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Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remissions

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Abstract

We developed a personalized cancer vaccine in which patient-derived acute myeloid leukemia (AML) cells are fused with autologous dendritic cells, generating a hybridoma that potently stimulates broad antitumor responses. We report results obtained from the first 17 AML patients, who achieved remission after chemotherapy and were then serially vaccinated to target minimal residual disease and prevent relapse. Vaccination was well tolerated and induced inflammatory responses at the site of administration, characterized by the dense infiltration of T cells. Vaccination was also associated with a marked rise in circulating T cells recognizing whole AML cells and leukemia-specific antigens that persisted for more than 6 months. Twelve of 17 vaccinated patients (71%; 90% confidence interval, 52 to 89%) remain alive without recurrence at a median follow-up of 57 months. The results demonstrate that personalized vaccination of AML patients in remission induces the expansion of leukemia-specific T cells and may be protective against disease relapse.

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SUPPLEMENTARY MATERIALS

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Author contributions

J.R. was the overall principal investigator of the clinical trial, designed the trial, provided the funding, analyzed the results, and wrote the paper. R.M.S. was the principal investigator of the clinical trial at the DFCI, reviewed the results, and reviewed the paper. R.J., J.D.L., J.A., M.M., K.L., S.J., J.I.Z., A.H., V.B., D.P.S., D.J.D., and I.G. enrolled patients to the trial and reviewed the manuscript. L.U. oversaw the vaccine production. P.S.D. was responsible for the vaccine production. E.L. and M.P.B. analyzed the data. D.S. performed and analyzed the immune correlates. D.N. and L.W. designed the study and provided the biostatistical analysis of the data. K.P., M.C., A.W., and L.C. manufactured the vaccines, characterized the vaccines, and performed the experiments. D.K. designed the trial and wrote the paper. D.A. designed the trial, enrolled the patients to the trial, provided the funding, and wrote the paper.

Competing interests: D.A., J.R., and D.K. are inventors on an international patent application (PCT/US2016/024980) held/submitted by Dana-Farber Harvard Cancer Center and Beth Israel Deaconess Medical Center that covers Compositions and Methods of Treating Acute Myeloid Leukemia. All other authors declare that they have no competing interests.

INTRODUCTION

Acute myeloid leukemia (AML) is a lethal hematological malignancy for which chemotherapy is rarely curative, particularly in older patients (1). In contrast to cytotoxic therapy, immune therapy offers an opportunity to broadly target the malignant cells and provide immunologic memory to prevent recurrence. The efficacy of cellular immunotherapy is supported by the observation that allogeneic hematopoietic stem cell transplantation is curative for a subset of patients because of the graft-versus-leukemia effect mediated by alloreactive lymphocytes (2). Conversely, allogeneic transplantation is associated with marked morbidity and mortality resulting from the lack of specificity of the alloreactive response, which results in graft-versus-host disease. An important area of investigation is therefore the development of strategies to induce immune responses that selectively eliminate leukemia cells.

AML offers an opportunity to explore the role of immunotherapy. The majority of patients achieve a complete remission after chemotherapy; however, cytotoxic therapy is curative for only a minority of patients, and most patients relapse with resistant disease. This therapeutic challenge is highlighted in AML patients 60 years and older. After achieving remission, only 15 to 20% of this population remains free of leukemia for 2 years (1, 3, 4). Even for patients under the age of 60, relapse after achieving chemotherapy-induced remission is common, with a 5-year survival rate of 34% for patients treated with intensive postremission chemotherapy (5). Allogeneic transplantation offers a potential for durable remission, particularly for patients with high-risk disease (2). However, allogeneic transplantation may not be feasible for many AML patients, as a result of age, comorbidities, availability of a suitable donor, and considerable treatment-associated morbidity and mortality.

We have developed a personalized vaccine in which patient-derived AML cells are fused with autologous dendritic cells (DCs), presenting a broad array of antigens in the context of DC-derived costimulation (6). In animal models, vaccination with DC/tumor fusions results in eradication of established metastatic disease (7–9). In phase I and II trials for patients with multiple myeloma (MM), we found that vaccination with DC/MM fusions is well tolerated and induces antitumor immune and clinical responses in a subset of patients (10, 11). Here, AML patients in remission were vaccinated with DC/AML fusions with the goal of eliciting a leukemia-specific immune response to immunologically eradicate residual disease. The results demonstrate that this personalized vaccine induces anti-AML cell immunity and provides protection against disease relapse.

