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Ute E. Burkhardt, … , Edwin P. Alyea, Catherine J. Wu

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Background. Patients with advanced hematologic malignancies remain at risk for relapse following reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (allo-HSCT). We conducted a prospective clinical trial to test whether vaccination with whole leukemia cells early after transplantation facilitates the expansion of leukemia-reactive T cells and thereby enhances antitumor immunity.

Methods. We enrolled 22 patients with advanced chronic lymphocytic leukemia (CLL), 18 of whom received up to 6 vaccines initiated between days 30 and 45 after transplantation. Each vaccine consisted of irradiated autologous tumor cells admixed with GM-CSF–secreting bystander cells. Serial patient PBMC samples following transplantation were collected, and the impact of vaccination on T cell activity was evaluated.

Results. At a median follow-up of 2.9 (range, 1–4) years, the estimated 2-year progression-free and overall survival rates of vaccinated subjects were 82% (95% CI, 54%–94%) and 88% (95% CI, 59%–97%), respectively. Although vaccination only had a modest impact on recovering T cell numbers, $CD8^+$ T cells from vaccinated patients consistently reacted against autologous tumor, but not alloantigen-bearing recipient cells with increased secretion of the effector cytokine IFN-γ, unlike T cells from nonvaccinated CLL patients undergoing allo-HSCT. Further analysis […]

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Autologous CLL cell vaccination early after transplant induces leukemia-specific T cells

Ute E. Burkhardt,[1,2] Ursula Hainz,[1,2] Kristen Stevenson,[3] Natalie R. Goldstein,[2] Mildred Pasek,[2] Masayasu Naito,[1,2] Di Wu,[1,2] Vincent T. Ho,[2] Anselmo Alonso,[1,2] Naa Norkor Hammond,[1,2] Jessica Wong,[1,2] Quinlan L. Sievers,[1,2] Ana Brusic,[1,2] Sean M. McDonough,[2] Wanyong Zeng,[1,2] Ann Perrin,[2] Jennifer R. Brown,[2,4] Christine M. Canning,[1,2,4] John Koreth,[2,4] Corey Cutler,[2,4] Philippe Armand,[2,4] Donna Neuberg,[3] Jeng-Shin Lee,[5] Joseph H. Antin,[2,4] Richard C. Mulligan,[5] Tetsuro Sasada,[1] Jerome Ritz,[1,2,4] Robert J. Soiffer,[2,4] Glenn Dranoff,[1,2,4] Edwin P. Alyea,[2,4] and Catherine J. Wu[1,2,4]

[1]Cancer Vaccine Center, [2]Department of Medical Oncology, and [3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. [4]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. [5]Harvard Gene Therapy Initiative, Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.

Background. **Patients with advanced hematologic malignancies remain at risk for relapse following reduced-intensity conditioning (RIC) allogeneic hematopoietic stem cell transplantation (allo-HSCT). We conducted a prospective clinical trial to test whether vaccination with whole leukemia cells early after transplantation facilitates the expansion of leukemia-reactive T cells and thereby enhances antitumor immunity.**

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Conclusion. **Our studies suggest that autologous tumor cell vaccination is an effective strategy to advance long-term leukemia control following allo-HSCT.**

Trial registration. **Clinicaltrials.gov NCT00442130.**

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Introduction

Graft-versus-leukemia (GvL) activity following allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents one of the most striking examples of effective human antitumor immunity and is the basis of curative responses observed in many patients with hematologic malignancies undergoing allo-HSCT (1, 2). Over the past decade, reduced-intensity conditioning (RIC) regimens have been developed to decrease toxicities related to allo-HSCT, thereby broadening the availability of this potentially curative therapeutic approach to patients of advanced age or with comorbidities. Since the RIC regimen alone is insufficient for eradicating leukemia, the effectiveness of RIC allo-HSCT relies entirely on the GvL response. Indeed, several studies have documented that RIC allo-HSCT results in substantial decreases in treatment-related toxicity, while preserving the potential for curative responses (3–5).

One disease for which the effectiveness of RIC allo-HSCT has been demonstrated is chronic lymphocytic leukemia (CLL), a malignancy of clonal mature B cells for which limited treatment options exist when in advanced stages. Early studies established that myeloablative allo-HSCT resulted in unacceptable morbidity in CLL patients, while RIC allo-HSCT could potentially provide an acceptable safety profile and effective leukemia control, even in patients with unfavorable clinical characteristics (6). However, CLL patients treated with RIC allo-HSCT remain at high risk for eventual disease progression (4, 7–9). Even with improvements in patient selection and supportive care, advanced CLL patients still have a 5-year progression-free survival rate of 64% (95% CI, 46%–78%) at best (10). Thus, developing strategies to enhance long-term leukemia control with minimal toxicity remains a high priority (11).

A mechanistic understanding of the basis of effective GvL responses following transplantation can provide clues as to what strategies can be implemented to advance long-term leukemia control following HSCT. We and others have demonstrated that

Conflict of interest: The authors have declared that no conflict of interest exists.
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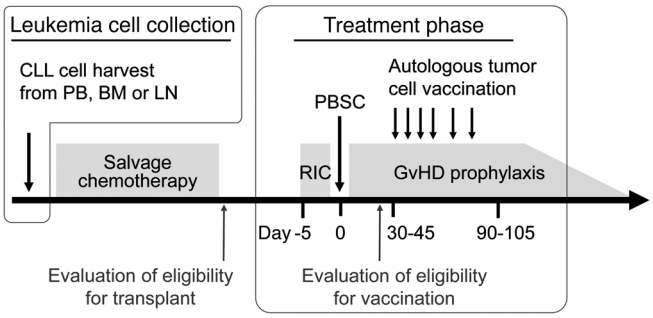

Figure 1
Clinical protocol schema. The study protocol was divided into two phases: (a) collection of autologous CLL cells from peripheral blood (PB), bone marrow (BM), or lymph node (LN), after which subjects underwent salvage chemotherapy; and (b) treatment, which included RIC, subsequent PBSC infusion, and posttransplant vaccination (up to 6 vaccine doses consisting of irradiated autologous tumor cells together with irradiated GM-CSF– secreting bystander cells) between posttransplant days 30 and 100, while maintaining stable GvHD prophylaxis.

GvL responses are initiated and sustained by the development of coordinated cellular and humoral immunity against tumor antigens and are not limited to a sole alloantigen response (12–15). These studies have further suggested that individual patients have unique profiles of immunogenic tumor antigens, likely reflecting the heterogeneity of the genetic alterations found in tumor cells from different patients as well as the diversity of HLA (12–15). Based on these principles, vaccination with autologous, irradiated leukemia cells is an attractive approach to expand leukemia-reactive T cells, since this cancer vaccine formulation reliably includes personal tumor antigens and can potentially elicit polyclonal $CD4^+$ and $CD8^+$ antitumor T cell responses (16).

This strategy is highly feasible in CLL, since leukemia cells from patients' blood, marrow, and lymph nodes can be readily procured for vaccine production. In the current study, we present the results of a phase I trial in which we tested the safety and feasibility of such an approach in patients with advanced CLL, while also testing the biologic effects of the vaccine. Whole leukemia cell vaccination was administered in the early posttransplant setting, since prior human studies have proven the safety of this type of approach following allo-HSCT (17), and preclinical studies have underscored the potential for rapid effector T cell expansion and augmented activity of tumor-reactive T cells in the setting of lymphopenia (18–21). Herein, we demonstrate that vaccination with irradiated autologous leukemia cells admixed with irradiated GM-CSF–secreting bystander cells (22) between days 30 and 100 after allo-HSCT is associated with the induction of polyfunctional CLL-specific T cell responses and promising clinical activity in patients with advanced CLL. Our studies thus suggest that this strategy can effectively promote a beneficial GvL response following RIC allo-HSCT.

