

Dendritic Cell Therapies for Hematologic Malignancies

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Dendritic cells (DCs) are potent antigen-presenting cells that constitute a major component of the immune system's role in the recognition, elimination, and tolerance of cancer. The unique immunologic capabilities of DCs have recently been harnessed for therapeutic use with the creation of DC-based anti-tumor vaccines, several of which have moved into testing in clinical trials for hematologic malignancies. This review summarizes how treatment strategies using DC-based anti-tumor vaccines are advancing immunotherapeutic options for these diseases.

Dendritic cells (DCs) consist of a network of immunomodulatory cells with the ability to present antigen to T lymphocytes and to induce powerful antigen-specific primary immune responses.¹ DCs may exert an anti-neoplastic effect by processing and presenting tumor antigen to autologous effector lymphocytes and thereby stimulating tumor-specific cytotoxicity. The presence of increased numbers of intratumoral DCs has been associated with improved clinical outcomes in human patients with non-small cell lung cancer and colorectal cancer, among other malignancies.^{2, 3} However, DCs can also exert an opposing, immunosuppressive, and tumor-sustaining effect in the context of malignancy, either by increased surface expression of immune checkpoint proteins such as PD-1⁴ or by the elaboration of tolerogenic substances such as indoleamine 2,3-dioxygenase.⁵ In this sense, DCs also participate in the fundamental immune tolerance of many tumors, as they are eventually able to evade the regulatory function of the immune system and grow without restriction. The field of cancer vaccines seeks to reverse such tumor-mediated immune tolerance by fostering the development of clinically meaningful tumor-specific immunity.

Dendritic Cell Tumor Vaccine Production: Rationale and Technical Considerations

The use of DCs as a platform for cancer vaccine development is based on their unique potency as antigen presenting cells with the capacity to induce a primary immune response. Mature DCs constitutively express co-stimulatory molecules, such as CD80 and CD86, which facilitate T lymphocyte immunoreactivity.⁶ Additionally, DCs have the ability to participate in “cross-presentation”, in which exogenous antigen is presented to CD8-positive cytotoxic T lymphocytes via the major histocompatibility complex (MHC) class I molecule.⁷ By preferentially engaging in cross-presentation, DCs are able to exert a direct cytotoxic effect on exogenous antigens expressed by tumor

cells. As a result, DCs represent a potentially critical platform to stimulate tumor specific immunity.

Fundamental issues regarding optimization of the DC model for tumor vaccination include: (1) choosing ideal single tumor antigen targets, (2) selecting the appropriate strategy for loading of single tumor antigens onto DCs, and (3) determining the role for multiple tumor antigens and/or whole-cell approaches. Each of these aspects of DC-tumor vaccine production will be discussed briefly below.

Single Tumor Antigen Identification

The selection of an appropriate target single tumor antigen is critical for the development of a vaccine strategy that preserves tumor specificity and immunologic efficacy. Common shared tumor antigens that have been explored in this setting include proteins/peptides otherwise expressed only during embryonic development (e.g., cancer testis antigens such as NY-ESO-1 and SP17),^{8, 9} peptides aberrantly or preferentially expressed by malignant cells (e.g., MUC1 in acute myelogenous leukemia and multiple myeloma¹⁰ or BCMA, which is selectively expressed by B-lymphocytes and plasma cells¹¹), or antigens truly unique to the tumor cell, such as the idiotype protein arising from the variable region of the immunoglobulin gene.¹² Additionally, intense research has recently focused on the use of tumor neoantigens—those generated by somatic alterations in the genomes of cancer cells as they acquire neoplastic characteristics—as an antigenic source for DC-tumor vaccination.¹³

Cancer testis antigens serve as attractive platforms for DC-tumor vaccine creation because of their limited expression on normal tissues and high expression by malignant hematologic cells, as well as the ease with which their mRNA can be incorporated into autologous DCs via electroporation. Liggins et al.¹⁴ have demonstrated that mRNA from at least eight cancer testis antigen genes—SP17, PRAME, CSAGE, PASD1, CAGE/DDX53, CTAGE1, HAGE/DDX43, and PLU-1/JARD1B—is expressed across numerous human B- and T cell lymphoma cell lines. A similar observation has been made regarding the expression of cancer testis antigens of the MAGE and SSX families in bone marrow biopsy specimens of

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**Table 1. Selected Clinical Trials of DC-Based Vaccines for Hematologic Malignancies**

| Disease | Number of Patients | DC Source | Tumor Antigen | Antigen Loading | Route of Administration | Immunologic Findings | Clinical Findings | Reference |
|--------------------------------|--------------------|-------------------------------|--|---|--|---|---|-----------|
| Follicular lymphoma | 35 | PBMCs | tumor idiotype | ex vivo pulse (co-culture) | intravenous | 65% anti-idiotype response | 22% regression of residual disease 70% without tumor progression at 43 months | 50 |
| Follicular lymphoma (relapsed) | 18 | peripheral blood monocytes | heat-shocked, irradiated tumor cells | ex vivo co-culture | subcutaneous, close to axillary and inguinal lymph nodes | objective clinical responses were associated with reduction in Tregs and increase in NK cells | 33% objective clinical response (16.7% complete response, 16.7% partial response) 44% stable disease | 59 |
| CML | 3 | PBMCs | NA | ex vivo | intra-dermal | 66% with delayed type hypersensitivity reaction to DCs | 33% with measurable anti-leukemic response at 20 months | 60 |
| CML | 6 | peripheral blood monocytes | NA | ex vivo | subcutaneous | increase in T lymphocyte immunogenicity | no clinical responses | 61 |
| CML | 10 | peripheral blood monocytes | NA | ex vivo | subcutaneous | 30% with expansion of T cells with specificity for leukemia-specific antigens | 40% with cytogenetic/molecular response | 62 |
| CLL | 12 | PBMCs | leukemia cell lysate | ex vivo co-culture | intra-dermal, close to axillary and inguinal lymph nodes | 33% with increased in CD8 ⁺ T lymphocytes against leukemia-associated antigens increase in IL-12 and decrease in Tregs noted to patients with clinical response | 41.7% with decreased peripheral blood leukemia cells 25% with stable disease 33% with disease progression | 64 |
| CLL | 15 | PBMCs | apoptotic tumor bodies | ex vivo co-culture | intra-dermal and intravenous | 66% with leukemia-specific immune response | no objective clinical responses | 65 |
| CLL | 9 | PBMCs (NB: allogeneic source) | leukemia cell lysate, tumor apoptotic bodies | ex vivo co-culture | intra-dermal, close to axillary and inguinal lymph nodes | 11.1% with expansion of cytotoxic T lymphocytes against leukemia-associated antigen | decrease in amount of circulating CLL cells in all patients | 63 |
| ATLL | 3 | PBMCs | tax peptide | ex vivo co-culture | subcutaneous | tax-specific cytotoxic T lymphocyte response in all patients | two patients with partial remission in first 8 weeks, one with subsequent complete remission | 66 |
| Multiple myeloma | 12 | PBMCs | tumor idiotype | ex vivo pulse | intravenous, with subcutaneous idiotype-KLH boosters | 16.7% anti-idiotype proliferative immune response | 16.7% with anti-idiotype proliferative immune response in complete remission at minimum follow-up of 16 months | 67 |
| Multiple myeloma | 26 | PBMCs | tumor idiotype | ex vivo pulse | intravenous | 15.4% anti-idiotype proliferative immune response | 65% alive at median follow-up of 30 months | 73 |
| Multiple Myeloma | 27 | PBMCs | tumor idiotype | ex vivo pulse | intravenous | NA | median overall survival 5.3 years (compared to 3.4 years in non-randomized control group) | 75 |
| Multiple myeloma | 12 | PBMCs | mRNA from MAGE3, Survivin, and BCMA | ex vivo pulse and electroporation with mRNA | intravenous and intra-dermal | 16.7% vaccine-specific T lymphocytes | 83% overall survival at 55 months (50% of those alive with stable disease) | 76 |
| Multiple Myeloma | 18 | PBMCs | whole tumor cell | ex vivo DC-tumor cell fusion | subcutaneous | 73.3% with expansion of circulating myeloma-reactive T cells | 68.9% with stable disease after vaccination | 77 |