RESULTS

Patient characteristics

The characteristics of the 19 AML patients who completed vaccine generation are summarized in Table 1. The median age was 63 years. Eleven patients had intermediate or high-risk disease, and eight patients had favorable risk disease (12) (table S1). Two patients completed vaccine generation but did not receive any vaccination because of relapsed AML ($n = 1$) or ongoing chemotherapy-related toxicity precluding initiation of vaccination ($n = 1$). Seventeen patients initiated vaccination; 16 patients received at least two doses of vaccine,

and 1 patient relapsed after having received only one dose of vaccine. The median time from achieving remission to initiating vaccination was 171 days (range, 73 to 197 days). The median time from completing postremission chemotherapy to initiating vaccination was 56 days (range, 38 to 118 days). Twenty-eight patients underwent tumor cryopreservation but did not complete vaccine generation (table S2).

Vaccine characterization

The mean yield of the DC and AML cells was 169×10^6 and 95×10^6 cells, respectively. The mean fusion efficiency (DC fused to AML cells), as determined by the percentage of cells that coexpressed distinct DC (CD80, CD86, and CD83) and AML (CD38, CD34, CD117, CD64, or MUC1) antigens, was 40%. The mean viability of the DC, AML, and fusion preparations was 90, 91, and 86%, respectively (table S3). In contrast to AML blasts, the DC and fusion preparations potently stimulated allogeneic T cell proliferation (mean stimulation indices, 3.7, 16.9, and 11.7, respectively; table S4). Fourteen of 17 patients received three doses of the vaccine, and two patients received two doses, one due to limitations of cell yields and one due to disease relapse before the third vaccine. One patient received one vaccine and relapsed before the second vaccine.

Adverse events

Vaccination was well tolerated, and no evidence of symptomatic autoimmunity was observed. Potential related adverse events were transient and of grade 1 to 2 intensity (Table 2). The most common adverse event was erythema, pruritis, and/or induration at the vaccine site. Biopsy of vaccine site reactions demonstrated a dense infiltrate of CD4 and CD8 T cells, consistent with recruitment of reactive T cell populations to the vaccine bed (Fig. 1).

Cellular immunologic response to vaccination

Vaccination with DC/AML fusions induced the expansion of leukemia-specific T cells in the peripheral blood and bone marrow, as determined by the percentage of CD4⁺ and CD8⁺ T cells expressing interferon- γ (IFN- γ) upon ex vivo exposure to autologous tumor lysate. Consistent with these findings, vaccination also resulted in the expansion of CD8⁺ T cells recognizing previously identified AML antigens quantified by pentamer analysis in human leukocyte antigen (HLA)-A2.1 patients. After recovery from consolidation chemotherapy, AML-specific T cells were nearly undetectable in the peripheral blood and bone marrow (Fig. 2). Vaccination resulted in a 5.4-fold increase in AML-specific CD4⁺ T cells ($P = 0.003$) and a 15.7-fold increase in AML-specific CD8⁺ T cells ($P = 0.0001$), comparing prevaccination numbers to peak numbers after vaccination ($n = 16$; Fig. 2, A to E, and fig. S1). Circulating leukemia-reactive T cells remained elevated 6 months after the last vaccination at the time of last assessment (fig. S1). In HLA-A*0201 patients, vaccination resulted in the expansion of peripheral blood T cells recognizing MUC1 (4.4-fold increase), WT1 (2.7-fold increase), and NY-ESO (3.8-fold increase) tumor antigens ($n = 4$; Fig. 3, A to E, and fig. S2). Vaccination was associated with a 4.7-fold increase in leukemic reactive CD8⁺ T cells in the bone marrow from before vaccination to 1 month after the last vaccination ($n = 5$; fig. S3A). An expansion in bone marrow-infiltrating T cells recognizing MUC1 (ninefold increase) and WT1 (eightfold increase) was observed from before vaccination to 1 month after vaccination ($n = 3$; fig. S3, B and C).

Quantification of regulatory T cell and PD-1–expressing T cells in the peripheral blood

Preclinical studies have demonstrated that, in addition to activated T cells, vaccination may result in the expansion of inhibitory cell populations that can mute clinical effects. Circulating regulatory T cell (T_{reg}) populations, defined as those coexpressing CD4/CD25 and FOXP3, were quantified before each vaccination and serially after vaccination. Circulating T_{regs} were present in low numbers before vaccination (mean, 7.18%; $n = 13$) and did not increase throughout the period of vaccination (mean, 5.57 and 5.43% before the second and third vaccine, respectively; $n = 13$; fig. S4A) or at the 6th month follow-up (mean, 7.04%; $n = 13$). Similarly, PD-1 expression on circulating CD4⁺ and CD8⁺ T cell populations was unchanged during the period of vaccination and follow-up (fig. S4B). No change was observed in the percentage of bone marrow–infiltrating T_{regs} after vaccination (fig. S4C).

