REGULAR ARTICLE

blood advances

GM-CSF secreting leukemia cell vaccination for MDS/AML after allogeneic HSCT: a randomized, double-blinded, phase 2 trial

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Key Points

- GVAX vaccination early after allogeneic HSCT was well tolerated but did not improve long-term disease-free survival after transplantation.
- This study highlights the challenges of conducting planned early posttransplant intervention trials after allogeneic HSCT.

Vaccination using irradiated, adenovirus transduced autologous myeloblasts to secrete granulocyte–macrophage colony-stimulating factor (GVAX) early after allogeneic hematopoietic stem cell transplantation (HSCT) can induce potent immune responses. We conducted a randomized phase 2 trial of GVAX after HSCT for myelodysplastic syndrome with excess blasts or relapsed/refractory acute myeloid leukemia. Myeloblasts were harvested before HSCT to generate the vaccine. Randomization to GVAX vs placebo (1:1) was stratified according to disease, transplant center, and conditioning. Graft-versus-host disease (GVHD) prophylaxis included tacrolimus and methotrexate. GVAX or placebo vaccination was started between day 30 and 45 if there was engraftment and no GVHD. Vaccines were administered subcutaneously/intradermally weekly × 3, then every 2 weeks × 3. Tacrolimus taper began after vaccine completion. A total of 123 patients were enrolled, 92 proceeded to HSCT, and 57 (GVAX, n = 30; placebo, n = 27) received at least 1 vaccination. No Common Toxicity Criteria grade 3 or worse vaccine-related adverse events were reported, but injection site reactions were more common after GVAX (10 vs 1; $P = .006$). With a median follow-up of 39 months (range, 9-89 months), 18-month progression-free survival, overall survival, and relapse incidence were 53% vs 55% ($P = .79$), 63% vs 59% ($P = .86$), and 30% vs 37% ($P = .51$) for GVAX and placebo, respectively. Nonrelapse mortality at 18 months was 17% vs 7.7% ($P = .18$), grade II to IV acute GVHD at 12 months was 34% vs 12% ($P = .13$), and chronic GVHD at 3 years was 49% vs 57% for GVAX and placebo ($P = .26$). Reconstitution of T, B, and natural killer cells was not decreased or enhanced by GVAX. There were no differences in serum major histocompatibility chain-related protein A/B or other immune biomarkers between GVAX and placebo. GVAX does not improve survival after HSCT for myelodysplastic syndrome/acute myeloid leukemia. This trial was registered at www.clinicaltrials.gov as #NCT01773395.

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Requests for data sharing may be submitted to Vincent T. Ho (Vincent_Ho@dfci.harvard.edu)

The full-text version of this article contains a data supplement.

Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is a potentially curative treatment option for patients with advanced myeloid malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). alloHSCT is, at its core, a form of immunotherapy as it relies on the "graft-versus-leukemia" effect mediated by the new donor-derived immune system. The field of transplantation has advanced, with improved HLA typing, less toxic conditioning regimens, superior graft-versus-host disease (GVHD) prophylaxis strategies, supportive care, and new antimicrobial treatments/surveillance, and thus transplant-related mortality has declined significantly over the last few decades.[1] Disease relapse has now emerged as the most prominent cause of transplant failure, especially in patients with high-risk myeloid malignancies.

A potential strategy to reduce relapse is to administer leukemia-specific vaccinations after transplant in hopes that the vaccination would stimulate/accelerate the development of cancer-specific immunity from the new donor immune system. Leukemia vaccination early after HSCT should also capitalize on the lymphopenic milieu created by the preparative regimen and surge of homeostatic cytokines such as interleukin-7 (IL-7), IL-12, and IL-15 that would be favorable toward activating immune responses.[2-5]

At our institution, we have previously shown that granulocyte–macrophage colony-stimulating factor (GM-CSF) production by whole-cell vaccines stimulates adaptive anticancer immune responses by inducing myeloid differentiation and dendritic cell cross-priming, and that vaccination with irradiated tumor cells engineered to secrete GM-CSF, collectively known as GVAX, stimulates potent, specific, and long-lasting antitumor immunity.[6] Phase 1/2 clinical trials of autologous GVAX vaccinations have reported tumor-specific immune responses in patients with melanoma, non–small cell lung cancer, and MDS/AML.[7-10] GVAX immune responses have been correlated with enhanced antigen presentation by recruited dendritic cells and macrophages, as well as improved coordinated cellular and humoral immunity by $CD4^+$, $CD8^+$ T lymphocytes, CD1a restricted natural killer (NK) cells, and B lymphocytes.[6,11-14] In murine transplant models, Teshima et al[15] have shown that GVAX elicits potent tumor-specific immunity when given 6 weeks after allogeneic transplantation.

We previously reported a pilot clinical trial testing the feasibility and safety of GVAX early after reduced-intensity conditioning (RIC) alloHSCT in patients with active MDS/refractory anemia with excess blasts or relapsed/refractory AML.[16] In this study, GVAX vaccination was well tolerated and did not elicit severe acute or chronic GVHD. Despite undergoing an RIC transplantation with active disease, 10 of 15 patients who started GVAX vaccination after transplant had durable responses, and 9 of 10 patients who completed all 6 vaccinations within the first 100 days achieved sustained long-term complete remissions. We also showed that immune responses in survivors correlated with declining levels of soluble major histocompatibility chain-related protein A and protein B (MICA and MICB, respectively) and antibody responses to a variety of antiangiogenic cytokines, including angiopoietin 1 and angiopoietin 2.[16,17]

Given these encouraging results, we conducted a follow-up study and hereby report the results of the phase 2, multicenter, randomized, double-blinded clinical trial testing GVAX vs placebo vaccination early after alloHSCT in patients with MDS–excess blasts and relapsed/refractory AML.