(Continued on next page)



Table 1. Continued

| Disease | Number of Patients | DC Source | Tumor Antigen | Antigen Loading | Route of Administration | Immunologic Findings | Clinical Findings | Reference |
|------------------|--------------------|-----------|------------------|---|-------------------------|---|--|-----------|
| Multiple myeloma | 36 | PBMCs | whole tumor cell | ex vivo DC-tumor cell fusion | subcutaneous | all evaluable patients with at least 2-fold expansion of myeloma-specific T lymphocytes | 47% CR/nCR 78% CR/VGPR 24% with PR converted to a CR after vaccination | 78 |
| AML | 10 | PBMCs | mRNA from WT1 | ex vivo pulse and electroporation with mRNA | intra-dermal | vaccinated patients showed increased levels of WT1-specific CD8 ⁺ T lymphocytes | 50% with molecular CR 20% with PR to a CR converted to a CR after vaccination | 85 |
| AML | 17 | PBMCs | whole tumor cell | ex vivo DC-tumor cell fusion | subcutaneous | 5.4-fold increase in AML-specific CD4 ⁺ T cells, and 15.7-fold increase in AML-specific CD8 ⁺ T cells | 71% alive without AML recurrence at median follow-up of 57 months | 89 |

CLL, chronic lymphocytic leukemia; KLH, keyhole limpet hemocyanin; NK, natural killer; CR, complete response; nCR, near complete response; VGPR, very good partial response; PR, partial response; AML, acute myelogenous leukemia; PBMCs, peripheral blood mononuclear cells.

human patients with multiple myeloma.^{15–17} Expression of such cancer testis antigens may vary across different disease states. For example, expression of the cancer testis antigens NY-ESO-1 and MAGEA3/A6 increases on leukemic blasts following treatment with the hypomethylating agent decitabine in human patients with acute myelogenous leukemia.¹⁸ Likewise, expression of the cancer testis antigen NXF2 is increased on neoplastic lymphocytes after the administration of decitabine in patients with chronic lymphocytic leukemia.¹⁹ Such variability in cancer testis antigen expression over time and following specific therapy has important implications not only for the choice of which of these antigens are best suited for incorporation into a DC-tumor vaccine, but also for the most appropriate timing of such vaccination. Several cancer testis antigen vaccines have already been tested in clinical trials for the hematologic malignancies, with variable degrees of immunologic and clinical success (Table 1). Additional cancer testis antigen-DC vaccines are currently in development (Table 2).

Tumor Antigen Loading

Loading strategies of single tumor antigens for DC-tumor vaccine production in the treatment of hematologic malignancies consist of *in vivo* and *ex vivo* methods. *In vivo* DC antigen loading for tumor-specific vaccination has been accomplished by using nanoparticles coated with antibodies specific to DC surface markers as the vehicle by which to carry tumor antigen and adjuvant immunostimulatory molecules to DCs.^{20, 21} This approach has several advantages compared to *ex vivo* manipulation, including decreased cost, improved ease of administration, and applicability in a variety of clinical practice environments, as well as the potential to target more DCs and different DC subsets than those available during *ex vivo* production.

By contrast, the *ex vivo* method of DC-tumor vaccine generation allows for more standardization and control of antigen loading onto DCs, as well as the ability to ensure that autologous DCs have fully matured prior to vaccination and thus have reached their complete

immunogenic capability. DC maturation can be stimulated *ex vivo* by exposing CD14-positive peripheral blood monocytes in culture to various cocktails of cytokines, including interleukin (IL)-1 β , IL-6, tumor necrosis factor alpha (TNF- α), and PGE2, or, more commonly, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and TNF- α .²² For this reason, monocyte derived precursor populations have been the most common source for autologous DC tumor vaccine production.

However, the ontogeny of human DCs is complex and has yet to be elucidated fully.²³ Myeloid/classic DC, plasmacytoid DC, monocyte-related DC, and Langerhans cell subtypes exert divergent biological effects as antigen presenting cells, including differential capacity for cross presentation,²⁴ cytokine production, and polarization toward Th1, Th2, Th17, or regulatory T cell (Treg) immunophenotypes under the influence of various microbiological and biochemical stimuli.^{25–28} Each of these alternative DC subtypes, including Langerhans cells,^{29–31} DCs derived from CD34-positive umbilical cord blood progenitor cells,^{32–35} and plasmacytoid DCs^{36–39} have been explored for use in DC vaccine development in different solid and hematologic malignancies. Further pre-clinical and clinical studies are necessary to determine precisely how the choice of DC subtype for vaccine production will impact vaccine yield and tumor-specific immunogenicity.

The Role of Multiple Tumor Antigens and Whole-Cell Approaches

A potential limitation of single antigen DC vaccines is their susceptibility to immune evasion due to the downregulation of antigen expression. Alternatively, loading of antigen derived from autologous whole tumor cells allows for the induction of a polyvalent response that can potentially target shared and patient-unique neoantigens. Whole-cell techniques for vaccine production include creation of DC-tumor fusion cells, as well as loading of DCs with cellular lysates,^{40, 41} apoptotic bodies,^{42, 43} tumor exosomes,^{44, 45} or whole-cell DNA or RNA.⁴⁶ Galea-Lauri et al.⁴⁷ have determined that

**Table 2. Currently Enrolling Clinical Trials of Autologous Dendritic Cell-Tumor Vaccines for the Hematologic Malignancies**

| Disease | Setting | Trial Design | Antigen Loading | Clinical Trials.gov | Reference |
|------------------|---|--|---|---------------------|---------------|
| Multiple myeloma | primary therapy, following autoSCT | vaccine and lenalidomide versus lenalidomide alone | whole cell DC-tumor fusion | NCT02728102 | ⁷⁹ |
| Multiple myeloma | primary therapy, prior to and following autoSCT | vaccine alone | adenovirus vector to load Survivin | NCT02851056 | ⁸¹ |
| Multiple myeloma | primary therapy, following autoSCT | vaccine and lenalidomide | electroporated mRNA for CT7, MAGE-A3, and WT1 | NCT01995708 | ⁸² |
| AML | primary therapy, following induction and consolidation chemotherapy | vaccine alone | electroporated mRNA for WT1 | NCT01686334 | ⁸⁶ |
| AML | primary therapy, following induction chemotherapy | vaccine alone | two leukemia antigens and one CMV antigen | NCT01734304 | ⁸⁷ |
| AML | primary therapy, following induction chemotherapy | vaccine alone | electroporated mRNA for WT1 and PRAME | NCT02405338 | ⁸⁸ |

autoSCT, autologous hematopoietic stem cell transplantation; SC, subcutaneous; CMV, cytomegalovirus; WT1, Wilms tumor protein; CT7, cancer testis 7 protein; MAGE-A3, melanoma-associated antigen gene-A3 protein; LMP-2, latent membrane protein 2.

DC-tumor whole-cell fusions induce a more potent cytotoxic T-lymphocyte response against human acute myelogenous leukemia (AML) cells in the in vitro setting than those DC vaccines created with tumor cell lysates or apoptotic bodies. Nevertheless, further research into the most appropriate whole-cell approach for tumor antigen loading remains necessary.

Our group has focused on using a whole-cell DC-tumor fusion approach for the creation of autologous DC vaccines in AML and multiple myeloma (Figure 1). Using this technique, patient-derived tumor cells are fused to autologous ex vivo-generated DCs by co-culture in the presence of polyethylene glycol. These DC-tumor cell fusions have several distinct immunologic advantages for vaccination in that they present numerous shared antigens and tumor neoantigens to immune effector cells and do so through both the MHC class I/CD8 (cytotoxic T lymphocyte) and MHC class II/CD4 (helper T lymphocyte) pathways.⁴⁸ This vaccine platform has proved to be successful in early phase clinical trials for AML and multiple myeloma, with larger phase III clinical trials to be performed in the near future.