Clinical response

The study population had a median age of 63 years. Thirteen of 19 patients who completed vaccine generation are in remission [68%; 90% confidence interval (CI), 51 to 86%]. Twelve of 17 patients who received at least one dose of vaccine remain alive and in remission (71%; 90% CI, 52 to 89%) at 16.7 to 66.5 months from initiating vaccination, with a median follow-up of 57 months. As a notable example, a 77-year-old female who relapsed within 1 year of primary induction chemotherapy underwent vaccination after achieving a second remission. She remains in remission at 53 months of follow-up from vaccination. No patient has relapsed 1 year after completing the vaccination. The 4-year progression-free survival (PFS) rate is 71% (90% CI, 52–89%). Notably, median PFS and overall survival (OS) have not been reached (Fig. 4).

DISCUSSION

AML offers a particular therapeutic challenge. The majority of patients achieve a remission after chemotherapy; however, only a small minority experience durable responses, particularly among patients over the age of 60. Only 15 to 20% of the population remains free of leukemia at 2 years (1, 3, 4). Even in patients under the age of 60 who achieve complete remission and receive intensive postremission chemotherapy, the 5-year survival rate is about 35% (5). The high rate of recurrence after induction chemotherapy is thought to arise from the persistence of clonal populations intrinsically resistant to cytotoxic injury, including the malignant stem cell population resulting in the repopulation of disease in 6 to 12 months. In contrast, the efficacy of cellular immunotherapy for AML is highlighted by the observation that allogeneic transplantation is potentially curative for a subset of patients because of the immunologic capacity of alloreactive lymphocytes to broadly eradicate the malignant clonal population (2). However, the application of this strategy is often limited by prohibitive treatment–associated morbidity and mortality due to damage to normal tissues in the context of graft-versus-host disease and other complications of therapy.

An important focus of investigation is therefore the development of AML vaccines to induce immune responses that will more selectively eliminate AML cells. However, to our knowledge, there are presently no vaccines that have shown promise in the treatment of

AML. Strategies to design AML vaccines have included single antigen-based approaches, such as WT1 peptide administered with adjuvant (13, 14), DCs loaded with tumor-associated antigens (15, 16), or the use of AML cells differentiated into DCs (17). Immune responses have been observed in several studies. In some patients, evidence of clinical effect was demonstrated with a reduction in peripheral blasts, and several patients have demonstrated prolonged survival of at least 3 years (16, 18–20). The results of the present study are more robust, with 70% of vaccinated patients demonstrating long-term remission. Both the potent immune response evoked by the DC/AML fusion cell vaccine and the incorporation of vaccination after cytoreductive chemotherapy likely account for the promising clinical outcomes observed in this study.

Notably, marked responses have been seen in a subset of patients with acute lymphocytic leukemia undergoing adoptive immunotherapy with chimeric antigen receptor T (CAR-T) cell therapy, in which persistence of the tumor-specific T cells has been associated with durable response (21). However, identifying a target that is broadly expressed on myeloid blasts, and one that is unique to the malignant myeloid blast population, to spare off target toxicity, has proved challenging. As a result, the application of CAR-T cell therapy to patients with AML has been limited to date.

Present studies describe a personalized vaccine in which patient-derived AML cells are fused with autologous DCs, incorporating antigens that capture the heterogeneity of the leukemia cell population and presenting them in the context of the potent antigen-presenting machinery of the DC. The fusion cell vaccine thus captures the clonal heterogeneity of the malignant population, including the presence of neoantigens, and creates a balanced cytotoxic and helper T cell response less susceptible to immune escape. Additionally, DC/AML fusions induce a polyclonal helper and cytotoxic T cell immune response that includes targeting of the leukemia stem cell population. We hypothesized that chemotherapy-induced remission would augment vaccine response by optimizing tumor cytoreduction and reducing the immunosuppressive effect on the bone marrow microenvironment and create a suitable platform to immunologically target persisting chemotherapy-resistant clones and achieve cures.

Our results support the efficacy of this vaccine in AML patients who achieve a remission after chemotherapy. Twelve of 17 patients (71%) remain in remission with a median of 57 months of follow-up, and no patient has relapsed more than 1 year after chemotherapy and vaccination. Patients remaining in remission include several patients over age 70. One patient who relapsed within 1 year of initial chemotherapy underwent vaccination after achieving a second chemotherapy-induced remission and remains in remission more than 4 years after completing chemotherapy. The long-term remission observed after chemotherapy and vaccination of a patient who had experienced early relapse after initial induction therapy is distinctly unusual in the absence of allogeneic transplantation, supporting the notion that this vaccine induces sustained responses and potentially cures.

