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Tumor-targeting vaccination instructs graft-vs.-tumor immune responses

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Abbreviations: DLI, donor lymphocyte infusion; GVHD, graft-vs.-host disease; GVT, graft-vs.-tumor; HSCT, hematopoietic stem cell transplantation; TRAMP, transgenic adenocarcinoma of the mouse prostate

Anticancer vaccines hold the potential to promote tumor eradication by immune effector cells. We have recently found dendritic cell-based vaccines to instruct graft-vs.-tumor responses following allogeneic hematopoietic stem cell transplantation and donor lymphocyte infusion. Vaccination was essential to elicit the intratumoral expression of interferon γ, promote local inflammation, and stimulate therapeutic T-cell infiltration.

Several strategies for the therapeutic vaccination of cancer patients are currently being developed, and several Phase II/III clinical studies are ongoing to evaluate the safety and efficacy of these approaches (http://www.clinicaltrials.gov). Often, the efficacy of anticancer vaccines is hindered by the lack of T cells expressing tumor-specific T-cell receptors (TCRs) of sufficient affinity, and/or by the presence of systemic or local mechanisms of tolerance and immunosuppression.[1] This is especially relevant for solid tumors, a setting in which an irregular blood flow and a dystrophic stroma, both of which limit T-cell extravasation and tumor infiltration, represent additional hurdles. Indeed, although active and adoptive immunotherapies have nowadays gained considerable momentum for the treatment of a limited number of neoplasms, such as melanoma and renal cell carcinoma,[2] they exert a limited therapeutic efficacy against most solid tumors.

To overcome tumor-elicited mechanisms of tolerance and restore immune competence, we have administered non-myeloablative allogeneic hematopoietic stem cell transplantation (HSCT) coupled to donor lymphocyte infusion (DLI) and a tumor-targeting vaccine to transgenic adenocarcinoma of the mouse prostate (TRAMP) mice[3] at advanced stages of the disease. Because of the thymic expression of tumor-associated antigens (promoting central tolerance) and the progressive development of neoplastic lesions, these mice are highly unresponsive to tumor-targeting vaccines,[4] and relatively resistant to both active and adoptive cell therapy.[5,6] We have recently shown that both central and peripheral T-cell tolerance could be overcome in tumor-bearing TRAMP mice by combining allogeneic HSCT/DLI with a tumor-targeting vaccine delivered post-transplantation.[7] Presumably, the efficacy of such an approach originated from the provision of a fresh repertoire of lymphocytes, allowed tumor-bearing animals to respond to therapeutic vaccination. In particular, we employed, as an anticancer vaccine, dendritic cells, which are known to be safe and to induce the expansion of both $CD4^+$ and $CD8^+$ T cells specific for tumor-associated antigens, pulsed with a MHC class I-restricted tumor-associated peptide. This turned out to be instrumental to initiate a graft-vs.-tumor (GVT) response that resulted in acute tumor debulking as well as in the amelioration of long-term survival rates.

By looking at the events dictated by the administration of our anticancer vaccine, we found it to be critical for the generation of sizeable numbers of interferon γ (IFNγ)-expressing effector T cells in the spleen, circulation and neoplastic lesions of tumor-bearing animals. High levels of these cells were associated with IFNγ-dependent immune responses at the tumor site, followed by robust infiltration of the neoplastic mass by $CD3^+$ lymphocytes and tumor debulking.[7] Actually, when HSCT and DLI were not given in combination with the dendritic cell-based vaccine, they failed to elicit tumor infiltration and exerted limited therapeutic effects (**Fig. 1**). Of note, tumor-infiltration as