Patients, materials, and methods

Patients

The clinical protocol was approved by the Scientific Review Committee, Biosafety Committee, the Institutional Review Board of the Dana-Farber/Harvard Cancer Center, and the US Food and Drug Administration (Investigational New Drug Application #17904; ClinicalTrials.gov identifier #NCT01773395). Informed consent was obtained from all subjects per the Declaration of Helsinki. This study enrolled patients at 3 transplant centers: Dana-Farber Cancer Institute/Brigham Women's Cancer Center, Massachusetts General Hospital, and Beth Israel Deaconess Medical Center. Patients were eligible for study enrollment if they were deemed to be an appropriate candidate for either myeloablative conditioning (MAC) or RIC HSCT and met all of the following criteria: age ≥18 years; MDS–refractory anemia with excess blasts or relapsed or refractory AML not in remission (defined as ≥5% marrow blast or ≥5% circulating blasts); available 8/8 or better matched related or unrelated donor (according to high-resolution typing) at HLA-A, HLA-B, HLA-C, and HLA-DRB1; and Eastern Cooperative Oncology Group performance status 0 to 2. Patients with uncontrolled infection, active central nervous system leukemic involvement, HIV positivity, or inadequate organ function (serum creatinine ≥2.0 mg/dL; alanine aminotransferase or aspartate aminotransferase ≥3 times the upper limit of normal; total bilirubin ≥2.0 mg/dL) were excluded. After enrollment, study subjects underwent leukemia cell harvests for GVAX vaccine generation via marrow aspiration, or peripheral blood draw with the goal of obtaining a minimum of 2×10^7 total myeloblasts. For subjects randomized (1:1 randomization) to the GVAX arm, harvested blasts were subjected to GVAX manufacture as detailed in the following section. Randomization was stratified according to disease, transplant center, conditioning intensity, and the intent of RIC (reduced intensity dose of busulfan/ fludarabine) vs MAC (myeloablative dose of busulfan/fludarabine) HSCT had to be declared at the time of enrollment. Patients were allowed to receive chemotherapy for treatment of their MDS or AML after vaccine blast harvest and before alloHSCT, at the discretion of the treating physician.

GVAX and placebo vaccine preparation

Myeloblasts harvested from the recipients randomized to receive GVAX were delivered to the Cell Manipulation Core Facility at the Dana-Farber Cancer Institute and introduced into short-term tumor culture in the presence of granulocyte colony-stimulating factor (G-CSF). Leukemic cells were transduced with a replication defective adenoviral vector encoding human GM-CSF, as previously reported.[7,18] After transduction, the tumor cells were washed and irradiated with 10 000 cGy to abolish its ability to proliferate but retain its ability to secrete GM-CSF. A small aliquot of the transduced cells was placed into culture for ~24 hours. Supernatant was harvested, and GM-CSF secretion was measured by using enzyme-linked immunosorbent assay. Routine sterility cultures and testing for endotoxin and mycoplasma contamination were performed before release for administration. Tumor cells for vaccination were cryopreserved and stored in liquid nitrogen. Six individual vaccine aliquots were prepared for each patient. Cell dose per aliquot was fixed for an individual patient, and the dosage was determined

by dividing the total cell yield following transduction into 6 aliquots. For total cell yields $>6 \times 10^7$, individual aliquots were capped at 1×10^7 cells per dose. For patients randomized to receive placebo, the harvest myeloblasts were stored for future research, and the placebo vaccine was made with a saline solution. To maintain the blinding for the study staff and patient, all vaccine/placebo syringes were covered with an opaque tape to mask the slight turbid appearance of the GVAX vaccine vs the clear saline placebo.

Allogeneic HSCT

The preparative regimen for RIC HSCT consisted of fludarabine 30 mg/m^2 per day IV $\times$ 4 (total 120 mg/m^2) and busulfan 0.8 mg/kg IV every 12 hours $\times$ 8 (total 6.4 mg/kg) from day −5 to −2. The MAC preparative regimen consisted of fludarabine 30 mg/m^2 per day IV $\times$ 4 (total 120 mg/m^2) and busulfan 0.8 mg/kg IV every 6 hours $\times$ 16 (total 12.8 mg/kg) from day −5 to −2. Unmanipulated G-CSF–mobilized peripheral blood stem cell or marrow product (at the discretion of the transplant physician) was infused on day 0. GVHD prophylaxis included tacrolimus starting day −3 (target serum trough level, 5-10 ng/mL) and "mini"-methotrexate 5 mg/m^2 on days 1, 3, 6, and 11. Taper of tacrolimus was allowed starting ~4 weeks after completion of GVAX vaccinations (approximately day 120). GM-CSF (Leukine, Partner Therapeutics) 250 mg/m^2 subcutaneously once daily was administered from day 12 until neutrophil engraftment. Infection prophylaxis included acyclovir for herpes simplex virus/varicella zoster virus and trimethoprim sulfamethoxazole or atovaquone for *Pneumocystis jirovecii* infection. Systemic antifungal prophylaxis was not routinely given. Cytomegalovirus (CMV) management after transplantation followed a pre-emptive treatment strategy, with weekly CMV viral load monitoring until day 100. Restaging bone marrow aspirate and biopsy were performed at ~30 days after HSCT, before initiation of GVAX or placebo vaccination. No planned posttransplant maintenance therapy was allowed on this study.

Vaccination administration

GVAX or placebo vaccination was initiated between day 30 and 45 after HSCT if the following criteria were met: no grade II to IV acute GVHD requiring systemic steroids; no uncontrolled acute infection; adequate hematologic recovery with an absolute neutrophil count $>500/\mu L$ off growth factors; platelet count >10 K/μL without transfusion; and no Common Toxicity Criteria (version 4.0) grade 3 or worse nonhematologic toxicity.

Patients not meeting the aforementioned criteria to start vaccination by day 45 after HSCT were removed from the study. Patients with persistent or progressive disease at day 30 were eligible to start vaccinations if there was no plan to administer cytoreductive therapy or accelerate the tacrolimus taper. A total of 6 vaccinations were planned. GVAX/placebo was administered as an intradermal/subcutaneous injection on the patient's limbs (on a rotating basis) weekly for the first 3 vaccinations, and every other week for vaccines 4 to 6. With this schedule, all vaccinations were to be completed before day 108 post-SCT. Patients remained on therapeutic dosing of tacrolimus to maintain trough serum levels between 5 and 10 ng/mL during the vaccination period. Taper of tacrolimus was allowed after vaccine completion. Vaccination was stopped if there was rapidly progressive disease requiring cytotoxic therapy and/or rapid tacrolimus withdrawal, unexpected severe toxicity, or if acute GVHD developed/progressed that required initiation of systemic corticosteroid therapy.