The clinical effects of the vaccine were observed in the context of sustained induction of AML-specific immunity, as measured by the expansion of AML-specific CD4⁺ and CD8⁺ T cells in the peripheral blood. A concomitant rise in leukemia-specific T cells in the bone

marrow supports the notion that the immune response is generated in this critical microenvironment. The specificity of the immune response is further supported by the expansion of T cells recognizing the leukemia-associated antigens WT1, MUC1, and NY-ESO. In this context, MUC1 is only expressed by leukemia stem cells, as compared to normal hematopoietic stem cells, consistent with the potential targeting of this self-renewing population (22). It is important to assess whether there is a correlation between the nature of the immune response to vaccination and clinical outcome. Here, five relapsed patients did not provide enough power to assess whether the immune response differed in the relapsed patients compared to the 12 patients who demonstrated sustained remission. The expansion of AML-specific T cells peaked at 2 months after vaccination and persisted at 6 months after treatment, the last time point measured. Thus, the role of booster vaccination in maintaining the response should be evaluated.

Vaccination with DC/AML fusions was well tolerated, with toxicity predominantly limited to reactions associated with recruitment of activated T cells into the vaccine site. This response stands in contrast to toxicities observed with nonspecific activation of the immune system in the setting of immune checkpoint inhibitors and the infusion of constitutively activated T cells, which include pneumonitis and the cytokine release syndrome, respectively (23–25).

Recent studies have demonstrated that up-regulation of immune checkpoint signaling through the PD-L1/PD-1 and Tim3 pathways induces tolerance in AML (26). Here, late relapse resulting from the reestablishment of tolerance also remains a possibility. In response to this concern, future work will need to combine vaccination with blockade of the PD-L1/PD-1 pathway. Moreover, the results reported here with the personalized AML vaccine will need to be confirmed in a multicenter randomized trial of postremission vaccination.

MATERIALS AND METHODS

Study design

The protocol was approved by the Dana-Farber Harvard Cancer Center Institutional Review Board, and is registered at <https://clinicaltrials.gov/> (NCT01096602). All patients provided written informed consent. The primary objective of the study was to assess the toxicity associated with treating AML patients with DC/AML fusion cells in the postchemotherapy setting. The secondary objective was to explore immunological response to DC/AML fusion vaccination in patients who have achieved a chemotherapy-induced remission and to define antitumor effects by determining time to disease progression. Patients with newly diagnosed or first relapsed AML were potentially eligible for collection and cryopreservation of primary leukemia samples. Patients with a history of autoimmune disease, evidence of symptomatic organ dysfunction, or other unstable comorbid medical illness were excluded. Patients who achieved complete remission after one to two courses of standard induction therapy but were deemed not appropriate for allogeneic transplantation and did not have ongoing grade 3 to 4 chemotherapy-related toxicity were assigned to the vaccine generation and administration phase of the protocol. Vaccines were generated during consolidation chemotherapy, with up to four cycles of postremission therapy permitted at the discretion of the treating physician. The vaccine was administered 1 to 3 months after the last cycle of

chemotherapy. Three doses of 5×10^6 fusion cells were administered at monthly intervals. No other anti-leukemia therapy was permitted. The study was not randomized or blinded; all patients who completed vaccine production and met eligibility criteria to initiate vaccination were treated with the DC/AML fusion vaccine. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 4.0. If 3 patients experienced treatment limiting toxicity, the study was to be suspended. Patients were monitored for 1 week after administration of vaccine during the period of immunotherapy and then monthly thereafter for 6 months during which time they were assessed for evidence of treatment-related toxicities with particular attention to autoimmune disease and cytopenias. Patients experiencing treatment-related grade 3 or higher toxicity were not eligible to receive further therapy. Immune response was measured by percentage of T cells that express IFN- γ as determined by the ratio of the maximum IFN- γ expression to the baseline level pretherapy.

Reagents for vaccine characterization and immunologic assays

The following were purchased from BD PharMingen: (i) Purified mouse anti-human monoclonal antibodies (mAbs) against HLA-DR, CD80, CD86, CD40, CD83, CD38, CD34, and CD117; (ii) CD14 phycoerythrin (PE)-conjugated mouse anti-human mAbs against CD4; (iii) fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (RPA-T4, IgG1) and anti-CD8 (RPA-T8, IgG1); (iv) FITC-, PE-conjugated matching isotype IgG1, IgG2a, and IgG2b controls; and (v) purified mouse monoclonal IgG1 (MOPC-21) isotype control. The mAb DF3 (anti-MUC1-N) has been described previously (27). Anti-human CD4 TC-conjugated, matching isotype control (IgG2a), PE-conjugated anti-human mAbs against IFN- γ (mouse IgG1-B27), and PE-conjugated matching isotype controls (rat IgG1-PE and mouse IgG1-PE) were purchased from Invitrogen. FITC-conjugated goat anti-mouse (IgG1) was purchased from Chemicon International.