DC-Tumor Vaccines in Clinical Trials for Hematologic Malignancies

DC-tumor vaccination has been explored in clinical trials for several hematologic malignancies, including indolent non-Hodgkin's lymphoma, chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), adult T cell leukemia/lymphoma), multiple myeloma, and AML (Table 1). Several notable examples of these studies are discussed below. While biologic potency has been demonstrated in many instances, the clinical efficacy of the DC-tumor vaccine treatment strategy for the hematologic malignancies continues to be investigated in ongoing studies (Table 2).

Indolent Non-Hodgkin's Lymphoma

In an initial trial of a DC vaccine pulsed with tumor-specific idiomotype for follicular lymphoma, a subset of patients developed a measurable anti-tumor immune response to the vaccine, and one of the patients

experienced complete tumor regression.⁴⁹ In a subsequent larger study by the same group, 35 patients with follicular lymphoma underwent DC vaccination using the tumor idiomotype vaccine platform following standard chemotherapy.⁵⁰ Immunologic response and regression of residual disease were noted in 65% and 22% of patients, respectively. Of note, 70% of patients in this study remained without tumor progression at a median follow-up of 43 months.

Idiomotype-based vaccines subsequently proved successful in a number of phase I and II clinical trials for follicular lymphoma,⁵¹⁻⁵⁵ but did not reach pre-specified clinical efficacy endpoints in three separate large phase III studies.⁵⁶⁻⁵⁸ Importantly, however, all of these larger trials administered tumor idiomotype alone as their method of vaccination, without autologous DCs. Therefore, further development and clinical study of idiomotype vaccines against follicular lymphoma using a DC platform may still be warranted.

Whole tumor cell techniques of antigen loading have also been studied in clinical trials of follicular lymphoma. For example, a pilot study of 18 patients with relapsed indolent follicular lymphoma demonstrated that vaccination with DCs loaded with autologous tumor cells that were heat-shocked and irradiated was associated with objective clinical response and stable disease in 6 (33%) and 8 (44%) patients, respectively.⁵⁹ Clinical responses were significantly associated with a reduction in Tregs and an increase in natural killer (NK) cells. This whole-cell approach to DC vaccine antigen loading against follicular lymphoma, therefore, also merits further clinical study.

Chronic Myelogenous Leukemia

Because of the success of immunologic approaches to the treatment of CML, namely the striking effectiveness of donor lymphocyte infusion following allogeneic hematopoietic stem cell transplantation, it was hypothesized that additional immunotherapeutic techniques may prove to be beneficial in inducing disease control for patients with CML. In 2003, Ossenkoppele et al.⁶⁰ reported the results of a pilot study of three patients with CML who were administered DCs that were obtained from autologous peripheral blood mononuclear cells.

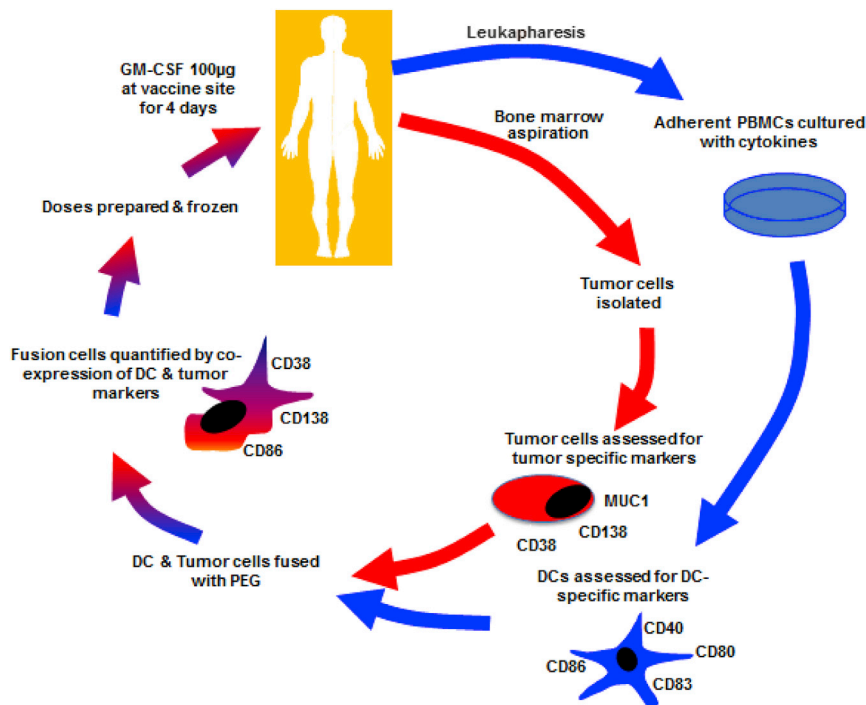


Figure 1. Production of Dendritic Cell-Myeloma Cell Fusion Vaccine

Key: The production of our dendritic cell-myeloma cell fusion vaccine begins with leukapheresis of the patient's peripheral blood mononuclear cells (PBMCs). PBMCs that are adherent to plates are then cultured with the cytokines IL-4, GM-CSF, and TNF- α , which induces DC differentiation and maturation with a characteristic immunophenotype (positive for CD86, CD80, CD40, and CD83). In parallel, bone marrow biopsy yields myeloma tumor cells, which are assessed for expression of myeloma-specific cell surface markers (CD38, CD138, and MUC1). The autologous mature DCs are then fused with the myeloma cells by co-culture in the presence of polyethylene glycol (PEG). The resultant fusion cells are quantified by co-expression of both DC and myeloma surface markers via flow cytometry and immunohistochemistry. The fusion vaccine is then prepared and frozen and is administered subcutaneously to the patient along with GM-CSF at the vaccine site for 4 days.

There were two of these patients that developed delayed type hypersensitivity reactions to CML-derived DCs, and one patient (33%) was found to have a sustained, leukemia-specific response after 20 months of follow up. There were six patients that were subsequently studied in a larger phase I trial of a DC-based vaccine for CML.⁶¹ Although no clinical responses were noted in this study, an increase in T cell immunogenicity to CML was observed. This spurred a further trial of DC vaccination, in which ten patients with chronic-phase CML were treated with DCs that had been harvested from autologous peripheral blood monocytes.⁶² In this series, three patients (30%) were found to have an expansion of T lymphocytes with specificity for leukemia-specific antigens and cytogenetic/molecular response was noted in four patients (40%). DC-based vaccination strategies therefore may represent a promising treatment option for CML in the future, particularly for patients with minimal residual disease following treatment with a potent BCR-ABL tyrosine kinase inhibitor.

Chronic Lymphocytic Leukemia

Because the clonal lymphocyte population in CLL represents the overwhelming preponderance of circulating nucleated cells, harvesting of DCs via leukapheresis of peripheral blood mononuclear cells can be technically challenging in patients with this disease. It is for this reason that allogeneic DCs—as opposed to autologous DCs, which have been used in the vast majority of other studies of DC-tumor vaccines for hematologic malignancies—were first used for the creation of a DC-based tumor vaccine against CLL. In a small pilot study of nine patients with early stage CLL (Rai 0-1), allogeneic DCs were obtained from healthy, unrelated donors and were then exposed to the study subjects' CLL tumor lysate and tumor apoptotic

bodies.⁶³ Administration of the resulting allogeneic DC-tumor vaccine led to a decrease in the amount of circulating CLL cells across all of the patients. Furthermore, one patient (11.1%) developed expansion of cytotoxic T lymphocytes directed against a leukemia-associated antigen.