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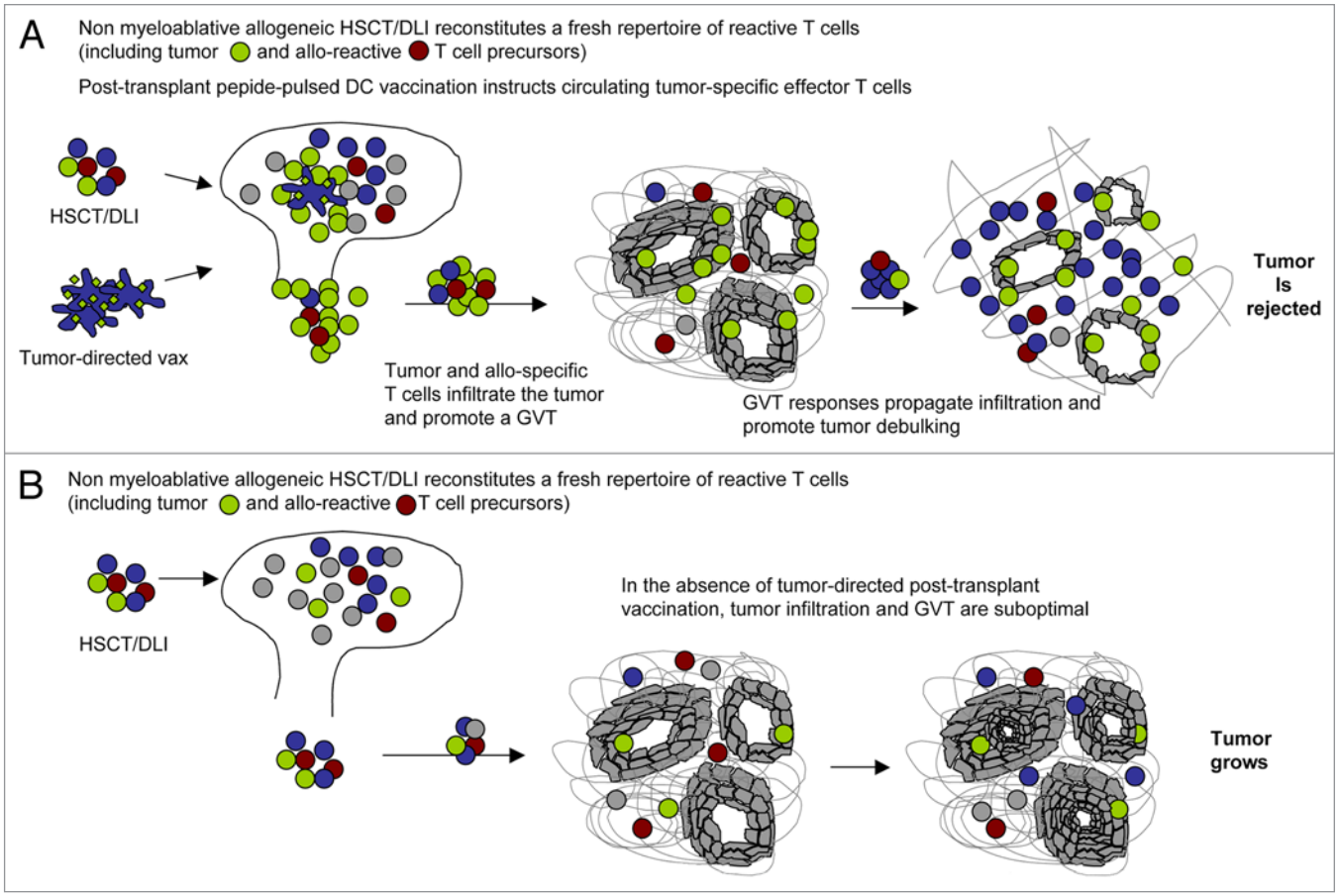

Figure 1. Dendritic cell-based anticancer vaccines delivered post-transplantation instructs graft-vs.-tumor responses. (**A**) Following non-myeloablative hematopoietic stem cell transplantation (HSCT), donor lymphocyte infusion (DLI) and tumor-targeting vaccination, in the form of peptide-pulsed dendritic cells (DCs), circulating tumor-specific interferon γ (IFNγ)-expressing effector T cells rapidly expand in secondary lymphoid organs and accumulate within prostate tumors. Therein, together with minor histocompatibility antigen-specific T lymphocytes, these cells support local inflammatory reactions and the recruitment of additional $CD3^+$ lymphocytes, a phenomenon that correlates with disease debulking and improved survival of tumor-bearing mice. (**B**) When HSCT/DLI is performed as a standalone immunotherapeutic intervention, tumor-specific effector cells are activated to suboptimal extent and fail to instruct robust graft-vs.-tumor effects.

triggered by this combinatorial regimen required not only tumor-directed effector T cells, but also minor histocompatibility antigen-specific immune responses, which were associated with increased T-cell recruitment to neoplastic lesions, enabling therapeutic GVT reactions.[7]

Since the administration of anticancer vaccines post-transplantation can ameliorate the efficacy of HSCT and promote GVT responses that are not accompanied (or are accompanied to limited extents) by graft-vs.-host disease (GVHD),[8] the clinical implementation of this combinatorial immunotherapeutic regimen might increase the benefit that individuals affected by solid tumors may obtain from allogeneic HSCT plus DLI.

It should be noted, however, that the beneficial effects of anticancer vaccines delivered post-transplantation might be limited by GVHD. While in a model of single minor histocompatibility antigen mismatch (HY alloreactivity) as well as in a model of multiple minor histocompatibility antigen-mismatched transplantation antigen-specific responses were properly evoked soon after vaccination,[7] the persistence of the cells mediating such responses was (at least to some extent) impaired in the presence of manifest GVHD,[9] consistent with previous observations.[10] To circumvent this issue, strategies that limit systemic GVHD at the time of vaccination should be developed. For instance, non-myeloablative conditioning and mixed bone marrow chimeras simultaneously favor the engraftment of DLI and limit the severity of GVHD. By this approach, we found therapeutic GVT responses in TRAMP mice in spite of manifest GVHD (manuscript in preparation).

The timing of vaccination, in particular relative to the time of T-cell infusion and to the degree of peripheral lymphopenia, might also constitute an important factor for the elicitation of proper T-cell responses to anticancer vaccines. Along similar lines, the form and route of vaccination are likely to have a major influence on the therapeutic efficacy of this immunotherapeutic intervention. Thus, the administration of dendritic cells pulsed ex vivo with tumor-associated antigens and optimally matured upon exposure to appropriate stimuli, providing them with lymph node-homing capacities, might constitute an optimal strategy to ensure antigen presentation at relevant

sites. Thus, further investigation is required to identify optimal vaccination protocols that might resist the negative influence of GVHD in humans.

As an alternative, anticancer vaccines should be administered in the context of cellular immunotherapies that do not cause GVHD, such as the infusion of autologous T cells derived from primary or metastatic tumors (tumor-infiltrating lymphocytes) or genetically modified to recognize tumor-associated antigens. In these scenarios, the toxicity associated with transplantation might be limited to some form of autologous GVHD, which may even specifically affect the tumor site and hence contribute to tumor rejection.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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