Vaccine generation, characterization, and administration

AML cells were collected by aspiration of 20 to 30 cm³ of bone marrow, 40 to 50 cm³ of peripheral blood in those patients with a high number of circulating blasts, or leukapheresis collections in patients requiring emergent cytoreduction because of the risk of leukostasis. Mononuclear cells were isolated by Ficoll density gradient centrifugation and cryopreserved in 10% dimethyl sulfoxide (DMSO)/90% autologous plasma. Patients eligible for vaccine generation underwent a single leukapheresis collection for DC generation and vaccine production. Adherent cells were cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) (1000 U/ml; Sanofi) and IL-4 (500 IU/ml; CellGenix) for 5 to 7 days and matured in the presence of tumor necrosis factor- α (25 ng/ml; CellGenix) for 2 to 3 days. The DC and thawed autologous AML preparations were analyzed by immunocytochemical staining and then co-cultured with polyethylene glycol to generate DC/tumor fusions, as previously described (11). DC/AML fusions were (i) quantified by determining the percentage of cells that coexpress specific DC (CD80, CD83, or CD86) and tumor-associated antigens (CD34, CD38, CD64, CD117, or MUC1) by immunohistochemical analysis, (ii) assessed for sterility, (iii) cryopreserved in autologous plasma (90%) and DMSO (10%) in single dose vials of 5×10^6 fusion cells, and (iv) stored frozen in the vapor phase of liquid nitrogen. At the time of administration, the fused cells were irradiated with 30 Gy and administered as a subcutaneous injection in the upper thigh at 4-week intervals.

for a total of three doses. GM-CSF (100 µg) was administered at the vaccine site on the day of vaccination and for 3 days thereafter. As a measure of their potency as antigen-presenting cells, the capacity of DC/AML fusion cells to stimulate allogeneic T cell proliferation was assessed. T cells were obtained from commercially available leukopak collections obtained from the Crimson Core Department of Pathology, Brigham and Woman's Hospital, Harvard Medical School. T cells (1×10^5) were cocultured with DC/AML fusion cells, DCs, or AML blasts at a ratio of 10:1 for 5 days. T cell proliferation was determined by measuring incorporation of [3 H]thymidine after overnight pulsing (1 µCi per well) of triplicate samples.

Vaccine induction of immunologic response

Patients underwent serial assessment to quantify circulating and bone marrow–derived T cells reactive with whole autologous AML cells and previously identified leukemia-associated antigens. Cryopreserved peripheral blood mononuclear cells (PBMCs) were collected before each vaccination and at 1, 3, and 6 months thereafter. After the last vaccination, PBMC samples were thawed, and 1×10^6 cells were cultured with lysate generated by repeated freeze thaw cycles of 1×10^5 autologous leukemia cells for 5 days. Cells were restimulated with autologous tumor lysate for 6 hours and cultured overnight with BD GolgiStop (1 µg/ml). We determined the intracellular expression of IFN-γ by CD4⁺ or CD8⁺ T cells by fluorescence-activated cell sorting (FACS) analysis of permeabilized cells. We also quantified leukemia-reactive T cells in the bone marrow before and 1 month after completion of vaccination in a subset of patients. In HLA-A2.1 patients, the number of circulating CD8⁺ T cells binding the MUC1 (antigenic peptide LLLLTVLTV), WT1 (antigenic peptide RMFPNAPYL), and NY-ESO (antigenic peptide SLLMWITQV) pentamers was determined by bi-dimensional FACS analysis using CD8-FITC and the corresponding pentamer-PE antibody.

Regulatory T cells were quantified by determining the expression of FOXP3 by CD4/CD25 cells using intracellular FACS analysis. PD-1 expression on circulating CD4 and CD8 T cell populations was assessed by flow cytometry. In one patient, vaccine site reactions underwent biopsy and immunocytochemical staining to assess infiltration of CD4 and CD8 T cells. Recruitment of native DCs was assessed by CD1a expression in the vaccine site.

Clinical disease assessment—Disease assessment was monitored by complete blood counts with differential and a bone marrow aspiration and biopsy a week before initiation of vaccination and 4 weeks and 3 and 6 months after completion of therapy. Cytogenetic evaluation was performed on bone marrow specimens in patients who had abnormal cytogenetics at presentation. Patients were monitored for disease relapse with assessments every 3 months.