Autologous DC-based vaccination against chronic lymphocytic leukemia was subsequently reported by Hus et al.⁶⁴ in 2008. In this study of 12 patients with early stage CLL (Rai stage 0–2), DC vaccine was produced by isolation of peripheral blood mononuclear cells, which were then co-cultured with leukemia cell lysates. Following vaccination, five patients (41.7%) demonstrated a decrease in the number of peripheral blood leukemia cells, three patients (25%) demonstrated stable disease, and four patients (33%) experienced disease progression. There were four patients (33%) that were found to have increased numbers of CD8-positive T lymphocytes directed against leukemia-specific antigens, and patients with clinical response were noted to have increased levels of the immunostimulatory cytokine IL-12, as well as decreased numbers of immunosuppressive Tregs. Palma et al.⁶⁵ subsequently reported a cohort of 15 patients with CLL who were treated with a DC vaccine that was produced with tumor apoptotic bodies. Although no objective clinical responses were noted in this study, ten patients (66%) developed leukemia-specific immune responses. The encouraging results of these early-phase trials indicate that DC-based tumor vaccination strategies may prove to have a role in the therapeutic armamentarium against CLL in the future.

Adult T Cell Leukemia/Lymphoma

In 2015, Suehiro et al.⁶⁶ reported an early phase clinical trial of a DC-tumor vaccine for patients with ATLL. For this study, a DC-tumor vaccine was created by pulsing autologous DCs with Tax, a peptide



product of human T cell leukemia virus type-I (HTLV-1), the causative viral agent of ATLL. Tax-specific cytotoxic T lymphocyte responses were identified in all three patients in this trial. Two of three patients experienced a partial remission within the first 8 weeks, and one of these patients converted to a complete remission subsequently. These two patients remained in remission 24 and 19 months after vaccination, with no need for further chemotherapy. The encouraging clinical outcome in this trial indicates the need for further study of this promising immunotherapy for ATLL.

Multiple Myeloma

The initial feasibility study of an idiotype-pulsed DC vaccine for the treatment of patients with multiple myeloma following autologous peripheral blood stem cell transplantation was reported by Reichardt et al.⁶⁷ from Stanford University in 1999. In this series, 12 patients were administered two intravenous infusions of idiotype-pulsed autologous DCs, with 5 monthly subcutaneous “boosters” of idiotype, keyhole limpet hemocyanin (KLH), and immune adjuvant. There were two patients (16.7%) that developed an idiotype-specific cellular immune response, and these patients remained in complete remission at a minimum follow-up of 16 months. Similar results were subsequently reported by groups in Italy,⁶⁸ Wales,⁶⁹ Australia,⁷⁰ Germany,⁷¹ and Arkansas,⁷² as well as within a larger cohort of patients from Stanford.⁷³ An idiotype-pulsed DC vaccine has also been demonstrated to lead to idiotype-specific T lymphocyte expansion in patients with earlier stages of myeloma.⁷⁴

In a subsequent phase II study performed at the Mayo Clinic, 27 patients with multiple myeloma who underwent consolidation therapy with autologous hematopoietic stem cell transplantation received idiotype-pulsed DCs (APC8020, Mylovenge) as post-transplantation adjunctive therapy between July 1998 and June 2001.⁷⁵ These patients were compared to a non-randomized, parallel group of control patients who underwent autologous hematopoietic stem cell transplantation for myeloma during that time, but were not vaccinated with APC8020. Comparison of these two groups revealed a statistically and clinically significant improvement in overall survival in the vaccine group (5.3 years) compared to the unvaccinated group (3.4 years, $p = 0.02$).

Others have reported a cancer testis antigen mRNA antigen-loading strategy for DC-myeloma vaccination. In a phase I study, Hobo et al.⁷⁶ obtained autologous monocyte-derived DCs from 12 patients with multiple myeloma who were treated with induction chemotherapy and high-dose melphalan with autologous hematopoietic stem cell transplantation. These DCs were pulsed with KLH and were electroporated with mRNA from the cancer testis antigen MAGE3, as well as SURVIVIN and B cell maturation antigen (BCMA). The DC-mRNA-loaded vaccines were then re-administered to the patients intravenously and intradermally. Two patients (16.7%) in this study developed vaccine-specific T lymphocyte responses.

Our group has developed a DC-tumor fusion vaccine model, whereby patient-derived myeloma cells are fused to ex vivo-generated DCs. In

a phase I study of 18 patients with advanced disease, vaccination resulted in the expansion of CD4-positive and CD8-positive myeloma-reactive T lymphocytes in 11 of 15 evaluable patients, with the majority of patients experiencing disease stabilization.⁷⁷ In a phase II trial of 36 subjects evaluating the DC-myeloma vaccine following autologous hematopoietic stem cell transplantation, 47% and 78% of patients experienced a complete response/near complete response and complete response/very good partial response, respectively.⁷⁸ Importantly, 24% of patients with a partial response following autologous hematopoietic stem cell transplantation were converted to complete response after DC-tumor fusion cell administration in the absence of any other therapy, consistent with the possibility that the vaccine targeted post-transplant residual disease. Based on these data, a randomized trial of DC-tumor fusion vaccination for multiple myeloma with lenalidomide maintenance versus lenalidomide maintenance alone is being conducted under the auspices of the CTN cooperative group (clinicaltrials.gov identifier: NCT02728102).⁷⁹ DC-myeloma fusion vaccination is also being studied in conjunction with blockade of PD-1, in an effort to augment vaccine effectiveness by immune checkpoint inhibition.⁸⁰

Several other trials of DC-myeloma vaccines are currently open to enrollment (Table 2), including those using an adenovirus vector to load the SURVIVIN antigen onto autologous DCs (clinicaltrials.gov identifier: NCT02851056),⁸¹ and those using Langerhans cells electroporated with the mRNA of the cancer testis antigens CT7 and MAGE-A3, as well as WT1, for antigen loading (clinicaltrials.gov identifier: NCT01995708).⁸²

AML

Various methods of antigen selection and loading have been employed for the creation of DC vaccines for therapeutic use in AML. These have included whole tumor cell-DC fusions, mRNA coding for particular tumor antigens, apoptotic tumor bodies,⁸³ and differentiation of leukemic blasts into autologous DCs.⁸⁴ Van Tendeloo et al.⁸⁵ developed a DC vaccine against AML by electroporating autologous DCs with the mRNA of *WT1*, an oncogene that is expressed in most cases of AML. In a phase I/II study of ten patients with AML, administration of this vaccine led to increases in *WT1*-specific T lymphocyte proliferation and *WT1*-specific interferon- γ -producing CD8-positive T lymphocytes.⁸⁵ Furthermore, molecular remission was induced in five of these patients (50%), and two patients who were in a partial remission were converted to complete remission after vaccination. Several clinical trials using electroporation of specific mRNAs as the method of DC vaccine construction against AML are currently ongoing (Table 2).^{86–88}

In a study of 17 patients with AML who were not candidates for allogeneic hematopoietic stem cell transplantation and achieved first complete remission after induction chemotherapy, our group demonstrated that vaccination with autologous DC/AML whole fusion cells induced the expansion of leukemia specific CD4-positive and CD8-positive T lymphocytes.⁸⁹ Remarkably, 12 of the patients (71%) remained alive without AML recurrence after a median



follow-up of 57 months. The encouraging results of this trial will lead to a randomized, phase III trial of this DC-AML whole-cell fusion vaccine in the near future.

Challenges and Future Directions: Augmenting and Standardizing the DC-Tumor Vaccine Response

In spite of the therapeutic excitement of DC-tumor vaccines against the hematologic malignancies, significant challenges to their widespread adoption in clinical practice remain. From a technical perspective, standardization of DC-tumor vaccine preparation is required for studies evaluating the relative potency of different approaches.⁹⁰ While increasingly sophisticated strategies to identify patient specific mutations/antigens for DC-tumor vaccine production have evolved, determining the immunologic relevance of such individual shared and neoantigens will be crucial. Furthermore, these newly identified antigens may show distinctive patterns of expression in different disease settings, with important implications for the use and timing of DC-tumor vaccines directed against those antigens.