Evaluation of toxicity and disease responses

Patients were monitored for local and systemic adverse reactions with weekly to twice weekly examinations and laboratory studies during the study period. Acute GVHD was graded according to the Keystone criteria.[19] Non-GVHD adverse events were reported according to the National Cancer Institute Common Toxicity Criteria version 4 guidelines. Disease responses were assessed by marrow aspiration and biopsies performed on the day of starting vaccine 1, one month after the last vaccination, and at 12 and 18 months after HSCT. Long-term follow-up beyond month 18 was conducted according to standard clinical care practice.

Assessment of biologic responses

Blood and marrow specimens were collected serially for biologic correlative research assessments on all patients enrolled in this study. Blood specimens were collected before HSCT, at the time of vaccine 1, monthly during the vaccination period, 1 month after the last vaccine, and at 6, 12, and 18 months after transplant.

Monitoring immune reconstitution after alloHSCT by flow cytometry. Peripheral blood samples were obtained at all the time points listed earlier to monitor recovery of CD3$^+$ T cells, CD4$^+$ conventional T cells (Tcon), CD4$^+$CD25$^+$CD127^{low} regulatory T cells (Treg), CD8$^+$ T cells, CD19$^+$ B cells, CD56$^+$ NK cells, and Treg/Tcon ratio. Immune phenotyping was performed by multicolor flow cytometry using directly conjugated monoclonal antibodies. Labeled cells were acquired in a FACSCanto II or LSRFortessa flow cytometer (BD Biosciences) and analyzed by using FACSDiva (BD Biosciences) or FlowJo software (Tree Star). Methods for staining, gating, and analysis strategies have been described previously.[20,21]

Detection of biomarkers associated with immune responses. Our previous pilot study had shown that long-term survivors after completing GVAX vaccinations had a decline in levels of circulating MICA and MICB in their blood, as well as development of antibodies against a variety of angiogenic cytokines that appeared to correlate with their response to vaccinations.[16] We therefore used Luminex kits (Bio-Techne Inc.) to assess for MICA and MICB along with an extended panel of markers that have been correlated with angiogenic cytokines, T-cell responses, NK cell status, soluble checkpoint markers, and neutrophilic chemokines, including Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2), CX3CL1, interferon-γ, 4-1BB, CD25, PDL1, IL-6, CXCL10, IL-8, G-CSF, IL-2, progranulin, hepatocyte growth factor, IL-1b, Tie2, IL12p70, IL-10, CCL4, CCL2, CXCL6, CXCL5, CXCL2, platelet-derived growth factor, and vascular endothelial growth factor. Assessments of these biomarkers were performed on banked plasma samples at various time points before HSCT, after transplant, and after vaccinations. Researchers performing the assays were blinded to the study arm assignment and clinical outcomes. Samples were run by using the Luminex FlexMap 3D platform; median fluorescent intensity values were extrapolated to standard curves for quantification, as previously reported.[22,23]

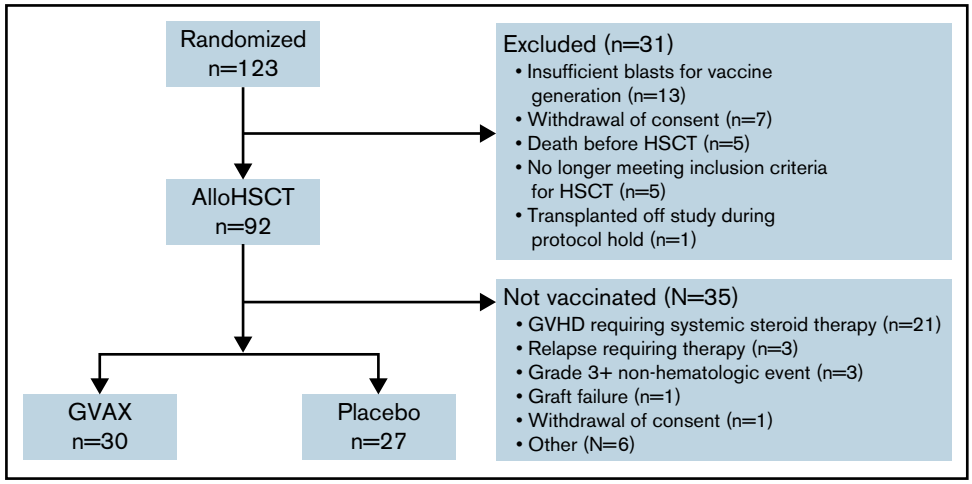

Figure 1. **Study flow diagram.**

Statistical analyses

Based on historical data and our previous study result,[16] we had projected sample size on the premise that the 18 months' progression-free survival (PFS) would be 26% in the placebo arm and 46% in the GVAX arm. Upon this assumption, the original target accrual goal for this trial was to have 106 patients starting vaccination, 53 per each arm and followed up for an additional 18 months. Using a 2-component cure rate for the null and a 3-component cure rate model for the alternative hypothesis,[24] the study would have 80% power to detect a 20% difference in PFS. The study protocol also included planned interim analysis for efficacy annually starting at 33% information time, and the interim results were reported annually to the Data and Safety Monitoring Board. At one of these annual planned interim analyses at the midway point of the study, no difference was found in the primary outcome, and it became increasingly clear that it would be futile to continue. Per the Data and Safety Monitoring Board recommendation, the study was terminated after 57 patients were vaccinated.