Statistical analysis—For analysis of immune response to vaccination, fold change of IFN-γ by CD4⁺ or CD8⁺ T cells between prevaccine measurement and 1 month after the last vaccination was summarized as median, mean, and SD. A paired *t* test was used to assess whether the ratio of expression of these markers differed from 1. PFS was defined as the time from the date of first vaccine to the date of relapse or death from any cause, and OS was defined as the date from first vaccine to the date of death. Kaplan-Meier method was

used to summarize the 4-year PFS rate and 5-year OS rate. Greenwood formula was used to estimate the standard error for calculating 90% CI. Statistical analysis was performed using Statistical Analysis Software (SAS/STAT) version 9.4 of the SAS System for Windows.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES AND NOTES

1. Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, Omura GA, Moore JO, Ross McIntyre O, Frei E, Cancer and Leukemia Group B. Intensive postremission chemotherapy in adults with acute myeloid leukemia. *N Engl J Med*. 1994; 331:896–903. [PubMed: 8078551]
2. Champlin R. Reduced intensity allogeneic hematopoietic transplantation is an established standard of care for treatment of older patients with acute myeloid leukemia. *Best Pract Res Clin Haematol*. 2013; 26:297–300. [PubMed: 24309534]
3. Kahl C, Krah R, Becker C, Al-Ali HK, Sayer HG, Schulze A, Herold M, Hänel M, Scholl S, Hochhaus A, Uharek L, Maschmeyer G, Haehling D, Junghans C, Peter N, Kämpfe D, Kettner E, Heinicke T, Fischer T, Kreibich U, Wolf HH, Niederwiese D. Long-term follow-up of the AML97 study for patients aged 60 years and above with acute myeloid leukaemia: A study of the East German Haematology and Oncology Study Group (OSHO). *J Cancer Res Clin Oncol*. 2016; 142:305–315. [PubMed: 26407768]
4. Goldstone AH, Burnett AK, Wheatley K, Smith AG, Michael Hutchinson R, Clark RE. Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: The results of the United Kingdom Medical Research Council AML11 trial. *Blood*. 2001; 98:1302–1311. [PubMed: 11520775]
5. Moore JO, George SL, Dodge RK, Amrein PC, Powell BL, Kolitz JE, Baer MR, Davey FR, Bloomfield CD, Larson RA, Schiffer CA. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B Study 9222. *Blood*. 2005; 105:3420–3427. [PubMed: 15572587]
6. Gong J, Koido S, Kato Y, Tanaka Y, Chen D, Jonas A, Galinsky I, DeAngelo D, Avigan D, Kufe D, Stone R. Induction of anti-leukemic cytotoxic T lymphocytes by fusion of patient-derived dendritic cells with autologous myeloblasts. *Leuk Res*. 2004; 28:1303–1312. [PubMed: 15475072]
7. Gong J, Avigan D, Chen D, Wu Z, Koido S, Kashiwaba M, Kufe D. Activation of antitumor cytotoxic T lymphocytes by fusions of human dendritic cells and breast carcinoma cells. *Proc Natl Acad Sci USA*. 2000; 97:2715–2718. [PubMed: 10688917]
8. Gong J, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nat Med*. 1997; 3:558–561. [PubMed: 9142127]
9. Gong J, Chen D, Kashiwaba M, Li Y, Chen L, Takeuchi H, Qu H, Rowse GJ, Gendler SJ, Kufe D. Reversal of tolerance to human MUC1 antigen in MUC1 transgenic mice immunized with fusions of dendritic and carcinoma cells. *Proc Natl Acad Sci USA*. 1998; 95:6279–6283. [PubMed: 9600956]
10. Rosenblatt J, Avivi I, Vasir B, Uhl L, Munshi NC, Katz T, Dey BR, Somaiya P, Mills H, Campigotto F, Weller E, Joyce R, Levine JD, Tzachanis D, Richardson P, Laubach J, Raje N,

Boussiotis V, Yuan YE, Bisharat L, Held V, Rowe J, Anderson K, Kufe D, Avigan D. Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. *Clin Cancer Res*. 2013; 19:3640–3648. [PubMed: 23685836]

