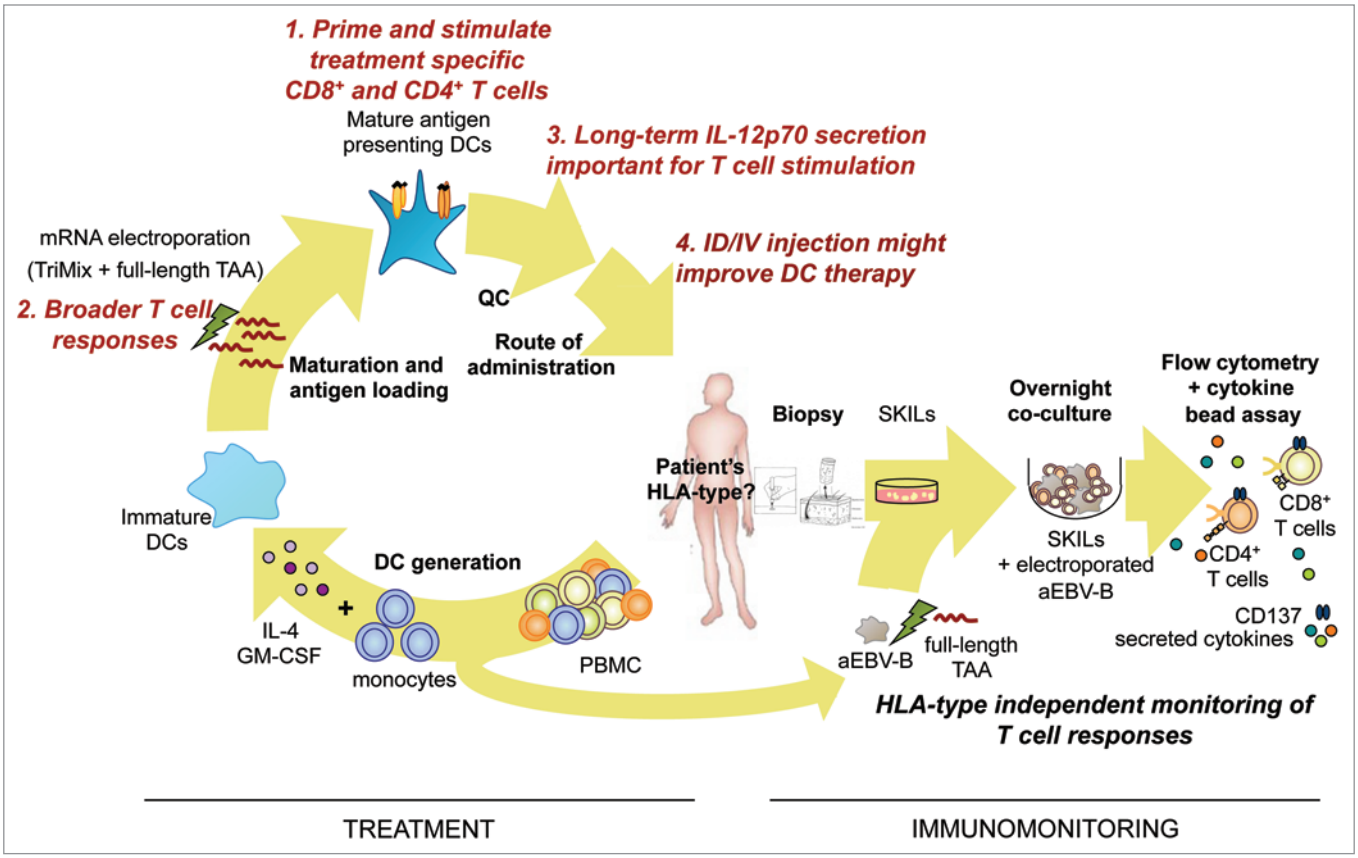

Figure 1. Overview of a DC therapy that avoids HLA restriction and its immunologic evaluation. DC generation: Peripheral blood mononuclear cells (PBMCs) are withdrawn from the patient. Monocytes are isolated and differentiated into immature DCs with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4). DC maturation and antigen loading is obtained through electroporation with mRNAs encoding TriMix (caTLR4, CD70 and CD40L) and a fusion protein with full-length tumor antigen (TA), respectively. After a quality control (QC) the DCs are administered intradermally or intradermally plus intravenously. On the right, a new method to monitor $CD8^+$ and $CD4^+$ T cells independently of the recognized epitope or the presenting HLA type is depicted. Skin infiltrating lymphocytes (SKILs) from a delayed-type hypersensitivity (DTH) skin biopsy are isolated and co-cultured with autologous B cells immortalized with the Epstein-Barr Virus and electroporated with the same antigen-encoding mRNA used to load DCs. This method has led to the following conclusions (in red): (1) TriMix-modified DCs prime and stimulate specific $CD8^+$ and $CD4^+$ T cells; (2) these T cells form a broad response both in terms of recognized epitopes and HLA types responsible for presentation, which is a direct consequence of the use of full-length TAs; (3) IL-12p70 secretion by the DCs appears to be important for T-cell stimulation; (4) a combined intradermal and intravenous administration might improve DC therapy.

Class II HLA molecules. Another patient mounted $CD4^+$ T cells recognizing the fusion region of this signal peptide with one of the TAs.[7] These observations point to two important notions. First, they provide definite proof that $CD8^+$ and $CD4^+$ T cells can be primed in vivo against the antigen encoded by the ex vivo generated mRNA that is loaded onto the DCs. Second, caution is required when fusion proteins are used, since the fusion region is immunogenic and the effect of these specific T cells is unknown (and hence potentially beneficial or harmful). Despite the induction of T-cell responses, no objective clinical responses were observed. In a follow-up trial, TriMix-DCs were injected both intradermally and intravenously in order to increase the T-cell distribution.[8] A Stage IV chemorefractory patient mounted an objective clinical response that was accompanied by an exceptionally broad immune response.[9]

On the whole, we overcame the HLA restriction as an eligibility criterion for clinical trials based on DC therapy and we demonstrated that mRNA-modified DCs hold a great potential for the treatment of melanoma patients (**Fig. 1**), de facto providing the basis for more patients to potentially benefit from DC therapy. Besides the stimulation of effector cells, the influence of TriMix-DCs on immune inhibition is the subject of further investigation (Pen JJ, article in preparation). Toll-like receptor matured DCs have been shown to resist suppression by regulatory T cells and to protect stimulated $CD8^+$ T cells in vitro and in mouse models.[3,4] Whether this holds true for melanoma patients is a future avenue to be explored. Furthermore, combinations with other immunomodulatory agents might contribute to break tumor tolerance and to improve antitumor activity. The combination of TriMix-DC and ipilimumab is currently being investigated in an Phase II study. Finally, efforts are dedicated to evade the generation of DCs ex vivo and to create an off-the-shelf product.[10] This would even broaden further the accessibility of DC treatment.

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