Baseline characteristics were reported descriptively and compared by using Fisher's exact test, χ^2 test, or Wilcoxon rank sum test, as appropriate. The primary end point was PFS; other end points of interest included overall survival (OS), relapse, and nonrelapse mortality (NRM). All time-to-event end points were measured from stem cell infusion to death (OS, NRM) or death or relapse (PFS, relapse). Patients who had persistent or relapsed disease after transplant but entered complete remission after vaccination and scheduled immune suppression taper were not considered as a treatment failure. OS and PFS were estimated by using the Kaplan-Meier method, and the log-rank test was used for group comparisons. Cumulative incidences of NRM and relapse were estimated in the competing risks framework considering relapse and NRM as a competing event, respectively; the Gray test was used for group comparison of cumulative incidences. Univariable and multivariable Cox regression analyses were performed to examine factors that are associated with PFS and OS. For the multivariable model, high-risk features or factors that were associated with $P < .1$ from univariable models were included. Risk factors considered in the regression analysis included treatment arm, age, patient sex, patient and donor sex combination, graft source, donor HLA type, conditioning intensity, sirolimus use as GVHD prophylaxis, disease status at alloHSCT, patient–donor CMV serostatus, HCT-CI score, and year of transplant. Before modeling, the linearity and proportional hazards assumptions and two-way interactions with the study were examined. For comparison of laboratory parameters, the Wilcoxon rank sum test was used. Multiplicity was not considered.

All *P* values were two-sided, and the significance level was set to .05. All analyses were performed by using SAS version 9.4 (SAS Institute, Inc.), and R version 3.6.1 (the CRAN project, www.cran.r-project.org). For correlation of GM-CSF secretion from the vaccine with clinical outcomes, GM-CSF level was dichotomized by using the classification and regression tree for survival data.[25,26]

Results

Patients and vaccine doses

A total of 123 patients were enrolled from 3 transplant centers in Boston from 2013 to early 2020. Of these, 92 proceeded to allogeneic transplantation after myeloblast harvest, and 57 (GVAX, n = 30; placebo, n = 27) received at least 1 vaccination starting between day 30 and 45 according to protocol. Among patients who underwent transplant who did not start vaccination, the primary reasons were GVHD requiring systemic steroid therapy (n = 21), relapse requiring therapy (n = 3), grade 3 or worse nonhematologic event (n = 3), graft failure (n = 1), and withdrawal of consent (n = 1). Six patients underwent transplant but did not start vaccination due to early study closure after futility analysis (Figure 1).

Patients who received at least 1 vaccination were considered evaluable for the primary end point. Baseline characteristics of these vaccinated patients are shown in Table 1. Baseline transplant and disease characteristics were well balanced between the 2 arms. Median marrow blast percentages at enrollment were 13% for the GVAX arm and 11% for the placebo arm; 93% of patients received peripheral blood stem cell as the graft source in both arms. Thirty-four of the 57 vaccinated patients proceeded to HSCT without intervening therapy after their marrow blast harvest for vaccine generation.

Table 1. Baseline characteristics

Characteristic	GVAX (n = 30)	Placebo (n = 27)	All (N = 57)	*P*
Age, median (range)	64 (27, 75)	63 (35, 74)	63 (27, 75)	.38
Patient sex, N (%)				.6
Female	11 (36.7)	12 (44.4)	23 (40.4)	
Male	19 (63.3)	15 (55.6)	34 (59.6)	
Donor age, median (range), y	28 (19, 67)	30 (19, 68)	28 (19, 68)	.17
Donor sex, N (%)				.15
Female	6 (20)	11 (40.7)	17 (29.8)	
Male	24 (80)	16 (59.3)	40 (70.2)	
Male recipient with female donor	2	4		.41
ECOG performance status, N (%)				.51
0	4 (13.3)	7 (25.9)	11 (19.3)	
1	18 (60)	15 (55.6)	32 (56.1)	
2	7 (23.3)	5 (18.5)	12 (21.1)	
Disease transplanted				.97
AML	11 (36.7)	10 (37)	21 (36.8)	
Second Complete Remission		1 (10)	1 (4.8)	
Induction failure	8 (72.7)	6 (60)	14 (66.7)	
Relapsed	2 (18.2)	3 (30)	5 (23.8)	
Untreated	1 (9.1)		1 (4.8)	
AML ELN risk category, N (%)				
Intermediate	5 (45.5)	4 (40)	9 (42.9)	
Adverse	6 (54.5)	6 (60)	12 (57.1)	
MDS	19 (63.3)	17 (63)	36 (63.2)	
Therapy-related MDS	3 (15.8)	4 (23.5)	7 (19.4)	
IPSS-R risk				
Good	10 (52.6)	8 (47.1)	18 (50)	
Intermediate	3 (15.8)	3 (17.6)	6 (16.7)	
Poor	4 (21.1)	2 (11.8)	6 (16.7)	
Very poor	2 (10.5)	4 (23.5)	6 (16.7)	
TP53 mutated				
No	14 (73.7)	10 (58.8)	28 (77.8)	
Yes	2 (10.5)	5 (29.4)	7 (19.4)	
Not done	3 (15.8)	2 (11.8)	5 (13.9)	
Cytoreductive therapy before HSCT, N (%)				
No	5 (16.7)	5 (18.5)	10 (17.5)	
Yes	25 (83.3)	22 (81.5)	47 (82.5)	
Marrow blasts at enrollment (%)				.28
Median (range)	13 (4, 60)	11 (4, 58)	12 (4, 60)	
Donor type				.37
Matched unrelated	24 (80)	18 (66.7)	42 (73.7)	
Matched sibling	6 (20)	9 (33.3)	15 (26.3)	
CMV serostatus				.72
R+/D+	4 (13.3)	6 (22.2)	10 (17.5)	
R+/D−	9 (30)	6 (22.2)	15 (26.3)	
R−/D+	4 (13.3)	5 (18.5)	9 (15.8)	
R−/D−	13 (43.4)	10 (37)	23 (40.4)	

ELN, European LeukemiaNet; IPSS-R, Revised International Prognostic Scoring System.

Table 1. (continued)

Characteristic	GVAX (n = 30)	Placebo (n = 27)	All (N = 57)	P
Conditioning				.68
MAC (myeloablative busulfan/ fludarabine)	15 (50)	15 (55.6)	30 (52.6)	
RIC (Reduced intensity busulfan/fludarabine)	15 (50)	12 (44.4)	27 (47.4)	
Graft source				1
Bone marrow	2 (6.7)	2 (7.4)	4 (7)	
Peripheral blood	28 (93.3)	25 (92.6)	53 (93)	

ELN, European LeukemiaNet; IPSS-R, Revised International Prognostic Scoring System.