Improvements in elucidating the complex interactions of the tumor microenvironment that induce effector T cell dysfunction and compromise migration and cytotoxic T lymphocyte mediated killing of tumor cells at the tumor bed are critical. While an individual disease setting may be dominated by a particular perturbation of immunity, hematologic malignancies typically manifest multiple areas of dysfunction that necessitate repair. DC vaccines may play a critical role in stimulating expansion of tumor-reactive lymphocytes, but other areas of immune dysregulation will need to be addressed in order for these cells to be maximally effective at the site of disease. Efforts to augment DC vaccine potency are now focusing on reversing these critical elements of the immunosuppressive milieu, including blockade of immunosuppressive accessory cells and reversal of alterations of biologic pathways that foster immune tolerance. Combining vaccination with immunomodulatory drugs such as lenalidomide or immune checkpoint blockade is being explored.^{91, 92} Future research efforts in this realm are also likely to include exploration of combinations of DC-tumor vaccination with various novel immunotherapy strategies, including chimeric antigen receptor (CAR) T lymphocyte therapy,⁹³ myeloid-derived suppressor cell (MDSC) inhibitors,⁹⁴ and therapies that deplete Tregs,⁹⁵ as well as lentiviral or retroviral gene therapy techniques for improved induction of anti-tumor immune response.^{96, 97}

While the current generation of DC-based vaccinations has demonstrated promise against the hematologic malignancies, further refinements in vaccine strategies are clearly needed to develop this promising area of investigation into a clinically meaningful therapy for patients with blood cancers.

REFERENCES

- Mellman, I., and Steinman, R.M. (2001). Dendritic cells: specialized and regulated antigen processing machines. *Cell* 106, 255–258.
- Dieu-Nosjean, M.C., Antoine, M., Danel, C., Heudes, D., Wislez, M., Poulot, V., Rabbe, N., Laurans, L., Tartour, E., de Chaisemartin, L., et al. (2008). Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J. Clin. Oncol.* 26, 4410–4417.
- Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., Tosolini, M., Camus, M., Berger, A., Wind, P., et al. (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 313, 1960–1964.
- Lim, T.S., Chew, V., Sieow, J.L., Goh, S., Yeong, J.P., Soon, A.L., and Ricciardi-Castagnoli, P. (2015). PD-1 expression on dendritic cells suppresses CD8(+) T cell function and antitumor immunity. *Oncolimmunology* 5, e1085146.
- Choe, J.Y., Yun, J.Y., Jeon, Y.K., Kim, S.H., Park, G., Huh, J.R., Oh, S., and Kim, J.E. (2014). Indoleamine 2,3-dioxygenase (IDO) is frequently expressed in stromal cells of Hodgkin lymphoma and is associated with adverse clinical features: a retrospective cohort study. *BMC Cancer* 14, 335.
- Caux, C., Vanbervliet, B., Massacrier, C., Azuma, M., Okumura, K., Lanier, L.L., and Banchereau, J. (1994). B70/B7-2 is identical to CD86 and is the major functional ligand for CD28 expressed on human dendritic cells. *J. Exp. Med.* 180, 1841–1847.
- Jung, S., Unutmaz, D., Wong, P., Sano, G., De los Santos, K., Sparwasser, T., Wu, S., Vuthoori, S., Ko, K., Zavala, F., et al. (2002). In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunity* 17, 211–220.
- Batchu, R.B., Moreno, A.M., Szmania, S.M., Bennett, G., Spagnoli, G.C., Ponnazhagan, S., Barlogie, B., Tricot, G., and van Rhee, F. (2005). Protein transduction of dendritic cells for NY-ESO-1-based immunotherapy of myeloma. *Cancer Res.* 65, 10041–10049.
- Dadabayev, A.R., Wang, Z., Zhang, Y., Zhang, J., Robinson, W.R., and Lim, S.H. (2005). Cancer immunotherapy targeting Sp17: when should the laboratory findings be translated to the clinics? *Am. J. Hematol.* 80, 6–11.
- Koido, S., Enomoto, Y., Apostolopoulos, V., and Gong, J. (2014). Tumor regression by CD4 T-cells primed with dendritic/tumor fusion cell vaccines. *Anticancer Res.* 34, 3917–3924.
- Carpenter, R.O., Evbuomwan, M.O., Pittaluga, S., Rose, J.J., Raffeld, M., Yang, S., Gress, R.E., Hakim, F.T., and Kochenderfer, J.N. (2013). B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin. Cancer Res.* 19, 2048–2060.
- Yi, Q., Szmania, S., Freeman, J., Qian, J., Rosen, N.A., Viswamitra, S., Cottler-Fox, M., Barlogie, B., Tricot, G., and van Rhee, F. (2010). Optimizing dendritic cell-based immunotherapy in multiple myeloma: intranodal injections of idiotype-pulsed CD40 ligand-matured vaccines led to induction of type-1 and cytotoxic T-cell immune responses in patients. *Br. J. Haematol.* 150, 554–564.
- Carreno, B.M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A.A., Ly, A., Lie, W.R., Hildebrand, W.H., Mardis, E.R., and Linette, G.P. (2015). Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* 348, 803–808.
- Liggins, A.P., Lim, S.H., Soilleux, E.J., Pulford, K., and Banham, A.H. (2010). A panel of cancer-testis genes exhibiting broad-spectrum expression in haematological malignancies. *Cancer Immunol.* 10, 8.
- Dhodapkar, M.V., Osman, K., Teruya-Feldstein, J., Filippa, D., Hedvat, C.V., Iversen, K., Kolb, D., Geller, M.D., Hassoun, H., Kewalramani, T., et al. (2003). Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. *Cancer Immunol.* 3, 9.
- Jungbluth, A.A., Ely, S., DiLiberto, M., Niesvizky, R., Williamson, B., Frosina, D., Chen, Y.T., Bhardwaj, N., Chen-Kiang, S., Old, L.J., and Cho, H.J. (2005). The cancer-testis antigens CT7 (MAGE-C1) and MAGE-A3/6 are commonly expressed in multiple myeloma and correlate with plasma-cell proliferation. *Blood* 106, 167–174.
- Taylor, B.J., Reiman, T., Pittman, J.A., Keats, J.J., de Bruijn, D.R., Mant, M.J., Belch, A.R., and Pilarski, L.M. (2005). SSX cancer testis antigens are expressed in most multiple myeloma patients: co-expression of SSX1, 2, 4, and 5 correlates with adverse prognosis and high frequencies of SSX-positive PCs. *J. Immunother.* 28, 564–575.
- Srivastava, P., Paluch, B.E., Matsuzaki, J., James, S.R., Collamat-Lai, G., Blagitko-Dorfs, N., Ford, L.A., Naqash, R., Lübbert, M., Karpf, A.R., et al. (2016). Induction of cancer testis antigen expression in circulating acute myeloid leukemia blasts following hypomethylating agent monotherapy. *Oncotarget* 7, 12840–12856.