Results

A phase I clinical trial investigating whole tumor cell vaccination following RIC allo-HSCT to stimulate anti-CLL responses. In order to enhance polyclonal leukemia-reactive T cell responses with the potential to target personal tumor antigens following allo-HSCT, we designed a clinical protocol in which patients with advanced CLL, defined as intolerance to or relapse within 24 months of a fludarabine-containing regimen, could receive irradiated autologous tumor cells in the early posttransplant period (Figure 1). Study participants were required to have adequate numbers of autologous $CD19^+CD5^+$ leukemia cells collected from peripheral blood, bone marrow, or lymph nodes banked for vaccine development, as well as sufficient tumor cytoreduction prior to transplantation (i.e., no site of adenopathy greater than 5 cm). The transplant preparative regimen consisted of reduced-intensity doses of fludarabine (30 $mg/m^2/day$) and busulfan (0.8 mg/kg/12 hours), administered for 4 days prior to infusion of unmanipulated G-CSF mobilized allogeneic peripheral blood stem cells (PBSCs) from a matched related or unrelated donor. Vaccination was initiated between posttransplant days 30 and 45 if patients demonstrated donor engraftment and no evidence of graft-versus-host disease (GvHD). Up to 6 vaccine doses, each consisting of 1×10^7 irradiated autologous tumor cells admixed with 1×10^7 irradiated K562 bystander cells secreting GM-CSF (GM-K562) (22), were administered subcutaneously/intradermally once a week for 3 doses and then every other week for the following 3 doses. As GvHD prophylaxis, all patients received mini-methotrexate (5 mg/m^2 on days 1, 3, 6, and 11 after allo-HSCT) and tacrolimus, which was maintained at therapeutic levels (5–10 ng/ml) during the entire period of vaccination without taper. Vaccination was halted with the occurrence of any grade 4 toxicity, GvHD requiring therapy, or disease progression.

Of 54 CLL patients from whom leukemia cells were collected and banked, 32 did not proceed to RIC allo-HSCT due to disease

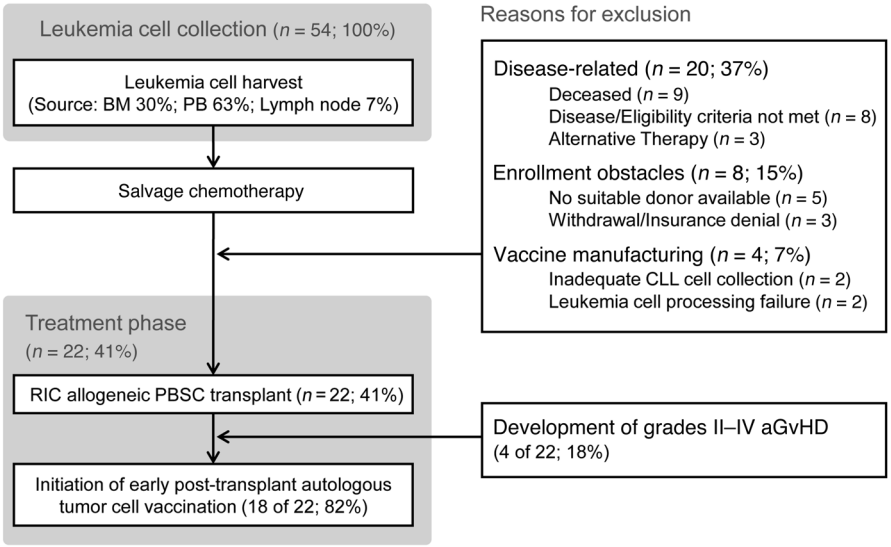

Figure 2
Number of participants and reasons for exclusion of study subjects from the treatment phase of the clinical trial.

Table 1
Clinical characteristics of the 22 study subjects entering the treatment phase of the study

	Eligible patients *n* (%)	Initiated vaccination *n* (%)
n	22	18
Patient characteristics		
Age at diagnosis (years; range)	52 (39, 70)	52 (39, 70)
Age ≥50 years	13 (59)	10 (56)
Male sex	17 (77)	15 (83)
Rai stage at transplantation		
0	2 (9)	2(11)
1	10 (45)	9 (50)
2–4	10 (46)	7 (39)
Number of prior therapies at transplantation		
0 or 1	0 (0)	0 (0)
2 or 3	12 (55)	9 (50)
4 or 5	6 (27)	4 (22)
6 or more	5 (25)	5 (25)
Prior allogeneic transplant	3 (14)	3 (17)
Cytogenetic abnormalities detected by FISH		
Del (13q)	15 (68)	12 (67)
Del (11q)	13 (59)	10(56)
Del (17p)	9 (41)	6 (33)
Trisomy 12	3 (14)	2 (11)
Complex karyotype	4 (18)	2 (11)
IGHV mutation status		
Unmutated, mutated, unknown	15 (68), 6 (27), 1 (5)	13 (72), 4 (22), 1 (6)
ZAP-70		
Positive, negative, unknown	12 (55), 2 (9), 8 (36)	11 (61), 2 (11), 5 (28)
Bone marrow involvement at transplantation (%; range)	10.0 (5.0, 80.0)	7.5 (5.0, 80.0)
Status at time of conditioning for transplant		
IF, CR, PR	1 (5), 3 (13), 18 (82)	1 (6), 3 (17), 14 (78)
Transplantation-related features		
Patient-donor sex-matched	11 (50%)	9 (50%)
Type of transplant		
MRD, URD	7 (32), 15 (68)	6 (33), 12 (67)
Time from diagnosis to transplantation (months; range)	71 (16, 280)	71 (16, 280)
Time from transplantation until first vaccination (days; range)	—	34.5 (31, 45)
Number of autologous tumor cell vaccinations after HSCT		
1–3	—	7 (39)
5–6	—	11 (61)

del, deletion; IF, induction failure.

progression (*n* = 20; 37%), enrollment obstacles (*n* = 8; 15%), or vaccine manufacturing failure (*n* = 4; 7%) (Figure 2). In total, 22 subjects (41%) were enrolled in the study for potential CLL/GM-K562 cell vaccination following RIC HSCT (clinical characteristics provided in Table 1). Seven of these patients were transplanted with matched related donors (MRDs) and 15 with matched unrelated donors (URDs). All patients had advanced disease, having undergone a median of 3 (range, 2–11) prior therapies. Furthermore, patients' leukemia cells frequently expressed biological markers associated with aggressive disease (23, 24). These included high expression of ZAP70 (16 of 22 subjects; 73%); unmutated immunoglobulin heavy-chain variable-region (*IGHV*) status (15 of 22 subjects, 68%); and high-risk cytogenetic features, including deletions in chromosomes 11q and 17p [sole del(11q), 9 of 22 (41%); sole del(17p), 5 of 22 (23%); del(11q and 17p), 4 of 22 (18%)] (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI69098DS1). Thirteen of 22 (59%) patients demonstrated persistent marrow disease involvement greater than or equal to 10% at the time of allo-HSCT (Supplemental Table 1).