Among vaccinated patients, 63% completed all 6 vaccines as planned in both arms; 9% received 5 vaccines; 5% each received 4, 3, and 2 vaccines; and 12% received 1 vaccination. The distribution of number of vaccines given in the GVAX and placebo arms was similar ($P = .2$). Primary reasons for not finishing all vaccinations were disease progression requiring additional therapy (45% GVAX, 60% placebo) or acute GVHD requiring systemic steroids (46% GVAX, 20% placebo).

The median number of cells per vaccine dose in the GVAX group was 2.1×10^6 (range, $0.22\text{-}10 \times 10^6$ cells per dose). The GM-CSF secretion data as measured by using enzyme-linked immunosorbent assay were available in 25 of the 30 patients who received at least 1 GVAX vaccine. The mean GM-CSF secretion was 421 ng/24 hours per 10^6 cells, with a median of 213.4 ng/24 hours per 10^6 cells (range, 3.05-2430 ng/24 hours per 10^6 cells). This level of secretion was higher than in the previous phase 1 trial in which the median GM-CSF secretion was 8.58 ng/24 hours per 10^6 cells (range, 0.4-600 ng/24 hours per 10^6 cells).[16] The reason for the higher secretion rate in the current cohort is not entirely clear, but it could potentially be a reflection of improved vector transduction efficiency as our laboratory gained experience over the years.

Vaccine toxicity and GVHD

GVAX vaccination was well tolerated. Only two grade 3 nonhematologic adverse events (hypoalbuminemia and hyperbilirubinemia) were reported in the 30 patients who received GVAX. Both adverse events were considered possibly related to vaccination. However, mild local injection site reactions were more common in GVAX compared with placebo vaccinations. These included pruritus, skin induration, and erythema multiforme in 10 GVAX patients, whereas only 1 patient on the placebo arm reported pruritus, and 1 patient had redness at the injection site ($P = .006$).

Grade II to IV acute GVHD at 1 year after HSCT was 34% in the GVAX group and 12% in the placebo group, but this difference did not reach statistical difference ($P = .13$). Incidence of grade III to IV GVHD was 16% in the GVAX arm and 0% in the placebo arm ($P = .09$). Cumulative incidence of chronic GVHD at 3 years was 47% with GVAX and 59% with placebo ($P = .26$). Cumulative incidence of moderate or severe chronic GVHD per the National Institutes of Health criteria was 23% for GVAX vs 33% for placebo ($P = .49$) (Table 2).

Relapse and survival after HSCT and vaccination

With a median follow-up time of 39 months (range, 9-89 months) after HSCT, the 18-month PFS (primary end point) was 53% for GVAX and 55% for placebo ($P = .79$). OS at 18 months was also similar: 63% for GVAX and 59% for placebo ($P = .86$). There was also no statistical difference in cumulative incidence of relapse in the GVAX vs placebo arms, although there was a trend toward higher NRM in the first year after transplant with GVAX (Figure 2; Table 2).

When we restricted the analysis of the primary end point (18-month PFS) to only patients who completed all 6 vaccinations, the results remained similar: 18-month PFS, 74% vs 82% for GVAX and placebo, respectively ($P = .54$). When the analysis was stratified according to conditioning intensity, there was also no difference between GVAX vs placebo after RIC ($P = .38$) or MAC ($P = .9$) for PFS and for OS (Figure 3).

Table 2. Study outcomes among patients receiving GVAX/placebo vaccinations after HSCT

Outcome	GVAX	Placebo	P
Grade II-IV acute GVHD at 1 y	34% (4-31)	12% (2.8-27)	.13
Grade III-IV acute GVHD at 1 y	16% (4.7-33)	0%	.09
Chronic GVHD at 3 y	47% (27-64)	59% (37-76)	.26
Moderate to severe chronic GVHD at 3 y	23% (10-40)	33% (16-52)	.42
18-mo PFS*	53% (34-69)	55% (35-72)	.79
18-mo OS	63% (43-77)	59% (38-75)	.86
18-mo NRM	17% (6-32)	7.7% (12-22)	.18
18-mo relapse	30% (15-47)	37% (19-55)	.51

Values in parentheses given as 95% confidence intervals. ECOG, Eastern Cooperative Oncology Group.
*Primary end point.

