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Post-transplant immunotherapy: combining cancer vaccines with hematopoietic stem cell transplantation

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"…hematopoietic stem cell transplantation appears to be an ideal scenario for immunotherapy in hematologic and maybe even solid tumors."

The immune infiltrate is a significant part of the tumor micromillieu, and the quantity and quality of the infiltrate have clearly been linked to disease progression and patient survival [1,2]. Correspondingly, immune-based targeting of cancer has been pursued for more than a century [3] and prophylactic vaccination has already proven successful, at least against pathogen-induced cancers. Therefore, it is now routinely used for the prevention of human papilloma virus (HPV)-induced cervical cancer and HBV-induced liver cancer. These excellent examples could be employed as models for further obvious applications such as HPV-associated anal and penis carcinoma and EBV-associated post-transplant lymphoproliferative disease, Burkitt or Hodgkin's lymphoma and even major cancers such as nasopharyngeal carcinoma in Asia (with an incidence of up to 30 out of 100,000 individuals affected by tumors) [4].

However, therapeutic vaccination, although effective in murine models and often reported to induce tumor-specific T-cell responses in humans, has not yet experienced its clinical breakthrough. On the contrary, after more than two decades and over 1500 patients treated with dendritic cell (DC)-based vaccines (the major therapeutic strategy in cancer immunotherapy), overall response rates remain in the range of spontaneous regressions [5]. Non-cell-based vaccines alike have been unable to meet the scientists', physicians' and – most importantly – patients' expectations [6].

This is surprising, as anticancer vaccination in mice has proven successful in many independent tumor models and immunologic settings. Furthermore, many tumor antigens and fundamental mechanisms of tumor evasion have been discovered and the knowledge has long entered the arena of immunotherapy [7].

"…therapeutic vaccination … has not yet experienced its clinical breakthrough."

What is the difference between the successful examples previously mentioned and the disappointing reality in advanced-stage cancer patients? First, the choice of tumor antigen (foreign/mutated vs self-antigens) skews towards tumor control in prophylactic antiviral vaccinations in humans and many murine tumor models. Second, immune surveillance has been shown to correlate negatively with tumor load [8]. The patients receiving anti-HBV or -HPV vaccinations and most of the murine models are free of tumor at the time of immunization and, thus, inhibitory mechanisms are not yet operative. Most importantly, the priming of an immune response against non-self antigens is an evolutionary necessity and, thus, much more powerful than the self-antigens chosen in many trials.

Therefore, the classic clinical scenario of advanced-stage patients with several kilograms of tumor burden that are vaccinated against self-antigens is a very challenging context indeed.

In this light, hematopoietic stem cell transplantation (HSCT) appears to be a unique setting for tumor vaccines: tumor load is generally required to be low before the patient is considered for

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transplantation and will further be reduced by the conditioning regimen; therefore, post-transplant levels of inhibitory cytokines and regulatory T cells (Tregs) appear to be low and there seems to be 'space' for the expansion of tumor-specific T cells. Furthermore, the context of allogeneic HSCT provides foreign antigens such as minor histocompatibility antigens (mHAGs) as promising targets.

Hence, what is the current status of anti-tumor vaccination in the HSCT setting and can these theoretical advantages be translated to a real therapeutic benefit?

Vaccination in the context of HSCT

In an ideal immunotherapist's world, large numbers of highly reactive anti-tumor lymphocytes are generated *in vivo* without being restricted by tolerance mechanisms. The reconstituting immune system after HSCT provides a unique approximation to this ideal scenario. The lymphopenic environment allows strong expansion of respective anti-tumor T cells in the presence of cytokines responsible for thymic-independent homeostatic T-cell proliferation such as IL-7, IL-15 and IL-21 [9]. In addition, pretransplant chemotherapy is known to be able to deplete regulatory cells [10]. Hence, although there is profound post-transplant immunosuppression and especially a lack of support by helper T cells, there are ways to exploit this scenario to mount powerful CD8 responses [11,12].

Against this background, both autologous and allogeneic strategies are attractive avenues to pursue specific immunologic targeting and lower the relapse rates of patients after HSCT. New developments in this field might indeed also revive transplantation for solid tumors [13,14].