Following HSCT, 4 of 22 (18%) study participants were precluded from vaccination due to the development of acute GvHD (aGvHD) before posttransplant day 45 (VAX0 group). The majority of patients (18 of 22; 82%) received a least one CLL/GM-K562 vaccine dose. Seven of 18 (39%) study participants received 1–3 (median 1) vaccines before developing aGvHD at a median of 49 days (range, 36–56) after HSCT, and thus did not continue to receive vaccines (VAX1–3 group). The remaining 11 of 18 (61%) vaccinated subjects received a median of 6 (range, 5–6) vaccines (VAX5–6 group) (Table 1 and Supplemental Table 1).

Posttransplant CLL/GM-K562 vaccines are safe and demonstrate promising clinical activity. Overall, the leukemia vaccines were well tolerated, but mild, transient erythema (lasting 2–16 days) at the injection sites was observed in at least 58% of the patients after each vaccine dose (Supplemental Table 2). Only 1 subject (Patient 3) developed a grade 4 event (neutropenia) with a possible attribution to autologous tumor cell vaccination, and vaccine treatment was subsequently stopped (Supplemental Table 3). At 1 year after HSCT, the incidence of grades II–IV aGvHD was similar between the 18 vaccinated subjects (39%; 95% CI, 17%–61%) and the 42 historical control CLL patients who underwent RIC allo-HSCT at our institution between 2004 and 2009 (31%; 95% CI, 18%–46%) (ref. 10 and Table 2). Similarly, we observed comparable incidence rates of 2-year cumulative chronic GvHD (cGvHD) between vaccinated study subjects (68%; 95% CI, 35%–87%) and historic controls from our center (63%; 95% CI, 48%–76%) (ref. 10 and Table 2).

The majority of patients who initiated vaccination (16 of 18; 89%) showed evidence of clinical response at 6 months after allo-HSCT. At this time, we observed 10 complete remissions (CRs) and 6 partial remissions (PRs). One of 18 patients had stable disease (SD) and 1 subject (Patient 9) who entered the trial with highly advanced disease initially responded to treatment with CR before disease relapse at 4 months after allo-HSCT (Supplemental Table 1). At a median follow-up of 2.9 years (range, 1–4 years), 13 of 18 patients (72%) have remained in continuous CR; 1 patient has maintained SD; 3 patients developed progressive disease (17%) at a median of 9 months (range, 4–15), 2 of whom died of disease at 10 and 16 months; and the cause of death in 1 patient is unknown

Table 2
Clinical outcome of the 22 study subjects entering the treatment phase of the study

	Eligible patients *n* (%)	Initiated vaccination *n* (%)
n	22	18
Developed aGVHD	12 (55)	9 (50)
Grade I	2 (9)	2 (11)
Grade II	9 (41)	7 (39)
Grades III–IV	1 (5)	0 (0)
Cumulative incidence of grades II–IV aGVHD at 1 year (%; 95% CI)	45 (24, 65)	39 (17, 61)
Median time to grades II–IV aGVHD (days; range)	46 (26, 188)	49 (36, 188)
Developed cGVHD	14 (64)	12 (67)
Limited	1 (5)	1 (6)
Extensive	13 (59)	11 (61)
Cumulative incidence of cGVHD at 2 years (%; 95% CI)	66 (38, 84)	68 (35, 87)
Median time to cGVHD (days; range)	314 (157, 756)	314 (157, 756)
Relapse	3 (14)	3 (17)
Death[A]	4 (18)	3 (17)
Overall survival at 2 years (%; 95% CI)	84 (58, 95)	88 (59, 97)
PFS at 2 years (%; 95% CI)	80 (54, 92)	82 (54, 94)

[A]Cause of death: disease (n = 2); severe GvHD (n = 1); unknown (n = 1) (see Supplemental Table 1).

(Supplemental Table 1). Overall, we observed an estimated 2-year progression-free survival (PFS) rate of 82% (95% CI, 54%–94%) and an overall survival rate of 88% (95% CI, 59%–97%) among the 18 vaccinated patients (Table 2).

Early posttransplant CLL/GM-K562 vaccination has a modest impact on T cell recovery. Donor-host chimerism analyses at 30 and 120 days after allo-HSCT revealed comparable donor engraftment of total PBMCs and $CD3^+$ T cells among VAX5–6, VAX1–3, and historical control patients who received RIC allo-HSCT for advanced CLL, but none of these patients received the vaccine or developed GvHD within the first 100 days after transplantation (Supplemental Table 4 and Supplemental Figure 1A). To determine the influence of early posttransplant CLL/GM-K562 vaccination on the kinetics of T cell immune reconstitution, we subjected freshly drawn peripheral blood samples to immunophenotyping at serial time points before and after HSCT between days 30 and 180. While the recovery of absolute T cell numbers and the balance between CD8 T cells and regulatory T cells ($CD4^+CD25^+CD127^-$) were not significantly altered in VAX5–6 or VAX1–3 patients compared with controls, we observed a differential expansion of $CD8^+$ versus $CD4^+$ T cells (VAX5–6 vs. control at day 60; P = 0.052) and an expansion of absolute effector memory T cell numbers (VAX5–6 vs. control at day 45; P = 0.041) in VAX5–6 patients between posttransplant days 45 and 60 (Supplemental Figure 1, B–E). On day 45, seven of eight VAX5–6 patients from whom samples were available for analysis had already received the first two vaccine doses and received the third dose before day 60.

Systemic tumor-specific T cell responses develop following CLL/GM-K562 vaccination. The presence and expansion of antigen-specific $CD8^+$ T cells have been associated with tumor regression following allo-HSCT or donor lymphocyte infusion (DLI) (13, 15, 25). We sought to determine whether tumor-specific $CD8^+$ T cell reactivity was enhanced by autologous tumor cell vaccination, beyond the effects of allo-HSCT alone. To this end, we established panels of cell targets for each study subject based on our current understanding of how the expression of target antigens can direct GvL or GvHD effects (26). These panels consisted of recipient tumor, recipient phytohemagglutinin-M–stimulated (PHA-stimulated) T cell blasts, and recipient skin fibroblasts and were used to test reactivity of serial pre- and post-HSCT patient $CD8^+$ T cell samples. As depicted in Figure 3A, reactivity against all three targets was interpreted to reveal reactivity directed predominantly against broadly expressed alloantigens. Reactivity limited to recipient tumor and PHA blasts was consistent with a response to alloantigens expressed on hematopoietic tissue, and reactivity restricted to autologous tumor was consistent with a tumor-specific response.

Figure 3B shows a representative example of the pattern of reactivity, detected by IFN-γ ELISpot, that we consistently observed among eight VAX5–6 patients whose T cell responses were tested (Figure 3C, left panel). In the example shown in Figure 3B, we detected $CD8^+$ T cell reactivity against autologous tumor cells, but not alloantigen-bearing recipient cells (PHA T cell blasts and fibroblasts), starting from posttransplant day 60 (after the patient received 3 of 6 vaccines). Among the eight VAX5–6 patients, a peak response against autologous tumor cells was reached at approximately day 60 after transplantation (days 60, 90, and 120: tumor vs. fibroblast $CD8^+$ T cell reactivity, P = 0.031, 0.031, and 0.031, respectively; day 60: tumor vs. PHA T cell blast $CD8^+$ T cell reactivity, P = 0.063) (Figure 3C, left panel). In contrast, circulating $CD8^+$ T cells isolated from control patients who underwent RIC allo-HSCT for advanced CLL but did not receive cancer vaccines or develop GvHD during the first 100 days following HSCT, demonstrated no increase in T cell reactivity against tumor-associated antigens or alloantigens within the observation period (day 60: VAX5–6 vs. control group $CD8^+$ T cell reactivity against autologous tumor, P = 0.035) (Figure 3C, right panel). Broad $CD8^+$ T cell reactivity, consistent with an alloantigen response, was observed in VAX1–3 study subjects who developed GvHD in the early posttransplant period (Figure 3C, middle panel). These results suggest that autologous tumor cell–based vaccination can potentially direct the specificity of reconstituting T cells against tumor antigens, even in the presence of immunosuppressive medication.