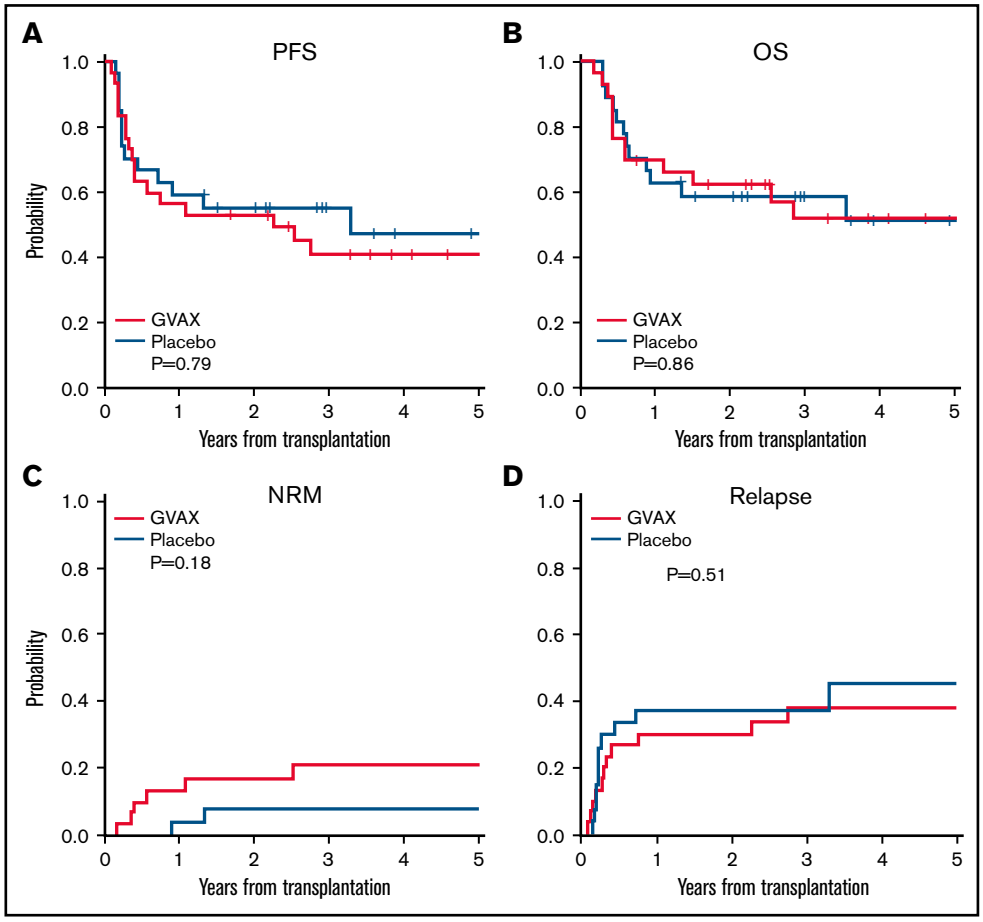

Figure 2. Treatment outcomes after HSCT and GVAX vs placebo vaccinations. PFS (A), OS (B), NRM (C), and relapse (D) after HSCT with GVAX vs placebo.

Most patients (83% and 82% of GVAX and placebo, respectively) received additional chemotherapy after myeloblast harvest and before transplant conditioning, and 23 of the 57 vaccinated patients had <5% marrow blasts at the time of alloHSCT conditioning. Minimal residual disease status on these patients was not available because this form of testing was not part of routine practice during the vast duration of this study period. When we compared patients who started transplant conditioning with excess marrow blasts (≥5%) vs those with <5% marrow blasts, there was also no difference in PFS or OS for those who received GVAX vs placebo.

Patients receiving a transplant for MDS had better PFS and OS than those undergoing transplant for AML, but their respective outcomes were similar in the GVAX and placebo groups. When the GVAX and placebo arms were combined, patients with MDS had a 3-year PFS of 56%, compared with 33% for patients with AML ($P = .03$). The difference in PFS was primarily driven by relapse (3-year cumulative incidence of relapse: 57% in AML vs 27% in MDS; $P = .018$).

Immune recovery after HSCT and vaccination

Posttransplant reconstitution of total white blood cells, absolute lymphocyte counts, CD4 and $CD8^+$ T cells, B cells, and NK cells was not adversely affected or enhanced by GVAX. Median absolute $CD4^+$ counts remained consistently above 200/μL starting 1 month after GVAX vaccination. B-cell recovery occurred between 5 and 9 months after vaccination, and NK cells recovered in both arms within the first 100 days of transplant. Treg/Tcon ratios appeared similar across all time points between the GVAX and placebo groups (Figure 4). We also analyzed recovery of dendritic cells and various differentiation subsets within Tcon, Treg, CD8 T cells, and NK cells and found no significant differences between the GVAX and placebo groups (supplemental Figure 1).

Plasma biomarker correlates

To assess whether GVAX vaccinated patients would exhibit different MICA/MICB or other immune biomarker profiles compared with HSCT patients who received placebo, we used a Luminex platform on 27 markers, including MICA, MICB, and other biomarkers of immune response. No distinguishable patterns were found after HSCT for patients who received GVAX vs placebo in any of the markers (supplemental Table 1). In terms of MICA and MICB, which appeared to correlate with disease burden and decreased after GVAX in the previous trial,[16] MICA levels in the current study significantly increased after first vaccination in both groups and remained increased until 1 year post-HCT ($P < .01$ at all time points compared with pre-HSCT), and there was no appreciable difference between GVAX vs placebo. There was also no difference in the MICA and MICB profiles for patients who relapsed after vaccination vs those who did not relapse.

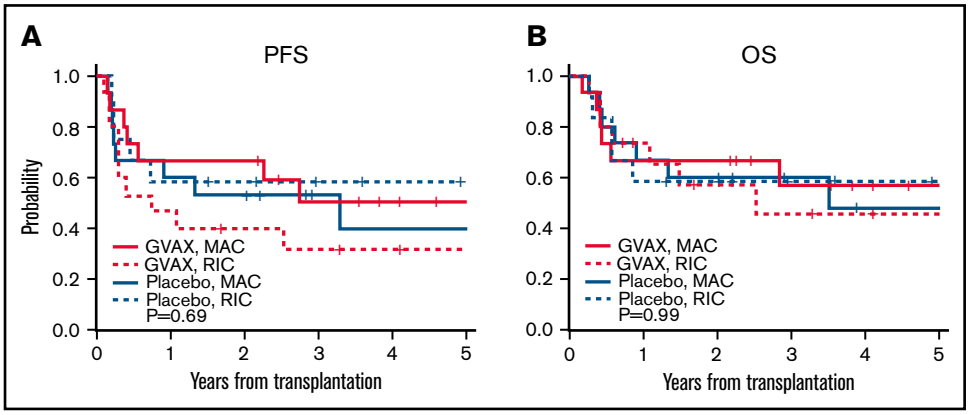

Figure 3. Transplant and vaccination outcomes stratified according to conditioning intensity. (A) PFS. (B) OS.

Correlation with vaccine GM-CSF secretion and outcomes

Because the rate of GM-CSF secretion from the vaccines generated on this trial have a wide range and are higher than those reported in the previous study in 2009,[16] we assessed for any association between the GM-SCF secretion rate with clinical outcomes after vaccination. Interestingly, we discovered that patients who received GVAX with low-level GM-CSF secretion (≤100 ng/mL per 24 hours; n = 10) had better outcomes compared with patients who received GVAX with high GM-SCF secretion (>100 ng/mL per 24 hours; n = 15). PFS and OS were significantly improved among patients who received low-secretion GM-CSF vaccines compared with those who received GVAX with high secretion (P = .009 for PFS; P = .0027 for OS). The 3-year estimate for relapse was also lower: 10% (95% confidence interval, 0.5-37) among the low GM-CSF vaccine recipients vs 47% (95% confidence interval, 19-70) in the high GM-CSF–secreting vaccine recipients, although this did not reach statistical significance (P = .09).