In autologous HSCT, unspecific immunostimulatory strategies used granulocyte–macrophage colony-stimulating factor (GM-CSF), IFN-γ or IL-2 (plus cyclosporine) to enhance the activity of reconstituting T cells and even mimic graft-versus-host (GvH) and graft-versus-tumor (GvT) effects known from allogeneic transplantation [15,16]. Proof-of-principle studies demonstrated feasibility and treatment response.

> "...both autologous and allogeneic strategies are attractive avenues to pursue specific immunologic targeting..."

Idiotype vaccination in the context of lymphoma and myeloma were among the first clinical vaccinations performed successfully, as comprehensively reviewed by de Cerio *et al.* [17]. Idiotype vaccination was also tested following HSCT and specific anti-idiotype immune responses were demonstrated for lymphoma and myeloma patients [18,19].

Intriguing autologous approaches have been introduced by groups from Stanford (CA, USA) and the NIH. The NIH group transferred autologous *in vitro*-expanded T cells derived from tumor-infiltrating lymphocytes (TILs) in combination with IL-2 to treat patients with metastatic melanoma after conditioning with total-body irradiation, fludarabine and cyclophosphamide. Objective response rates of 50–70% were achieved [13]. This procedure is based on the successful expansion of CTLs recognizing relevant tumor-associated antigens as well as on the reduction of Tregs by radio/chemotherapy. In a further-developed experimental system, they were able to evade these limitations. Here, instead of using pre-existing TILs, genetically engineered peripheral blood lymphocytes carrying T-cell receptor chains specific for a melanoma antigen were transferred into patients. Sustained levels of these engineered cells could be observed in two patients with objective regression of metastatic melanoma lesions [20].

> "Allogeneic hematopoietic stem cell transplantation itself demonstrates the power of the immunologic approach to treat even cancers refractory to chemotherapy."

The Stanford group initially injected CG-enriched oligonucleotides (CpGs) intratumorally after chemotherapeutic treatment, resulting in anti-tumor CD8 responses and the induction of remission in preclinical and clinical lymphoma models [21]. In a murine system, they have now further developed this approach to enhance the anti-tumor effect by transferring tumor-specific CD8 lymphocytes from a vaccinated host into a syngeneic recipient followed by post-transplant booster vaccinations – a procedure they termed immunotransplantation [22]. In a similar manner, Kochenderfer *et al.* induced vigorous CD8 responses after autologous transplantation using tumor peptide vaccines in conjunction with CpG and IL-2 [23].

Allogeneic HSCT itself demonstrates the power of the immunologic approach to treat even cancers refractory to chemotherapy. The finding that mere infusion of donor lymphocytes (DLIs) could re-induce remission after relapse following allogeneic transplantation paved the way to exploit the GvT effect [24]. Over the past decade, this strategy has been refined by a reduction of the chemotherapeutic aspect of the conditioning regimen prior to transplant. This shift towards the immunologic effect exerted by donor T cells allowed the extension of the indication for transplant and significantly decreased transplant-related mortality [25]. Furthermore, it instigated numerous studies to improve the efficacy of the anti-tumor response through graft manipulation.

In general, anti-tumor activity can either be induced against unspecific allo-antigens or specific tumor- or leukemia-associated antigens (TAA/LAA). The efficacy of targeting TAA/LAA is especially well studied for epitopes derived from myeloid leukemia-associated antigens such as proteinase (PR)3, Wilms tumor (WT)-1 and BCR-ABL [26]. Immunotherapeutic strategies either focus on the *ex vivo* generation of TAA/LAA-specific cytotoxic T cells (CTLs), which have been used successfully in relapse after allotransplantation [27], or on using BCR-ABL, PR3 or WT1-targeted vaccines [28].

In particular, alloreactive T cells evoke a strong immune response. Unfortunately, these alloantigen-induced immune responses (GvT responses) are often linked to the occurrence of GvH disease (GvHD) because tumor cells as well as the recipient's

healthy organ cells are detected as 'foreign'. In this case, immunosuppressive therapy will have to be commenced or maintained, thereby diminishing the GvT effect.