Polyfunctional tumor-reactive $CD8^+$ T cell responses are induced following CLL/GM-K562 cell vaccination. Recent studies have suggested that polyfunctional T cells, which produce multiple cytokines and chemokines in response to antigen stimulation, are a key indicator of the efficacy of antiviral and antitumoral cell–mediated immune responses (27, 28). In addition to characterizing IFN-γ responses (described above), we also performed simultaneous assessment of secreted GM-CSF, TNF-α, IL-2, and IL-10 from culture supernatants after 24-hour coculture of serial pre- and post-HSCT $CD8^+$ T cell samples with recipient leukemia cells or recipient alloantigen targets. Consistent with the IFN-γ ELISpot data, $CD8^+$ T cells isolated from VAX5–6 patients (n = 6) showed significantly increased reactivity and augmented secretion of the effector cytokines GM-CSF (posttransplant days 60, 90, and 120: VAX5–6

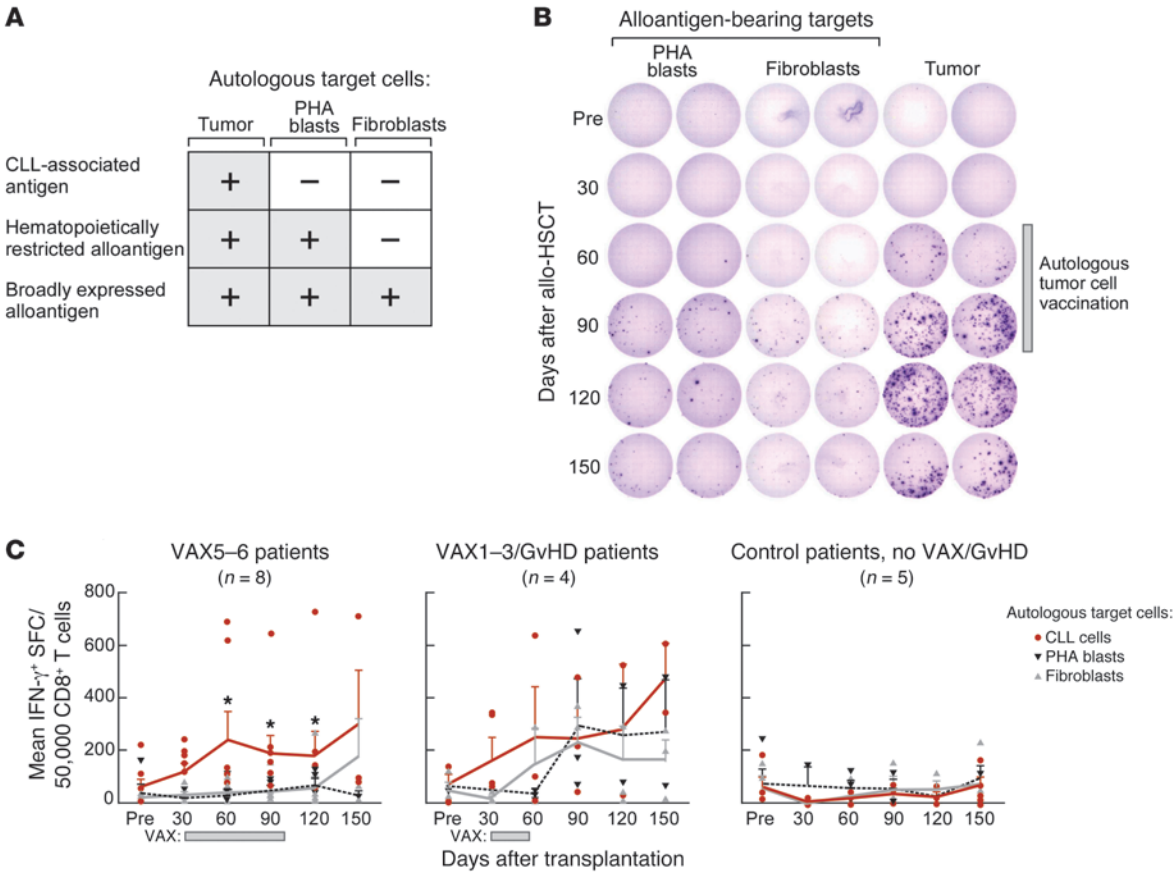

Figure 3

CLL-specific CD8+ T cell immunity evolving in CLL patients treated with autologous tumor cells early after allo-HSCT. (**A**) Schema of the target cell panel used to distinguish antigen specificities of responding T cells. (**B**) Representative ELISpot experiment examining serial pre- and post-HSCT CD8+ T cell samples obtained from a VAX5–6 patient (Patient 9) for reactivity against autologous tumor cells or alloantigen-bearing recipient cells (PHA T cell blasts and fibroblasts). (**C**) Depiction of the mean ± SEM tumor- or alloantigen-specific IFN-γ spot production of CD8+ T cells isolated from VAX5–6 (n = 8), VAX1–3/GvHD (n = 4), or control patients (with RIC allo-HSCT for advanced CLL, but without vaccine or GvHD within the first 100 days after transplantation; n = 5). Individual values are indicated by symbols. Bars denote the period of vaccine administration. *P < 0.05, tumor versus fibroblast CD8+ T cell reactivity; 2-sided Wilcoxon matched-pairs, signed-rank test. SFC, spot-forming cells.

vs. control group CD8+ T cell reactivity, P = 0.014, 0.028 and 0.052, respectively), TNF-α (days 60 and 90: VAX5–6 vs. control group, P = 0.035 and 0.052), and IL-10 (days 60, 90, and 120: VAX5–6 vs. control group, P = 0.040, 0.088 and 0.049; day 60: VAX5–6 vs. VAX1–3/GvHD group, P = 0.033) in response to stimulation with autologous CLL cells compared with CD8+ T cells obtained from VAX1–3/GvHD patients (n = 4) or control patients who received RIC allo-HSCT for advanced CLL but did not receive the vaccine or develop GvHD within the first 100 days after transplantation (n = 5) (Figure 4). Of note, VAX1–3/GvHD patients received additional systemic corticosteroids for GvHD therapy, which may have affected the cytokine secretion capability of the CD8+ T cells obtained from this patient cohort.