11. Rosenblatt J, Vasir B, Uhl L, Blotta S, MacNamara C, Somaiya P, Wu Z, Joyce R, Levine JD, Dombagoda D, Yuan YE, Francoeur K, Fitzgerald D, Richardson P, Weller E, Anderson K, Kufe D, Munshi N, Avigan D. Vaccination with dendritic cell/tumor fusion cells results in cellular and humoral antitumor immune responses in patients with multiple myeloma. *Blood*. 2011; 117:393–402. [PubMed: 21030562]
12. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Löwenberg B, Bloomfield CD. Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010; 115:453–474. [PubMed: 19880497]
13. Brayer J, Lancet JE, Powers J, List A, Balducci L, Komrokji R, Pinilla-Ibarz J. WT1 vaccination in AML and MDS: A pilot trial with synthetic analog peptides. *Am J Hematol*. 2015; 90:602–607. [PubMed: 25802083]
14. Di Stasi A, Jimenez AM, Minagawa K, Al-Obaidi M, Rezvania K. Review of the results of WT1 peptide vaccination strategies for myelodysplastic syndromes and acute myeloid leukemia from nine different studies. *Front Immunol*. 2015; 6:36. [PubMed: 25699052]
15. Subklewe M, Geiger C, Lichtenegger FS, Javorovic M, Kvalheim G, Schendel DJ, Bigalke I. New generation dendritic cell vaccine for immunotherapy of acute myeloid leukemia. *Cancer Immunol Immunother*. 2014; 63:1093–1103. [PubMed: 25186611]
16. Van Tendeloo VF, Van de Velde A, Van Driessche A, Cools N, Anguille S, Ladell K, Gostick E, Vermeulen K, Pieters K, Nijs G, Stein B, Smits EL, Schroyens WA, Gadisseur AP, Vrelust I, Jorens PG, Goossens H, de Vries IJ, Price DA, Oji Y, Oka Y, Sugiyama H, Berneman ZN. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted dendritic cell vaccination. *Proc Natl Acad Sci USA*. 2010; 107:13824–13829. [PubMed: 20631300]
17. Vogt V, Schick J, Ansprenger C, Braeu M, Kroell T, Kraemer D, Köhne CH, Hausmann A, Buhmann R, Tischer J, Schmetzer H. Profiles of activation, differentiation-markers, or β -integrins on T cells contribute to predict T cells' antileukemic responses after stimulation with leukemia-derived dendritic cells. *J Immunother*. 2014; 37:331–347. [PubMed: 24911794]
18. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, Elisseeva OA, Oji Y, Kawakami M, Ikegame K, Hosen N, Yoshihara S, Wu F, Fujiki F, Murakami M, Masuda T, Nishida S, Shirakata T, Nakatsuka S, Sasaki A, Uda K, Dohy H, Aozasa K, Noguchi S, Kawase I, Sugiyama H. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci USA*. 2004; 101:13885–13890. [PubMed: 15365188]
19. Schmitt M, Schmitt A, Rojewski MT, Chen J, Giannopoulos K, Fei F, Yu Y, Götz M, Heyduk M, Ritter G, Speiser DE, Gnjatich S, Guillaume P, Ringhoffer M, Schlenk RF, Liebisch P, Bunjes D, Shiku H, Dohner H, Greiner J. RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. *Blood*. 2008; 111:1357–1365. [PubMed: 17978170]
20. Keilholz U, Letsch A, Busse A, Asemisen AM, Bauer S, Blau IW, Hofmann WK, Uharek L, Thiel E, Scheibenbogen C. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. *Blood*. 2009; 113:6541–6548. [PubMed: 19389880]
21. Maude SL, Teachey DT, Porter DL, Grupp SA. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood*. 2015; 125:4017–4023. [PubMed: 25999455]
22. Stroopinsky D, Rosenblatt J, Ito K, Mills H, Yin L, Rajabi H, Vasir B, Kufe T, Luptakova K, Arnason J, Nardella C, Levine JD, Joyce RM, Galinsky I, Reiter Y, Stone RM, Pandolfi PP, Kufe D, Avigan D. MUC1 is a potential target for the treatment of acute myeloid leukemia stem cells. *Cancer Res*. 2013; 73:5569–5579. [PubMed: 23867470]

23. Topalian SL, Stephen Hodi F, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kolli GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366:2443–2454. [PubMed: 22658127]
24. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, Bagg A, Marcucci KT, Shen A, Gonzalez V, Ambrose D, Grupp SA, Chew A, Zheng Z, Milone MC, Levine BL, Melenhorst JJ, June CH. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. *Sci Transl Med*. 2015; 7:303ra139.
25. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS, Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase 1 dose-escalation trial. *Lancet*. 2015; 385:517–528. [PubMed: 25319501]
26. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, Murphy WJ, Azuma M, Anderson AC, Kuchroo VK, Blazar BR. Coexpression of Tim-3 and PD-1 identifies a CD8⁺ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood*. 2011; 117:4501–4510. [PubMed: 21385853]
27. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma*. 1984; 3:223–232. [PubMed: 6094338]

Fig. 1. Biopsy of vaccine site reaction

A dense mononuclear cell infiltrate is present at the vaccine site (left panel).

Immunohistochemical staining demonstrates an infiltrate of CD4⁺ and CD8⁺ T cells and CD1a⁺ immature DCs at the biopsy site. Scale bar, 50 μ m.

Fig. 2. Expansion of leukemia-specific CD4⁺ and CD8⁺ T cells after vaccination

(A) A representative example of intracellular expression of IFN- γ by CD8⁺ T cells before and serially after vaccination is shown. (B and C) The percentages of CD8⁺ (B) and CD4⁺ (C) T cells expressing IFN- γ for individual study patients before initiating vaccination (white), and peak response after vaccination (black) are shown. (D and E) Mean percentages of leukemia-specific T cells before vaccination and peak after vaccination. Mean percentages of CD8⁺ (D) and CD4⁺ (E) T cells expressing IFN- γ after ex vivo exposure to autologous tumor lysate are shown before initiating vaccination and at the peak time after vaccination. The results are presented as means $\pm$ SEM from 16 vaccinated patients.