Mismatched minor histocompatibility antigens present on tumor cells or only on cells of the hematopoietic system have been shown to induce GvT effects without significant GvHD [29]. This strategy has now been taken to a new level by Goulmy's group from Leiden who targeted the mHAG HA-1 by HA-1-mismatched CTLs and induced remissions in murine solid tumors aberrantly expressing HA-1 [14]. In a second report, they were able to induce HA-1 expression in previously HA-1-negative tumors by the DNA-hypomethylating compound 5-aza-2′-deoxycytidine [30], raising hopes that specific mHag-directed therapy could even revive allotransplantation in solid tumor patients.

Another strategy to benefit from a defined mismatch situation is to use donor-versus-recipient natural killer (NK) cell alloreactivity. Lack of inhibitory signals based on the absence of certain recipient HLA class I ligands for donor-inhibitory killer Ig-like receptors (KIRs) enable NK cells to eradicate leukemia cells as well as host antigen-presenting cells (APCs), thus reducing the incidence of GvHD [31].

Donor lymphocyte infusion therapy has been a reasonably successful post-transplant immunotherapy since its discovery [24]. However, response rates vary significantly depending on the disease and are generally rather low. There are different strategies to improve DLI efficacy. *Ex vivo* activation via CD3/CD28-coated beads improved DLI efficacy in chronic myeloid leukaemia patients without significantly increasing GvHD [32]. A combination of DLI with antibodies or immunomodulatory agents such as interferon might increase responses and enable the reduction of DLI dosing to lower GvHD rates [33,34]. The vaccination/priming of donors [35,36] is another method that should be further evaluated.

Improving the response

All these immunotherapeutic strategies are based on intriguing ideas. However, translation into routine use will very much depend on their efficacy in clinical tumor regression.

Based on advancing immunologic knowledge, many groups work on improving the anti-tumor response with new strategies. Effectiveness of the Toll-like receptor 9 ligand CpG in the induction of vaccine responses was mentioned earlier. Eckl-Dorna *et al.* report how this CpG effect can now be directed to B cells via B-cell receptor-dependent uptake of CpG/antigens to induce antibody responses [37]. In mice, CpGs also activate invariant NK T cells [38], a subset of lipid-reactive innate lymphocytes previously shown to mount anti-tumor responses in humans after activation by CD1d-expressing DCs [39,40].

Invariant NKT cell activation itself leads to the transactivation of NK T cells, another cell type of interest. In a murine system, these activated NK T cells help to turn so-called myeloid-derived suppressor cells into more immunogenic APCs [41], boosting the CTL response and reducing Treg numbers.

Modification of peripheral tolerance mechanisms is also pursued by work on Treg depletion. A recombinant IL-2/diphtheria toxin conjugate (denileukin) has been successfully used to deplete FOXP3-positive Tregs and induce remission in metastases in mice and humans [42,43]. Alternative methods to decrease Treg numbers or activity is to use gemcitabine [44], fludarabine [45] or cyclophosphamide chemotherapy [46]. New transplant strategies omitting calcineurin inhibitors such as post-transplant cyclophosphamide as single-agent GvHD prophylaxis may allow an early expansion of CTLs and decrease the risk for relapse [47].

Dendritic cells are often used as APCs to prime and expand tumor-specific T-cells *in vivo* or *ex vivo*. However, the generation of sufficient numbers of pure DCs for repetitive, potentially life-long vaccinations, as suggested in some murine models, is often not feasible and too expensive [48]. Therefore, alternative strategies have been studied. Among these, CD40-activated B cells and artificial APCs appear to be a promising alternative, as they can be generated at the highest purity and in virtually unlimited amounts [49,50]. Using antigen-loaded CD40-activated B cells to expand specific donor lymphocytes *ex vivo* prior to infusion followed by *in vivo* boosters is a strategy we find especially intriguing.

Taken together, HSCT appears to be an ideal scenario for immunotherapy in hematologic and maybe even solid tumors. However, successful and clinically feasible concepts need to be established. The identification of the best allo-antigens and the specific prevention of GvHD are among the major challenges that must be addressed. Nevertheless, current experimental strategies are promising and first clinical benefit may be seen in the near future.

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