Concomitant with the peak response of VAX5–6 CD8+ T cells against autologous tumor cells in IFN-γ ELISpot assays (Figure 3), the amount of GM-CSF secreted by VAX5–6 CD8+ T cells in response to stimulation with tumor cells was highest on day 60 following HSCT and early posttransplant vaccination (Figure 4A). However, while CD8+ T cells isolated from VAX5–6 patients exhibit increased IFN-γ secretion in response to autologous tumor cells, but not to alloantigen-bearing recipient cells (Figure 3), this tumor-specific T cell reactivity pattern was not observed for the secretion of other effector cytokines. CD8+ T cells obtained from VAX5–6 patients responded to autologous CLL cells and PHA T cell blasts, but not to skin-derived fibroblasts, with the secretion of high levels of GM-CSF and TNF-α (posttransplant day 60: tumor vs. fibroblast CD8+ T cell reactivity, GM-CSF, P = 0.094; TNF-α, P = 0.031; IL-10, P = 0.063; day 90: GM-CSF, P = 0.063, TNF-α, P = 0.031) (Figure 4A and Supplemental Figure 2, A and D). These results suggest that vaccination induces a mixed CD8+ T cell population that is capable of conducting a broad variety of effector functions upon recognition of hematopoietically associated alloantigens or CLL-associated antigens.

Reactivity to CLL-specific targets is induced by CLL/GM-K562 cell vaccination. To confirm that a proportion of T cells induced by vaccination indeed recognizes antigens with expression restricted to CLL cells, we used limiting dilution to isolate CD8+ T cell clones from bulk tumor-reactive T cells of 4 vaccinated study subjects at a median of 140 (range, 96–172) days after allo-HSCT (Table 3). We subsequently tested these CD8+ T cell clones for reactivity

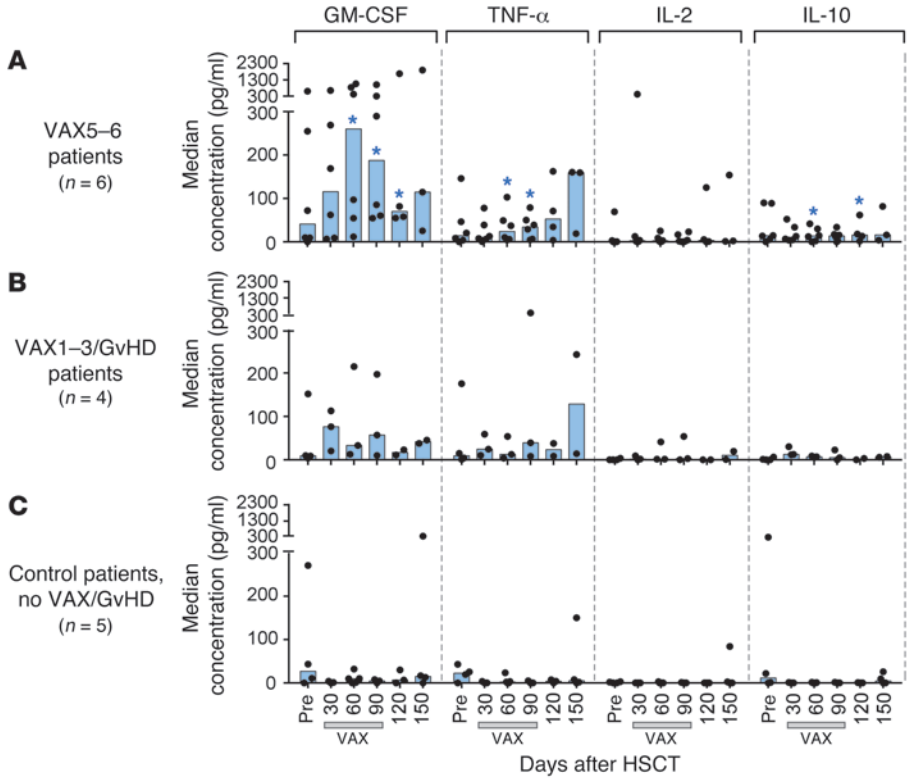

Figure 4
Polyfunctional tumor-reactive CD8+ T cell responses are induced following early posttransplant CLL/GM-K562 cell vaccination. Functional capacity of CD8+ T cells isolated at serial pre- and post-HSCT time points from (**A**) VAX5–6 (n = 6), (**B**) VAX1–3/GvHD (n = 4) study subjects, or (**C**) control patients (with RIC allo-HSCT for advanced CLL, but without vaccine or GvHD within the first 100 days after transplantation; n = 5) in response to autologous tumor cells was analyzed by simultaneous assessment of secreted GM-CSF, TNF-α, IL-2, and IL-10. Bars represent the median concentration of the corresponding cytokine in pg/ml. Individual values are depicted as black circles. *$P < 0.05$ (VAX5–6 vs. control group and VAX5–6 vs. VAX1–3/GvHD group CD8+ T cell reactivity; 2-sided Wilcoxon rank-sum test).

against recipient tumor, PHA T cell blasts, and fibroblasts to determine their antigen restriction (Figure 5). A median of 17% (range, 13%–33%) of the T cell clones solely reacted against CLL-specific antigens, 22% (range, 13%–35%) responded to recipient alloantigens with hematopoietic cell–restricted expression, and 4% (range, 0%–11%) of the T cell clones showed reactivity against alloantigens expressed by hematopoietic and nonhematopoietic recipient tissue (Table 3). These results indicate that early posttransplant autologous tumor cell–based vaccination induced reactivity to CLL-specific targets.

Discussion

As toxicities related to allogeneic transplantation improve through the development of RIC regimens and better supportive care, disease relapse has become the primary driver of treatment failure, especially in patients with more advanced hematologic malignancies. Strategies to augment GvL responses without exacerbating GvHD are of utmost importance if we are to advance the application of RIC allo-HSCT (11). To address this challenge, we devised a strategy in which patients with advanced CLL were vaccinated early after transplantation with irradiated autologous tumor cells together with GM-CSF as a cytokine adjuvant, with the goal of focusing the reconstituting donor T cells toward the elimination of malignant cells. We observed promising clinical activity and a favorable toxicity profile among the 18 vaccinated study subjects. While all study subjects were required to have a lymphadenopathy of less than 5 cm at the time of transplantation (a known feature associated with relative improved transplant outcome; refs. 9, 10), we also notably identified the emergence of polyclonal CLL-specific CD8+ T cell populations capable of a broad array of effector functions following this immunotherapeutic intervention. Consistent with prior studies demonstrating the induction of CLL-restricted T cells following RIC allo-HSCT (13), our studies now reveal that tumor cell vaccination can further expand this leukemia-specific population and thereby potentially improve outcomes after transplantation. Our findings thus demonstrate the feasibility of using immunotherapeutic strategies that aim to manipulate the specificity of T cell reactivity toward malignant cells following RIC allo-HSCT.

Our study adds to a growing body of clinical studies that established safety, feasibility, and biological activity of whole tumor cell–based vaccination for patients with hematologic malignancies (17, 29, 30). A number of features unique to our vaccination approach may have contributed to the observed expansion of leukemia-reactive CD8+ T cell responses. First, by using whole autologous tumor cells as the immunogen, our vaccine could potentially provide not only a broad range of tumor-associated or tumor-specific antigens to stimulate B and T cell responses, but also an immunogen content that is personalized. This is a favorable strategy, since only a few CLL-specific antigens are known, a high degree of molecular heterogeneity between individuals with CLL exists (31), and the complexity and diversity of HLA ensures vast immune epitope heterogeneity.