19. Dubovsky, J.A., McNeel, D.G., Powers, J.J., Gordon, J., Sotomayor, E.M., and Pinilla-Ibarz, J.A. (2009). Treatment of chronic lymphocytic leukemia with a hypomethylating agent induces expression of NXF2, an immunogenic cancer testis antigen. *Clin. Cancer Res.* *15*, 3406–3415.
20. Bol, K.F., Tel, J., de Vries, I.J., and Figdor, C.G. (2013). Naturally circulating dendritic cells to vaccinate cancer patients. *OncoImmunology* *2*, e23431.
21. van Broekhoven, C.L., Parish, C.R., Demangel, C., Britton, W.J., and Altin, J.G. (2004). Targeting dendritic cells with antigen-containing liposomes: a highly effective procedure for induction of antitumor immunity and for tumor immunotherapy. *Cancer Res.* *64*, 4357–4365.
22. Curti, A., Isidori, A., Ferri, E., Terragna, C., Neyroz, P., Cellini, C., Ratta, M., Baccarani, M., and Lemoli, R.M. (2004). Generation of dendritic cells from positively selected CD14+ monocytes for anti-tumor immunotherapy. *Leuk. Lymphoma* *45*, 1419–1428.
23. Collin, M., McGovern, N., and Haniffa, M. (2013). Human dendritic cell subsets. *Immunology* *140*, 22–30.
24. Dudziak, D., Kamphorst, A.O., Heidkamp, G.F., Buchholz, V.R., Trumppfeller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H.W., Park, C.G., et al. (2007). Differential antigen processing by dendritic cell subsets in vivo. *Science* *315*, 107–111.
25. Mohamadzadeh, M., Olson, S., Kalina, W.V., Ruthel, G., Demmin, G.L., Warfield, K.L., Bavari, S., and Klaenhammer, T.R. (2005). Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization. *Proc. Natl. Acad. Sci. USA* *102*, 2880–2885.
26. Gori, S., Vermeulen, M., Remes-Lenicov, F., Jancic, C., Scordo, W., Ceballos, A., Towstyka, N., Bestach, Y., Belli, C., Sabbione, F., et al. (2017). Acetylcholine polarizes dendritic cells toward a Th2-promoting profile. *Allergy* *72*, 221–231.
27. Linehan, J.L., Dileepan, T., Kashem, S.W., Kaplan, D.H., Cleary, P., and Jenkins, M.K. (2015). Generation of Th17 cells in response to intranasal infection requires TGF- β 1 from dendritic cells and IL-6 from CD301b+ dendritic cells. *Proc. Natl. Acad. Sci. USA* *112*, 12782–12787.
28. George, M.M., Subramanian Vignesh, K., Landero Figueroa, J.A., Caruso, J.A., and Deepe, G.S., Jr. (2016). Zinc induces dendritic cell tolerogenic phenotype and skews regulatory T cell-Th17 balance. *J. Immunol.* *197*, 1864–1876.
29. Ratzinger, G., Baggers, J., de Cos, M.A., Yuan, J., Dao, T., Reagan, J.L., Münz, C., Heller, G., and Young, J.W. (2004). Mature human Langerhans cells derived from CD34+ hematopoietic progenitors stimulate greater cytolytic T lymphocyte activity in the absence of bioactive IL-12p70, by either single peptide presentation or cross-priming, than do dermal-interstitial or monocyte-derived dendritic cells. *J. Immunol.* *173*, 2780–2791.
30. Chung, D.J., Romano, E., Pronschinske, K.B., Shyer, J.A., Mennecozzi, M., St Angelo, E.T., and Young, J.W. (2013). Langerhans-type and monocyte-derived human dendritic cells have different susceptibilities to mRNA electroporation with distinct effects on maturation and activation: implications for immunogenicity in dendritic cell-based immunotherapy. *J. Transl. Med.* *11*, 166.
31. Romano, E., Cotari, J.W., Barreira da Silva, R., Betts, B.C., Chung, D.J., Avogadri, F., Fink, M.J., St Angelo, E.T., Mehrara, B., Heller, G., et al. (2012). Human Langerhans cells use an IL-15R- α /IL-15/pSTAT5-dependent mechanism to break T-cell tolerance against the self-differentiation tumor antigen WT1. *Blood* *119*, 5182–5190.
32. Ueno, H., Tcherepanova, I., Reygrobelle, O., Laughner, E., Ventura, C., Palucka, A.K., and Banchereau, J. (2004). Dendritic cell subsets generated from CD34+ hematopoietic progenitors can be transfected with mRNA and induce antigen-specific cytotoxic T cell responses. *J. Immunol. Methods* *285*, 171–180.
33. Banchereau, J., Palucka, A.K., Dhodapkar, M., Burkeholder, S., Taquet, N., Rolland, A., Taquet, S., Coquery, S., Wittkowski, K.M., Bhardwaj, N., et al. (2001). Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res.* *61*, 6451–6458.
34. Palucka, A.K., Dhodapkar, M.V., Paczesny, S., Burkeholder, S., Wittkowski, K.M., Steinman, R.M., Fay, J., and Banchereau, J. (2003). Single injection of CD34+ progenitor-derived dendritic cell vaccine can lead to induction of T-cell immunity in patients with stage IV melanoma. *J. Immunother.* *26*, 432–439.
35. Palucka, A.K., Dhodapkar, M.V., Paczesny, S., Ueno, H., Fay, J., and Banchereau, J. (2005). Boosting vaccinations with peptide-pulsed CD34+ progenitor-derived dendritic cells can expand long-lived melanoma peptide-specific CD8+ T cells in patients with metastatic melanoma. *J. Immunother.* *28*, 158–168.
36. Meyer-Wentrup, F., Benitez-Ribas, D., Tacken, P.J., Punt, C.J., Figdor, C.G., de Vries, I.J., and Adema, G.J. (2008). Targeting DCIR on human plasmacytoid dendritic cells results in antigen presentation and inhibits IFN- α production. *Blood* *111*, 4245–4253.
37. Dzionek, A., Sohna, Y., Nagafune, J., Cella, M., Colonna, M., Facchetti, F., Günther, G., Johnston, I., Lanzavecchia, A., Nagasaka, T., et al. (2001). BDCA-2, a novel plasmacytoid dendritic cell-specific type II C-type lectin, mediates antigen capture and is a potent inhibitor of interferon alpha/beta induction. *J. Exp. Med.* *194*, 1823–1834.
38. Matta, B.M., Castellana, A., and Thomson, A.W. (2010). Tolerogenic plasmacytoid DC. *Eur. J. Immunol.* *40*, 2667–2676.
39. Krug, A., Towarowski, A., Britsch, S., Rothenfusser, S., Hornung, V., Bals, R., Giese, T., Engelmann, H., Endres, S., Krieg, A.M., and Hartmann, G. (2001). Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur. J. Immunol.* *31*, 3026–3037.
40. González, F.E., Gleisner, A., Falcón-Beas, F., Osorio, F., López, M.N., and Salazar-Onfray, F. (2014). Tumor cell lysates as immunogenic sources for cancer vaccine design. *Hum. Vaccin. Immunother.* *10*, 3261–3269.
41. Hong, S., Li, H., Qian, J., Yang, J., Lu, Y., and Yi, Q. (2012). Optimizing dendritic cell vaccine for immunotherapy in multiple myeloma: tumour lysates are more potent tumour antigens than idiotype protein to promote anti-tumour immunity. *Clin. Exp. Immunol.* *170*, 167–177.
42. Spisek, R., Chevallier, P., Morineau, N., Milpied, N., Avet-Loiseau, H., Harousseau, J.L., Meflah, K., and Gregoire, M. (2002). Induction of leukemia-specific cytotoxic response by cross-presentation of late-apoptotic leukemic blasts by autologous dendritic cells of nonleukemic origin. *Cancer Res.* *62*, 2861–2868.
43. Kokhaei, P., Choudhury, A., Mahdian, R., Lundin, J., Moshfegh, A., Osterborg, A., and Mellstedt, H. (2004). Apoptotic tumor cells are superior to tumor cell lysate, and tumor cell RNA in induction of autologous T cell response in B-CLL. *Leukemia* *18*, 1810–1815.
44. Hao, S., Bai, O., Li, F., Yuan, J., Laferte, S., and Xiang, J. (2007). Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and anti-tumor immunity. *Immunology* *120*, 90–102.
45. Näslund, T.I., Gehrman, U., Qazi, K.R., Karlsson, M.C., and Gabrielsson, S. (2013). Dendritic cell-derived exosomes need to activate both T and B cells to induce anti-tumor immunity. *J. Immunol.* *190*, 2712–2719.
46. Milazzo, C., Reichardt, V.L., Müller, M.R., Grünebach, F., and Brossart, P. (2003). Induction of myeloma-specific cytotoxic T cells using dendritic cells transfected with tumor-derived RNA. *Blood* *101*, 977–982.
47. Galea-Lauri, J., Darling, D., Mufti, G., Harrison, P., and Farzaneh, F. (2002). Eliciting cytotoxic T lymphocytes against acute myeloid leukemia-derived antigens: evaluation of dendritic cell-leukemia cell hybrids and other antigen-loading strategies for dendritic cell-based vaccination. *Cancer Immunol. Immunother.* *51*, 299–310.
48. Parkhurst, M.R., DePan, C., Riley, J.P., Rosenberg, S.A., and Shu, S. (2003). Hybrids of dendritic cells and tumor cells generated by electrofusion simultaneously present immunodominant epitopes from multiple human tumor-associated antigens in the context of MHC class I and class II molecules. *J. Immunol.* *170*, 5317–5325.
49. Hsu, F.J., Benike, C., Fagnoni, F., Liles, T.M., Czerwinski, D., Taidi, B., Engleman, E.G., and Levy, R. (1996). Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* *2*, 52–58.
50. Timmerman, J.M., Czerwinski, D.K., Davis, T.A., Hsu, F.J., Benike, C., Hao, Z.M., Taidi, B., Rajapaksa, R., Caspar, C.B., Okada, C.Y., et al. (2002). Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. *Blood* *99*, 1517–1526.
51. Bhandi, M., Gocke, C.D., Kobrin, C.B., Benko, F.A., Sternas, L.A., Pennington, R., Watson, T.M., Reynolds, C.W., Gause, B.L., Duffey, P.L., et al. (1999). Complete molecular remissions induced by patient-specific vaccination plus granulocyte-macrophage colony-stimulating factor against lymphoma. *Nat. Med.* *5*, 1171–1177.
52. Barrios, Y., Cabrera, R., Yáñez, R., Briz, M., Plaza, A., Forés, R., Fernández, M.N., and Díaz-Espada, F. (2002). Anti-idiotypic vaccination in the treatment of low-grade B-cell lymphoma. *Haematologica* *87*, 400–407.