Fig. 3. Expansion of MUC1, WT1, NY-ESO, survivin pentamer⁺ cells after vaccination
 CD8⁺ T cells binding the MUC1, WT1, NY-ESO, and survivin pentamers were quantified at serial time points (before each of three vaccinations and at 1, 3, and 6 months after the third vaccination) in patients who are HLA-A2.1. Binding to a control pentamer was quantified in parallel, and the control value was subtracted from that obtained for the indicated pentamer. **(A)** A representative example demonstrating the percentage of circulating pentamer⁺ cells before and after vaccination is shown. **(B to E)**. Percentages of circulating T cells recognizing MUC1 **(B)**, WT1 **(C)**, NY-ESO **(D)**, and survivin **(E)** before (white bar) and peak after vaccination (black bar) for individual patients are shown.

Fig. 4. PFS and OS

Median PFS (A) and OS (B) have not been reached for the 17 patients who received vaccination.

Table 1

Patient demographics. MEC, mitomycin, etoposide, and cytarabine; HiDAC, high-dose cytarabine; MiDAC, intermediate dose Ara-C; CR, complete remission; MDS, myelodysplastic syndrome; t-AML, therapy related AML.

Study number	Age	Gender	Disease status at presentation	WHO classification	Induction regimen(s)	Consolidation	Current status	Time to relapse (months)	Duration of CR (months)
PA2	37	F	Initial diagnosis	Acute myelomonocytic leukemia	7 + 3	Four cycles of HiDAC	CR		71.6
PA3	76	M	Initial diagnosis	AML with maturation	7 + 3	Three cycles of MiDAC	CR		71
PA5	69	M	Initial diagnosis	Acute monocytic leukemia	7+3	Three cycles of MiDAC	CR		69.4
PA6	66	M	Initial diagnosis	AML with minimal differentiation	7 + 3 and MEC	One cycle HiDAC, one cycle MiDAC	Relapse	5.8	
PA8	32	M	Initial diagnosis	Acute myelomonocytic leukemia	7+3	Four cycles of HiDAC	CR		68.2
PA9	54	F	Initial diagnosis	Acute monocytic leukemia	7+3	Four cycles of HiDAC	Relapse	8.1	
PA10	56	F	Initial diagnosis	t-AML	7+3	Four cycles of HiDAC	CR		58.3
PA11	77	F	First relapse (after remission of 1 year)	Acute myelomonocytic leukemia	MEC	One cycle of MiDAC	CR		59.8
PA13	76	M	Initial diagnosis	AML with MDS-related changes	Two cycles of decitabine	Four cycles of decitabine	Relapse	9.1	
PA14	28	F	Initial diagnosis	AML with maturation	7 + 3 and 5 + 2	Four cycles of HiDAC	Relapse	14	
PA16	64	M	Initial diagnosis	AML with t(8;21)(q22;q22); RUNX1-RUNX1T1	7+3	Four cycles of HiDAC	CR		58.3
PA18	62	F	Initial diagnosis	AML without maturation	7+3	Three cycles of HiDAC	CR		54.6
PA21	56	M	Initial diagnosis	AML with t(8;21)(q22;q22); RUNX1-RUNX1T1	7+3	Four cycles of HiDAC	CR		57.1
PA23	74	F	Initial diagnosis	AML with mutated NPM1	7+3	One cycle of HiDAC	Relapse	5.9	
PA24	67	M	Initial diagnosis	AML with minimal differentiation	7+3	Three cycles of MiDAC	CR		51.9
PA26	72	M	Initial diagnosis	Acute myelomonocytic leukemia	7+3	Four cycles of HiDAC	Relapse	3.8	
PA29	59	F	Initial diagnosis	t-AML	7+3	Four cycles of HiDAC	CR		26
PA36	56	M	Initial diagnosis	AML with mutated NPM1	7+3	Three cycles of HiDAC	CR		23.4
PA38	63	M	Initial diagnosis	AML with t(8;21)(q22;q22); RUNX1-RUNX1T1	7+3	Four cycles of MiDAC	CR		21.7

Table 2

Adverse events.

Adverse events	Grade	Number of Episodes
Vaccine site reaction	1	31
	2	3
Leukopenia	1	5
Urticaria	1	4
Eosinophilia	1	3
Elevated thyroid-stimulating hormone	1	3
Thrombocytopenia	1	3
Pruritis	1	2
	2	1
Increased monocytes	1	1
Arthralgia	1	1
Myalgia	1	1