Second, the timing of vaccination following allo-HSCT may have been critical. The early weeks after transplantation have been conventionally regarded as a controversial platform for immunotherapy due to the immunosuppressive effect of GvHD prophylaxis and incomplete immune reconstitution, while later time periods have been demonstrated to be safe for tumor vaccination (32). Preclinical models, however, have demonstrated the potential to manipulate host antitumor immunity early after HSCT. In particular, early posttransplant immunization with whole tumor

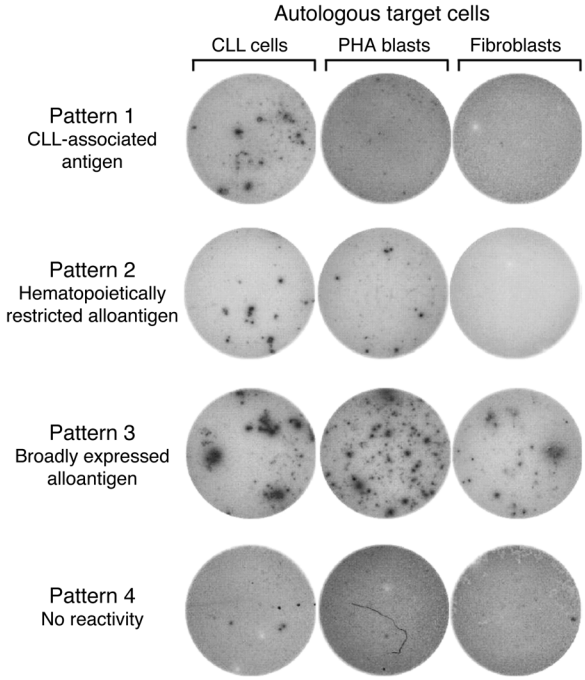

Figure 5
Reactivity patterns of T cell clones obtained from CLL/GM-K562–vaccinated patients. T cell clones obtained by limiting dilution from posttransplant tumor-reactive T cell pools show different patterns of reactivity on IFN-γ ELISpot assays, namely, (a) sole reactivity against CLL-associated antigens; (b) reactivity against hematopoietically restricted alloantigens; (c) reactivity against broadly expressed alloantigens; or (d) absent reactivity against the tested target cells.

cell vaccines resulted in sustained amplification and activation of tumor-specific T cell responses (18, 21). Furthermore, a recent phase I study demonstrated minimal toxicity and immunogenicity of tumor cell vaccination initiated between posttransplant days 30 and 45 in patients with high-risk acute myeloid leukemia or myelodysplasia, despite the administration of a calcineurin inhibitor–based GvHD prophylaxis regimen (17). This study, together with our current results, supports the idea that whole tumor–cell vaccination in the early posttransplant period can be beneficial. In particular, this approach takes advantage of donor T cells, which are undergoing rapid homeostatic expansion in the setting of lymphopenia after conditioning (20) and are not functionally compromised by immunosuppressive effects mediated through high tumor burden or chemotherapy. Although our study regimen had only a modest impact on recovering T cell subpopulations, reactivity of IFN-γ^+CD8$^+$ T cells from vaccinated patients was consistently directed against autologous tumor cells, but not alloantigen-bearing, nonmalignant recipient cells at the time of clinical response, demonstrating the specificity of our vaccine. Whether these observations translate into qualitatively improved immune recovery that in turn aids in immune competence and enhanced relapse control awaits further study.

Third, our vaccine incorporated GM-CSF as a cytokine adjuvant. The adjuvant effects of GM-CSF have been well demonstrated in a number of preclinical and clinical studies, and GM-CSF has become an attractive vaccine component because of its low-toxicity profile (16, 17, 29, 32–34). Irradiated autologous whole tumor cells modified to secrete GM-CSF as well as irradiated allogeneic GM-CSF–secreting vaccines derived from established tumor cell lines have been explored in clinical trials for multiple tumor types (17, 29, 32–34). Several studies have suggested that paracrine production of GM-CSF can alter the local microenvironment surrounding the tumor cell inoculum by stimulating the recruitment, maturation, and function of dendritic cells (17, 33–36), implying the augmentation of tumor antigen presentation. Despite its promise, this strategy was unavailable to lymphoid malignancies until the development of GM-CSF–secreting bystander cells (22), since primary human lymphoid tumor cells are poorly transducible using conventional methodologies. Hence, the GM-K562 cells that we used in this study represent a reliable and standardized source of GM-CSF that broadens the availability of this active cytokine adjuvant (37).

Recent studies have suggested that GM-CSF not only plays a critical role in the priming and effector phase of immune responses, but paradoxically, also serves a regulatory role in dampening responses and mediating immune homeostasis (38–41). We observed evidence of both pro- and antiimmune effects in vaccinated study subjects. On one hand, we observed a rise in circulating CLL-reactive CD8$^+$ T cells that secreted a broad profile of effector cytokines, including GM-CSF, TNF-α, IL-2, and IL-10, in response to autologous tumor cells, equipping the tumor-specific CD8$^+$ T cell population with multiple functions required for effective antitumor immunity (28). On the other hand, we also observed a decline in vaccine-associated T cell responses at day 90 within the vaccination cycle, even with vaccination continuation. At the same time, we did not observe an excessive incidence or severity of GvHD (grades I and II, but not grades III and IV aGvHD was observed among the 18 vaccinated study subjects). Thus, we speculate that in the early posttransplant setting, GM-CSF delivered by GM-K562 cells may play a beneficial role in balancing the stimulation of antitumor immunity without exacerbating GvHD.

In summary, our findings suggest that autologous whole tumor cell–based vaccination is an exportable strategy to tip the balance between leukemia-specific and alloantigen-reactive immunity in favor of GvL. Further randomized studies with expanded patient

Table 3
Reactivity to CLL-specific targets is induced by CLL/GM-K562 cell vaccination

Patient	5	6	9	12
Days after HSCT	130	149	96	172
Number of T cell clones (%)				
CLL-associated antigen	4 (17)	15 (33)	37 (18)	4 (13)
Hematopoietically restricted antigen	7 (29)	16 (35)	30 (15)	4 (13)
Broadly expressed antigen	0 (0)	5 (11)	2 (1)	2 (7)
No reactivity	13 (54)	10 (21)	137 (66)	20 (67)
Total	24 (100)	46 (100)	206 (100)	30 (100)

Specificity analysis of T cell clones isolated from 4 distinct vaccinated patients by IFN-γ ELISpot revealed that 13%–33% of the T cell clones responding to autologous tumor cells have reactivity restricted to autologous leukemia cell targets.

cohorts are required to determine whether these promising results of tumor-specific T cell activation will translate into a true clinical benefit for patients with advanced CLL.

Methods

Clinical trial. Patients were eligible for study enrollment if they had advanced CLL, defined as intolerance to fludarabine or no response, or relapse within 24 months of a fludarabine-containing regimen. Eligible subjects had an (8 of 8) HLA-matched related or unrelated donor available. $CD19^+CD5^+$ tumor cells were harvested for vaccine generation from peripheral blood, marrow, or lymph node, and were cryopreserved in aliquots of 1×10^7 tumor cells per vial under GMP conditions until the time of vaccine administration (DF/HCC Cell Processing Lab). Patients were eligible to undergo HSCT if they lacked sites of adenopathy greater than 5 cm. The conditioning regimen consisted of fludarabine (30 mg/m^2/day) and busulfan (0.8 mg/kg/12 hours) administered between pretransplant days –5 and –2. Donor PBSCs were harvested using G-CSF. The stem cell product consisted of a target cell dose of 5×10^6 $CD34^+$ cells/kg. GvHD prophylaxis consisted of methotrexate (5 mg/m^2 i.v. on days 1, 3, 6, and 11) and tacrolimus (at 0.05 mg/kg/p.o., b.i.d., adjusted to maintain levels between 5 to 10 ng/ml, starting on day –3). Between posttransplant days 30 and 45, patients were eligible for vaccination if they were neutrophil- and platelet-engrafted and lacked GvHD requiring systemic therapy. One vaccination cycle comprised 6 vaccine doses, each consisting of 1×10^7 autologous tumor cells admixed with 1×10^7 clinical-grade GM-K562 cells (Harvard Gene Therapy Initiative; see Supplemental Materials and Methods) irradiated at 100 Gy.