Discussion

Through its immune-modulatory effects, enforced GM-CSF production via adenovirus transfected autologous tumor cells stimulates adaptive antitumor immunity, and these whole-cell vaccines, collectively known as GVAX, have generated enthusiasm as a cancer vaccination strategy over the last 2 decades. Phase 1/2 clinical trials of GVAX in multiple solid and hematologic cancers have shown frequent dense infiltrates of B7-1–expressing dendritic cells that induced cellular and humoral responses at the injection sites and, in many cases, enhanced tumor infiltration of lymphocytes.[7,18,27-29] Furthermore, GVAX seemed to induce antibodies against multiple angiogenic cytokines that correlated with improved outcomes, as well as antibodies against MICA, a ligand for the activating NK cell receptor NKG2D, thereby overcoming potential immune escape mechanisms mediated through soluble MICA.[11,14,17] Despite these immune signals, overall sustained clinical responses from standalone GVAX trials have largely been disappointing, leading investigators to combine GVAX with other therapies that could augment the vaccine response.

The addition of GVAX after alloHSCT represents a logical extension of such a strategy for patients with MDS/AML because antileukemic immunity mediated by the donor graft is crucial for achieving durable remission, and vaccination early after transplant could also capitalize on the lymphodepletion achieved with the conditioning regimen. Our initial pilot study[16] found that this approach is feasible, and the encouraging clinical results among patients who completed all 6 vaccinations led us to pursue the current multicenter, double-blinded, randomized trial.

Unfortunately, the results of the current study showed no improvement in PFS or OS at 18 months after HSCT with GVAX vs placebo. GVAX vaccination was generally well tolerated and elicited mild local skin reactions in one-third of patients. There was no statistical difference in acute and chronic GVHD, relapse, or NRM. Our results further add to the literature of recent randomized GVAX clinical trials in advanced pancreatic and prostate cancer, which have also reported largely negative results.[30-33] In the advanced pancreatic cancer trial in which patients were randomized to receive GVAX plus ipilimumab vs FOLFIRINOX-based chemotherapy, 42 subjects who received ipilimumab + GVAX had an OS of 9.38 months, compared with 42 subjects who received FOLFIRINOX who had an OS of 14.7 months.[32]

Although there is some selection bias because our evaluable study population had to make it through transplant to be eligible to start GVAX/placebo, survival outcomes were still overall encouraging for both groups, with PFS of 53% in the GVAX group and 55% in the placebo group at 18 months after transplant. This is higher than what we had anticipated based on historical data for patients who underwent alloHSCT for advanced MDS or relapsed/refractory AML, in whom we would have anticipated a long-term PFS of ≤30%. This improvement may reflect better selection of patients for transplant in this trial, as well as therapeutic advances over the last decade with hypomethylating agents as cytoreductive "bridging" therapy for patients with excess blast MDS, (which result in higher rates of complete remission or near remissions by the time of HSCT) and newer AML treatments such as venetoclax. This contrasts with the previous phase 1 pilot study[16] conducted in the early 2000s when these therapies were not available, and patients started their transplant conditioning with higher marrow blasts/disease burden. As such, it is possible that any potential incremental benefit from vaccination is no longer discernible because the

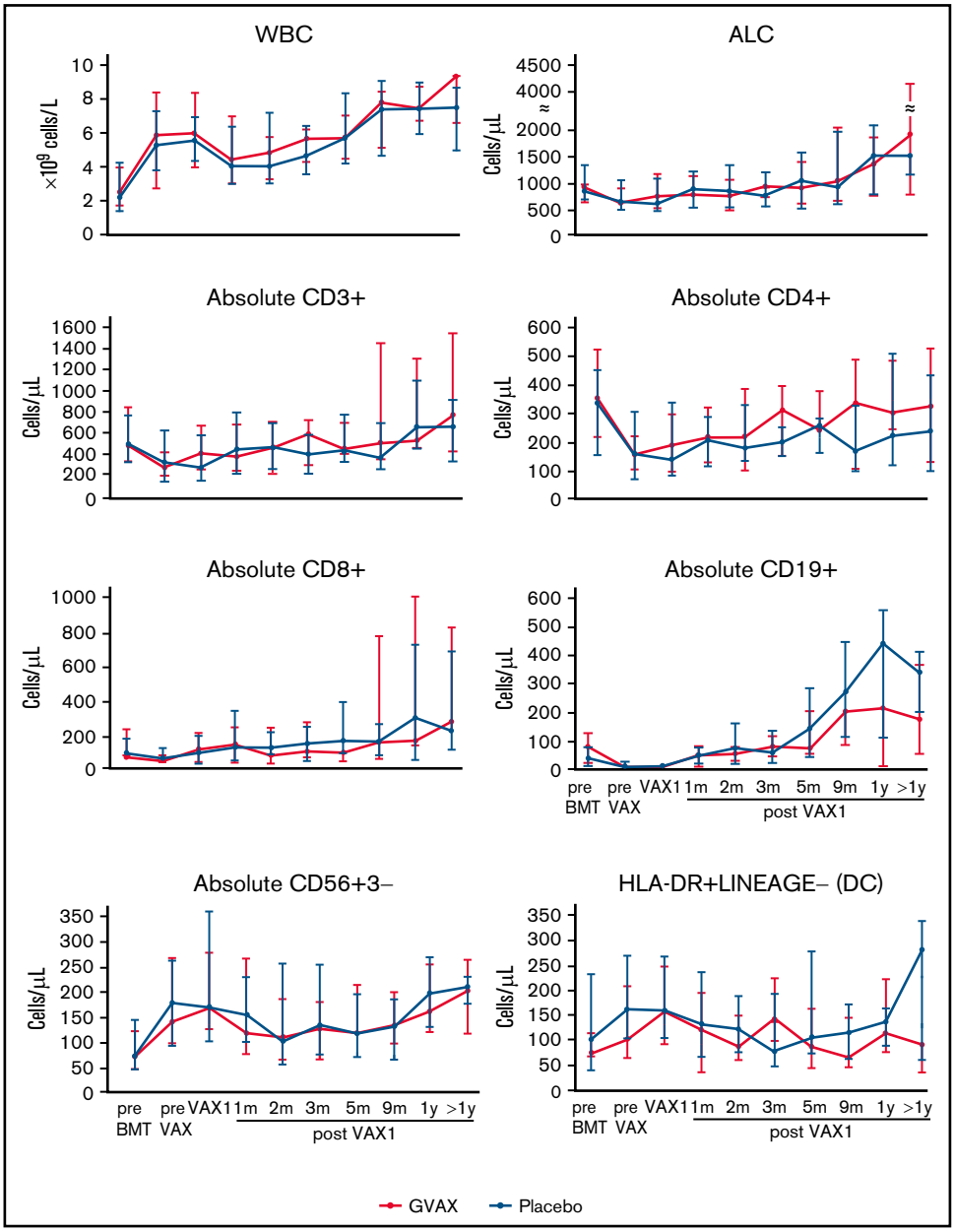

Figure 4. Reconstitution of immune cell subsets after HSCT and GVAX vs placebo vaccinations. ALC, absolute lymphocyte count; BMT, bone marrow transplantation; WBC, white blood cell count.

baseline survival in the control cohort is now improved with currently available care.