53. Neelapu, S.S., Baskar, S., Gause, B.L., Kobrin, C.B., Watson, T.M., Frye, A.R., Pennington, R., Harvey, L., Jaffe, E.S., Robb, R.J., et al. (2004). Human autologous tumor-specific T-cell responses induced by liposomal delivery of a lymphoma antigen. *Clin. Cancer Res.* *10*, 8309–8317.
54. Inogès, S., Rodríguez-Calvillo, M., Zabalegui, N., López-Díaz de Cerio, A., Villanueva, H., Soria, E., Suárez, L., Rodríguez-Caballero, A., Pastor, F., García-Muñoz, R., et al.; Grupo Español de Linfomas/Trasplante Autólogo de Medula Ósea study group; Programa para el Estudio y Tratamiento de Hemopatías Malignas study group (2006). Clinical benefit associated with idiotype vaccination in patients with follicular lymphoma. *J. Natl. Cancer Inst.* *98*, 1292–1301.
55. Bertinetti, C., Zirikli, K., Heining-Mikesch, K., Ihorst, G., Dierbach, H., Waller, C.F., and Veelken, H. (2006). Phase I trial of a novel intradermal idiotype vaccine in patients with advanced B-cell lymphoma: specific immune responses despite profound immunosuppression. *Cancer Res.* *66*, 4496–4502.
56. Freedman, A., Neelapu, S.S., Nichols, C., Robertson, M.J., Djulbegovic, B., Winter, J.N., Bender, J.F., Gold, D.P., Ghalib, R.G., Stewart, M.E., et al. (2009). Placebo-controlled phase III trial of patient-specific immunotherapy with mitumprotimut-T and granulocyte-macrophage colony-stimulating factor after rituximab in patients with follicular lymphoma. *J. Clin. Oncol.* *27*, 3036–3043.
57. Schuster, S.J., Neelapu, S.S., Gause, B.L., Janik, J.E., Muggia, F.M., Gockerman, J.P., Winter, J.N., Flowers, C.R., Nikcevic, D.A., Sotomayor, E.M., et al. (2011). Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. *J. Clin. Oncol.* *29*, 2787–2794.
58. Levy, R., Ganjoo, K.N., Leonard, J.P., Vose, J.M., Flinn, I.W., Ambinder, R.F., Connors, J.M., Berinstein, N.L., Belch, A.R., Bartlett, N.L., et al. (2014). Active idiotype vaccination versus control immunotherapy for follicular lymphoma. *J. Clin. Oncol.* *32*, 1797–1803.
59. Di Nicola, M., Zappasodi, R., Carlo-Stella, C., Mortarini, R., Pupa, S.M., Magni, M., Devizzi, L., Matteucci, P., Baldassari, P., Ravagnani, F., et al. (2009). Vaccination with autologous tumor-loaded dendritic cells induces clinical and immunologic responses in indolent B-cell lymphoma patients with relapsed and measurable disease: a pilot study. *Blood* *113*, 18–27.
60. Ossenkoppele, G.J., Stam, A.G., Westers, T.M., de Grujil, T.D., Janssen, J.J., van de Loosdrecht, A.A., and Scheper, R.J. (2003). Vaccination of chronic myeloid leukemia patients with autologous in vitro cultured leukemic dendritic cells. *Leukemia* *17*, 1424–1426.
61. Litzow, M.R., Dietz, A.B., Bulur, P.A., Butler, G.W., Gastineau, D.A., Hoering, A., Fink, S.R., Letendre, L., Padley, D.J., Paternoster, S.F., et al. (2006). Testing the safety of clinical-grade mature autologous myeloid DC in a phase I clinical immunotherapy trial of CML. *Cytotherapy* *8*, 290–298.
62. Westermann, J., Kopp, J., van Lessen, A., Hecker, A.C., Baskaynak, G., le Coutre, P., Döhner, K., Döhner, H., Dörken, B., and Pezzutto, A. (2007). Vaccination with autologous non-irradiated dendritic cells in patients with bcr/abl+ chronic myeloid leukaemia. *Br. J. Haematol.* *137*, 297–306.
63. Hus, I., Roliński, J., Tabarkiewicz, J., Wojas, K., Bojarska-Junak, A., Greiner, J., Giannopoulos, K., Dmoszyńska, A., and Schmitt, M. (2005). Allogeneic dendritic cells pulsed with tumor lysates or apoptotic bodies as immunotherapy for patients with early-stage B-cell chronic lymphocytic leukemia. *Leukemia* *19*, 1621–1627.
64. Hus, I., Schmitt, M., Tabarkiewicz, J., Radej, S., Wojas, K., Bojarska-Junak, A., Schmitt, A., Giannopoulos, K., Dmoszyńska, A., and Roliński, J. (2008). Vaccination of B-CLL patients with autologous dendritic cells can change the frequency of leukemia antigen-specific CD8+ T cells as well as CD4+CD25+FoxP3+ regulatory T cells toward an antileukemia response. *Leukemia* *22*, 1007–1017.
65. Palma, M., Hansson, L., Choudhury, A., Näsman-Glaser, B., Eriksson, I., Adamson, L., Rossmann, E., Widén, K., Horváth, R., Kokhaei, P., et al. (2012). Vaccination with dendritic cells loaded with tumor apoptotic bodies (Apo-DC) in patients with chronic lymphocytic leukemia: effects of various adjuvants and definition of immune response criteria. *Cancer Immunol. Immunother.* *61*, 865–879.
66. Suehiro, Y., Hasegawa, A., Iino, T., Sasada, A., Watanabe, N., Matsuoka, M., Takamori, A., Tanosaki, R., Utsunomiya, A., Choi, I., et al. (2015). Clinical outcomes of a novel therapeutic vaccine with Tax peptide-pulsed dendritic cells for adult T cell leukaemia/lymphoma in a pilot study. *Br. J. Haematol.* *169*, 356–367.
67. Reichardt, V.L., Okada, C.Y., Liso, A., Benike, C.J., Stockerl-Goldstein, K.E., Engleman, E.G., Blume, K.G., and Levy, R. (1999). Idiotype vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma—a feasibility study. *Blood* *93*, 2411–2419.
68. Massaia, M., Borriore, P., Battaglio, S., Mariani, S., Beggiano, E., Napoli, P., Voena, C., Bianchi, A., Coscia, M., Besostri, B., et al. (1999). Idiotype vaccination in human myeloma: generation of tumor-specific immune responses after high-dose chemotherapy. *Blood* *94*, 673–683.
69. Lim, S.H., and Bailey-Wood, R. (1999). Idiotype protein-pulsed dendritic cell vaccination in multiple myeloma. *Int. J. Cancer* *83*, 215–222.
70. Cull, G., Durrant, L., Stainer, C., Haynes, A., and Russell, N. (1999). Generation of anti-idiotype immune responses following vaccination with idiotype-protein pulsed dendritic cells in myeloma. *Br. J. Haematol.* *107*, 648–655.
71. Titzer, S., Christensen, O., Manzke, O., Tesch, H., Wolf, J., Emmerich, B., Carsten, C., Diehl, V., and Bohlen, H. (2000). Vaccination of multiple myeloma patients with idiotype-pulsed dendritic cells: immunological and clinical aspects. *Br. J. Haematol.* *108*, 805–816.
72. Yi, Q., Desikan, R., Barlogie, B., and Munshi, N. (2002). Optimizing dendritic cell-based immunotherapy in multiple myeloma. *Br. J. Haematol.* *117*, 297–305.
73. Liso, A., Stockerl-Goldstein, K.E., Auffermann-Gretzinger, S., Benike, C.J., Reichardt, V., van Beckhoven, A., Rajapaksa, R., Engleman, E.G., Blume, K.G., and Levy, R. (2000). Idiotype vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Biol. Blood Marrow Transplant.* *6*, 621–627.
74. Röllig, C., Schmidt, C., Bornhäuser, M., Ehninger, G., Schmitz, M., and Auffermann-Gretzinger, S. (2011). Induction of cellular immune responses in patients with stage-I multiple myeloma after vaccination with autologous idiotype-pulsed dendritic cells. *J. Immunother.* *34*, 100–106.
75. Lacy, M.Q., Mandrekar, S., Dispenzieri, A., Hayman, S., Kumar, S., Buadi, F., Dingli, D., Litzow, M., Wettstein, P., Padley, D., et al. (2009). Idiotype-pulsed antigen-presenting cells following autologous transplantation for multiple myeloma may be associated with prolonged survival. *Am. J. Hematol.* *84*, 799–802.
76. Hobo, W., Strobbe, L., Maas, F., Fredrix, H., Greupink-Draaisma, A., Esendam, B., de Witte, T., Preijers, F., Levenga, H., van Rees, B., et al. (2013). Immunogenicity of dendritic cells pulsed with MAGE3, Survivin and B-cell maturation antigen mRNA for vaccination of multiple myeloma patients. *Cancer Immunol. Immunother.* *62*, 1381–1392.
77. Rosenblatt, J., Vasir, B., Uhl, L., Blotta, S., Macnamara, C., Somaiya, P., Wu, Z., Joyce, R., Levine, J.D., Dombagoda, D., et al. (2011). Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. *Blood* *117*, 393–402.
78. Rosenblatt, J., Avivi, I., Vasir, B., Uhl, L., Munshi, N.C., Katz, T., Dey, B.R., Somaiya, P., Mills, H., Campigotto, F., et al. (2013). Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. *Clin. Cancer Res.* *19*, 3640–3648.
79. ClinicalTrials.gov. (2016). Dendritic cell/myeloma fusion vaccine for multiple myeloma (BMT CTN 1401). <https://clinicaltrials.gov/ct2/show/NCT02728102>.
80. Rosenblatt, J., Glotzbecker, B., Mills, H., Vasir, B., Tzachanis, D., Levine, J.D., Joyce, R.M., Wellenstein, K., Keefe, W., Schickler, M., et al. (2011). PD-1 blockade by CT-011, anti-PD-1 antibody, enhances ex vivo T-cell responses to autologous dendritic cell/myeloma fusion vaccine. *J. Immunother.* *34*, 409–418.
81. ClinicalTrials.gov. (2016). Survivin vaccine: multiple myeloma autologous hematopoietic cell transplant (HCT). <https://clinicaltrials.gov/ct2/show/NCT02851056>.
82. ClinicalTrials.gov. (2016). CT7, MAGE-A3, and WT1 mRNA-electroporated autologous Langerhans-type dendritic cells as consolidation for multiple myeloma. <https://clinicaltrials.gov/ct2/show/NCT01995708>.
83. Kitawaki, T., Kadowaki, N., Fukunaga, K., Kasai, Y., Maekawa, T., Ohmori, K., Itoh, T., Shimizu, A., Kuzushima, K., Kondo, T., et al. (2011). Cross-priming of CD8(+) T cells in vivo by dendritic cells pulsed with autologous apoptotic leukemic cells in immunotherapy for elderly patients with acute myeloid leukemia. *Exp. Hematol.* *39*, 424–433.e2.
84. Nourizadeh, M., Masoumi, F., Memarian, A., Alimoghaddam, K., Moazzeni, S.M., Yaghmaie, M., and Hadjati, J. (2014). In vitro induction of potent tumor-specific cytotoxic T lymphocytes using TLR agonist-activated AML-DC. *Target. Oncol.* *9*, 225–237.