The CLL/GM-K562 vaccines were administered once a week for 3 doses and then every other week for 3 doses. At the time of vaccination, GM-K562 cells and autologous tumor were thawed in a 37°C water bath, washed, irradiated at 100 Gy, and resuspended in 1 milliliter of sterile saline. Vaccines were administered to the patient's arms or thighs on a rotating basis. Half of the vaccine dose was administered subcutaneously and half intradermally. Vaccination was halted if there was any evidence of disease relapse or progression requiring additional therapy, grades II–IV aGvHD, extensive chronic GvHD requiring systemic steroid therapy, or any grade 4 toxicity. Two patients initiated a second vaccine cycle, analogous to the first cycle, at days 207 and 271 after allo-HSCT, respectively.

Patient samples. Heparinized blood was obtained from study participants, normal volunteers, and control group patients enrolled under IRB-approved protocols at the Dana-Farber Cancer Institute. Clinical characteristics and transplant-related features of the control patient cohort are provided in Supplemental Table 4. PBMCs were isolated from whole blood specimens collected from study subjects with Ficoll-Paque PLUS (GE Healthcare) and stored in 10% DMSO in vapor-phase liquid nitrogen until further use. Clinical factor, chimerism, and immunophenotyping analyses were preformed using standard methods as described in the Supplemental Materials and Methods.

Generation of tissue targets for detection of T cell responses. $CD5^+CD19^+$ tumor cells collected from patients were activated for 48 hours using CD40L-Tri (0.5 μg/ml) (42) in Iscove's modified Dulbecco's medium (IMDM) (Life Technologies) supplemented with 10% human AB serum (Gemini Bioproducts), 15 μg/ml gentamycin, and 2 ng/ml IL-4 (R&D Systems). Recipient PHA T cell blasts were generated by exposing recipient pre-HSCT PBMCs to 1% PHA (Life Technologies) in IMDM supplemented with 10% human AB serum and 10 mM HEPES (Life Technologies) for 72 hours and by subsequent expansion of the cells in the presence of 100 U/ml IL-2 (R&D Systems). Recipient fibroblast lines were generated by the Cell Culture Core lab of the Harvard Skin Disease Research Center from 5-mm-diameter skin punch biopsies and subsequently cultured in a 1:1 mixture of M199 and M106 medium containing 15% FBS, 10 ng/ml EGF, and 0.4 μg/ml hydrocortisone (EMD Millipore), as described (15).

Detection of T cell responses. T cell responses were detected in cryopreserved CLL patient PBMC samples. PBMCs were thawed, seeded at 2×10^6 cells per well, and stimulated with 0.5×10^6 CD40L-activated irradiated CLL B cells per well and 10 ng/ml IL-7 (R&D Systems) for 1 week. IL-2 (100 U/ml; R&D Systems) was added on day 3 of culture. $CD8^+$ T cells were isolated by magnetic cell sorting (Miltenyi Biotec) according to the manufacturer's instructions and rested overnight in IMDM supplemented with 20% FBS. ELISpot assays were performed using three different sources of irradiated autologous target cells (1×10^5 cells per well): recipient CD40L-activated CLL B cells, recipient PHA T cell blasts, or recipient fibroblasts, which were coincubated with 1×10^5 $CD8^+$ T cells per well in duplicate on ELISpot plates (EMD Millipore) for 24 hours. IFN-γ secretion was detected using capture (15 μg/ml) and detection (1 μg/ml) antibodies (Mabtech AB) and imaged on an ELISpot reader (ImmunoSpot Series Analyzer; Cellular Technology). For some study subjects, bulk $CD8^+$ T cells were cloned by limiting dilution on feeder cells (irradiated allogeneic PBMCs [and 5:1 autologous EBV cells (15) in the case of T cells obtained from Patient 5] with 100 U/ml recombinant human IL-2 and 1% PHA) after confirming their CLL reactivity by IFN-γ ELIspot assays as described above. Following expansion, we performed ELISpot assays to test the clones (10^4 cells per well) for reactivity against irradiated CD40L-activated CLL B cells, PHA T cell blasts, or fibroblasts (5×10^5 cells per well).

Multiplexed cytokine analyses. We thawed and presensitized cryopreserved serial pre- and post-HSCT T cells in vitro with autologous tumor for 7 days, as described above. Subsequently, we restimulated samples overnight with a panel of tissue targets (described above). Supernatants were collected and cryopreserved at –80°C until analysis. We analyzed culture supernatants as well as standards in duplicates for various cytokines (GM-CSF, TNF-α, IL-2, and IL-10) using multiplex bead–based Luminex technology (Milliplex Map human cytokine/chemokine kit; EMD Millipore) according to the manufacturer's recommendations. In brief, microspheres, each with a unique spectral signature, were coated with specific cytokine capture antibodies. After incubation with the culture supernatant sample, we used a biotinylated detection antibody followed by a streptavidin-PE conjugate to detect captured cytokines. The fluorescence of each bead was measured with a Luminex 200-bead array instrument (Luminex), and the median fluorescence intensity for each cytokine was recorded. We calculated cytokine levels against the standards using Upstate BeadView software (EMD Millipore). Values below the minimal detectable concentration (MinDC) given by the manufacturer were set at 0 pg/ml (MinDC plus 2SD for GM-CSF, TNF-α, IL-2, and IL-10 is 0.1, 18.9, 0.6, and 0.5 pg/ml, respectively).

Statistics. A two-sided Wilcoxon rank-sum test was used to test for differences in donor chimerism, recovery of immune cell subpopulations, and T cell activation status, as well as for differences in the amount of secreted effector cytokines (IFN-γ, GM-CSF, TNF-α, IL-2, and IL-10) from $CD8^+$ T cells in response to stimulation with autologous tumor cells, PHA T cell blasts, or fibroblasts between the three patient cohorts. To test for differences in effector cytokine secretion from $CD8^+$ T cells obtained from VAX5–6, VAX1-3/GvHD, or control group patients in response to the three different target cell types, we used a 2-sided Wilcoxon signed-rank test. Differences were considered significant if P was less than 0.05. No adjustments were made for multiple comparisons.

Overall survival (OS) was calculated as the time from RIC allo-HSCT to death from any cause using the Kaplan-Meier method; those patients who were alive were censored at the date of their last follow-up visit. PFS was calculated from the time of HSCT to disease relapse, progression, or death from any cause, whichever occurred first and was censored at the date of

the last follow-up visit using the Kaplan-Meier method. CR, PR, and SD were assessed by complete blood counts, CT scans, and bone marrow biopsy evaluation and were defined according to the National Cancer Institute Working Group criteria for CLL (43). The cumulative incidence of acute or chronic GvHD was calculated from the time of HSCT to the time of GvHD with relapse/progression and death included as competing events.

Study approval. Our phase I clinical trial received approval from the IRB and biosafety committees of the Dana-Farber Cancer Institute/Harvard Cancer Center, the National Institute of Health Recombinant DNA Advisory Committee (NIH RAC), and the FDA, and was registered at www.clinicaltrials.gov (NCT00442130). Informed written consent was obtained from all participants.