85. Van Tendeloo, V.F., Van de Velde, A., Van Driessche, A., Cools, N., Anguille, S., Ladell, K., Gostick, E., Vermeulen, K., Pieters, K., Nijs, G., et al. (2010). Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. *Proc. Natl. Acad. Sci. USA* *107*, 13824–13829.
86. Clinical Trials.gov. (2016). Efficacy study of dendritic cell vaccination in patients with acute myeloid leukemia in remission (WIDEA). <https://clinicaltrials.gov/ct2/show/NCT01686334>.
87. Clinical Trials.gov. (2016). DC vaccination for postremission therapy in AML. <https://clinicaltrials.gov/ct2/show/NCT01734304>.
88. Clinical Trials.gov. (2016). DC vaccination for post-remission therapy in AML. <https://clinicaltrials.gov/ct2/show/NCT02405338>.
89. Rosenblatt, J., Stone, R.M., Uhl, L., Neuberger, D., Joyce, R., Levine, J.D., Arnason, J., McMasters, M., Luptakova, K., Jain, S., et al. (2016). Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remissions. *Sci. Transl. Med.* *8*, 368ra171.
90. Ni, M., Hoffmann, J.M., Schmitt, M., and Schmitt, A. (2016). Progress of dendritic cell-based cancer vaccines for patients with hematological malignancies. *Expert Opin. Biol. Ther.* *16*, 1113–1123.
91. Luptakova, K., Rosenblatt, J., Glotzbecker, B., Mills, H., Stroopinsky, D., Kufe, T., Vasir, B., Arnason, J., Tzachanis, D., Zwicker, J.I., et al. (2013). Lenalidomide enhances anti-myeloma cellular immunity. *Cancer Immunol. Immunother.* *62*, 39–49.
92. Clinical Trials.gov. (2016). Blockade of PD-1 in conjunction with the dendritic cell/AML vaccine following chemotherapy induced remission. <https://clinicaltrials.gov/ct2/show/NCT01096602>.
93. Nagle, S.J., Garfall, A.L., and Stadtmauer, E.A. (2016). The promise of chimeric antigen receptor engineered T cells in the treatment of hematologic malignancies. *Cancer J.* *22*, 27–33.
94. de Haas, N., de Koning, C., Spilgies, L., de Vries, I.J., and Hato, S.V. (2016). Improving cancer immunotherapy by targeting the STATE of MDSCs. *Oncoimmunology* *5*, e1196312.
95. Butt, A.Q., and Mills, K.H. (2014). Immunosuppressive networks and checkpoints controlling antitumor immunity and their blockade in the development of cancer immunotherapeutics and vaccines. *Oncogene* *33*, 4623–4631.
96. Stripecke, R., Cardoso, A.A., Pepper, K.A., Skelton, D.C., Yu, X.J., Mascarenhas, L., Weinberg, K.I., Nadler, L.M., and Kohn, D.B. (2000). Lentiviral vectors for efficient delivery of CD80 and granulocyte-macrophage colony-stimulating factor in human acute lymphoblastic leukemia and acute myeloid leukemia cells to induce antileukemic immune responses. *Blood* *96*, 1317–1326.
97. Sundarasetty, B.S., Singh, V.K., Salguero, G., Geffers, R., Rickmann, M., Macke, L., Borchers, S., Figueiredo, C., Schambach, A., Gullberg, U., et al. (2013). Lentivirus-induced dendritic cells for immunization against high-risk WT1(+) acute myeloid leukemia. *Hum. Gene Ther.* *24*, 220–237.