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Address correspondence to: Catherine J. Wu, Dana-Farber Cancer Institute, Harvard Institutes of Medicine, Room 420, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA. Phone: 617.632.5943; Fax: 617.632.3351; E-mail: cwu@partners.org.

1. Horowitz MM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990;75(3):555–562.
2. Wu CJ, Ritz J. Induction of tumor immunity following allogeneic stem cell transplantation. *Adv Immunol*. 2006;90:133–173.
3. Brown JR, et al. Predictors of improved progression-free survival after nonmyeloablative allogeneic stem cell transplantation for advanced chronic lymphocytic leukemia. *Biol Blood Marrow Transplant*. 2006;12(10):1056–1064.
4. Dreger P, et al. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood*. 2010;116(14):2438–2447.
5. Shea T, et al. Reduced-intensity allogeneic transplantation provides high event-free and overall survival in patients with advanced indolent B cell malignancies: CALGB 109901. *Biol Blood Marrow Transplant*. 2011;17(9):1395–1403.
6. Dreger P, et al. Reduced-intensity conditioning lowers treatment-related mortality of allogeneic stem cell transplantation for chronic lymphocytic leukemia: a population-matched analysis. *Leukemia*. 2005;19(6):1029–1033.
7. Khouri IF, et al. Nonmyeloablative allogeneic stem cell transplantation in relapsed/refractory chronic lymphocytic leukemia: long-term follow-up, prognostic factors, and effect of human leukocyte histocompatibility antigen subtype on outcome. *Cancer*. 2011;117(20):4679–4688.
8. Schetelig J, et al. Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European Group for Blood and Marrow Transplantation analysis. *J Clin Oncol*. 2008;26(31):5094–5100.
9. Sorror ML, et al. Five-year follow-up of patients with advanced chronic lymphocytic leukemia treated with allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *J Clin Oncol*. 2008;26(30):4912–4920.
10. Brown JR, et al. Long-term follow-up of reduced-intensity allogeneic stem cell transplantation for chronic lymphocytic leukemia: prognostic model to predict outcome. *Leukemia*. 2013;27(2):362–369.
11. Porter DL, et al. NCI First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Report from the Committee on Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant*. 2010;16(11):1467–1503.
12. Marina O, et al. Serologic markers of effective tumor immunity against chronic lymphocytic leukemia include nonmutated B-cell antigens. *Cancer Res*. 2010;70(4):1344–1355.
13. Nishida T, et al. Development of tumor-reactive T cells after nonmyeloablative allogeneic hematopoietic stem cell transplant for chronic lymphocytic leukemia. *Clin Cancer Res*. 2009;15(14):4759–4768.
14. Biernacki MA, et al. Novel myeloma-associated antigens revealed in the context of syngeneic hematopoietic stem cell transplantation. *Blood*. 2012; 119(13):3142–3150.
15. Zhang W, et al. Graft-versus-leukemia antigen CML66 elicits coordinated B-cell and T-cell immunity after donor lymphocyte infusion. *Clin Cancer Res*. 2010;16(10):2729–2739.
16. Dranoff G, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A*. 1993; 90(8):3539–3543.
17. Ho VT, et al. Biologic activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. *Proc Natl Acad Sci U S A*. 2009;106(37):15825–15830.
18. Borrello I, Sotomayor EM, Rattis FM, Cooke SK, Gu L, Levitsky HI. Sustaining the graft-versus-tumor effect through posttransplant immunization with granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing tumor vaccines. *Blood*. 2000;95(10):3011–3019.
19. Gattinoni L, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. *J Exp Med*. 2005;202(7):907–912.
20. Mirmonsef P, et al. Escape from suppression: tumor-specific effector cells outcompete regulatory T cells following stem-cell transplantation. *Blood*. 2008;111(4):2112–2121.
21. Teshima T, et al. Tumor cell vaccine elicits potent antitumor immunity after allogeneic T-cell-depleted bone marrow transplantation. *Cancer Res*. 2001; 61(1):162–171.
22. Brusic A, et al. Detecting T-cell reactivity to whole cell vaccines: Proof of concept analysis of T-cell response to K562 cell antigens in CML patients. *Oncoimmunology*. 2012;1(7):1095–1103.
23. Dohner H, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000; 343(26):1910–1916.
24. Rassenti LZ, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med*. 2004;351(9):893–901.
25. Marijt WA, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. *Proc Natl Acad Sci U S A*. 2003; 100(5):2742–2747.
26. Wu CJ, Ritz J. Revealing tumor immunity after hematopoietic stem cell transplantation. *Clin Cancer Res*. 2009;15(14):4515–4517.
27. Betts MR, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood*. 2006;107(12):4781–4789.
28. Perales MA, et al. Phase I/II study of GM-CSF DNA as an adjuvant for a multipeptide cancer vaccine in patients with advanced melanoma. *Mol Ther*. 2008; 16(12):2022–2029.
29. Borrello IM, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cellular immunotherapy in combination with autologous stem cell transplantation (ASCT) as postremission therapy for acute myeloid leukemia (AML). *Blood*. 2009;114(9):1736–1745.
30. Rousseau RF, et al. Immunotherapy of high-risk acute leukemia with a recipient (autologous) vaccine expressing transgenic human CD40L and IL-2 after chemotherapy and allogeneic stem cell transplantation. *Blood*. 2006;107(4):1332–1341.
31. Wang L, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011;365(26):2497–2506.
32. Higano CS, et al. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. *Cancer*. 2008;113(5):975–984.
33. Jaffee EM, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol*. 2001; 19(1):145–156.
34. Soiffer R, et al. Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J Clin Oncol*. 2003;21(17):3343–3350.
35. Kielian T, Nagai E, Ikubo A, Rasmussen CA, Suzuki T. Granulocyte/macrophage-colony-stimulating factor released by adenovirally transduced CT26 cells leads to the local expression of macrophage

inflammatory protein 1alpha and accumulation of dendritic cells at vaccination sites in vivo. *Cancer Immunol Immunother*. 1999;48(2–3):123–131.
36. Mach N, Gillessen S, Wilson SB, Sheehan C, Mihm M, Dranoff G. Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand. *Cancer Res*. 2000;60(12):3239–3246.
37. Borrello I, Sotomayor EM, Cooke S, Levitsky HI. A universal granulocyte-macrophage colony-stimulating factor-producing bystander cell line for use in the formulation of autologous tumor cell-based vaccines. *Hum Gene Ther*. 1999;10(12):1983–1991.
38. Jinushi M, Hodi FS, Dranoff G. Enhancing the clinical activity of granulocyte-macrophage colony-stimulating factor-secreting tumor cell vaccines. *Immunol Rev*. 2008;222:287–298.
39. Serafini P, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res*. 2004; 64(17):6337–6343.
40. Filipazzi P, et al. Identification of a new subset of myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine. *J Clin Oncol*. 2007; 25(18):2546–2553.
41. Jinushi M, Nakazaki Y, Dougan M, Carrasco DR, Mihm M, Dranoff G. MFG-E8-mediated uptake of apoptotic cells by APCs links the pro- and anti-inflammatory activities of GM-CSF. *J Clin Invest*. 2007;117(7):1902–1913.
42. Naito M, et al. CD40L-Tri, a novel formulation of recombinant human CD40L that effectively activates B cells. *Cancer Immunol Immunother*. 2013; 62(2):347–357.
43. Cheson BD, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*. 1996;87(12):4990–4997.