We were also disappointed to find that patients who received GVAX in this trial did not exhibit any difference in circulating MICA or MICB levels after vaccination compared with placebo. In our previous pilot study,[16] there seemed to be a correlation between circulating MICA and MICB levels in the plasma that reflected disease burden, and these levels declined after vaccination in patients who attained long-term remission. In the current trial, we did not observe a similar pattern. This difference may reflect the fact that patients in the current trial are entering transplant with a lower disease burden and thus expected to have lower circulating levels of soluble MICA/B, which are putatively shed from leukemic cells.

Beyond MICA/B, we were disappointed in not finding any obvious correlation in a large panel of immune biomarkers with GVAX compared with placebo. The explanation for this is unclear. It could be that our vaccine population was too small, or the vaccine exerted limited biologic activity, or that any inducible immune biomarker signals were not discernible above the background noise in patients early after allogeneic transplantation. Our ability to assess biomarker trends over time was also limited by the fact that at later time points, particularly 6 months and 1 year or beyond after vaccine

completion, the number of samples/data points available dropped off significantly.

Our finding of a wider and higher range of GM-CSF secretion from the GVAX vaccines generated in this trial relative to the previous phase 1 trial[16] led us to investigate whether the GM-CSF secretion could affect vaccine efficacy and account for the lack of response in the current trial. Interestingly, we found that patients who received vaccines with low GM-CSF secretion (≤100 ng/mL per 24 hours) had a significantly improved PFS and OS compared with those who received GVAX with high GM-CSF secretion. These results are in line with previous studies which showed that high-dose GM-CSF–secreting vaccines actually impair antigen-specific T-cell responses by inducing $Gr1^{+}/CD11b^{+}$ myeloid suppressor cells.[34,35] In this study, patients who received low GM-CSF–secreting GVAX seem to have superior survival compared with those receiving placebo, but our interpretation should be taken with caution because of the small sample size. Nonetheless, these intriguing results suggest that it may be preferable to restrict GM-CSF secretion in future autologous tumor cell vaccines, as higher GM-CSF secretion could paradoxically blunt vaccine activity.

Although the current study was terminated after a planned interim analysis, this remains one of the largest randomized, placebo-controlled cancer vaccination trials conducted in the alloHSCT setting to date. This study shows that GVAX vaccination is feasible and can be administered in patients within the first 100 days of transplantation, while they are on full immune suppression with tacrolimus as GVHD prophylaxis. This study also highlights the challenges of conducting an autologous cellular vaccine trial in alloHSCT patients, or potentially any clinical trial that requires the study subject to be able to proceed to HSCT, survive the transplant process, and retain reasonable performance status without GVHD or other complications before starting the study intervention.

Highlighting these challenges, our study took almost 7 years to accrue. There was a high attrition rate after initial enrollment/myeloblast harvest, especially from disease progression or failure to maintain fitness/eligibility to proceed to alloHSCT, and development of GVHD or other early complications after transplant that precluded vaccine initiation, as only 57 of the 123 patients enrolled ultimately started the vaccination. Future studies testing the addition of autologous cancer cell vaccines after alloHSCT will need to focus on maximizing the ability of enrolled patients to proceed to transplant and minimizing dropout after transplantation because of GVHD, early transplant complications, or early disease relapse.

In summary, this randomized, placebo-controlled trial adding autologous leukemia cells transduced to secrete GM-CSF as a vaccine in patients with advanced MDS/AML showed that the early post-alloHSCT period is a feasible platform for a cancer vaccination strategy. GVAX vaccination seemed to be safe but was not associated with any improvement in relapse or relapse-free survival after HSCT. It is possible that the absence of activity in this trial could be related to the higher GM-CSF secretion, which could paradoxically blunt immune responses. Further research efforts to improve GVAX efficacy may focus on strategies to augment dendritic cell activation while minimizing the tolerogenic effects of higher levels of GM-CSF, such as with controlling the rate of GM-CSF secretion and coadministration of adjuvants, Toll-like receptor 7 agonists, or agonists of the stimulator of interferon genes (STING) pathways. Combination therapy of GVAX with new checkpoint blockade agents may also hold promise.

Despite the disappointing clinical results of this and other GVAX randomized trials to date, these studies continue to teach us much about the subtleties of tumor immunity, and highlight the challenges of performing large randomized autologous leukemia vaccination trials, especially in the alloHSCT setting. A deeper understanding of the checks and balances regulating GVAX-mediated immune responses is needed to define its potential as a cancer vaccine in the future.

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Authorship

Contribution: R.J.S., G.D., H.T.K., and V.T.H. conceived the study; V.T.H. wrote the study protocol, oversaw the conduct of the trial as the study Principal Investigator, contributed patients to the study, interpreted the results, and wrote the manuscript; H.T.K. analyzed and interpreted the study results and assisted in writing the manuscript; J.B. was the primary research nurse for the trial, coordinated patient schedules, and collected adverse event data for the study; I.G. helped coordinate and performed marrow blast harvests; Jerome Ritz supervised the cell manipulation core facility where all vaccine manufacture, sample banking, and immune reconstitution studies were performed; H.D. supervised the laboratory staff in the manufacture and release of the vaccine/placebo; C.R. and A.W. performed the immune reconstitution studies; O.P. enumerated the number of myeloblasts after all harvests to enable the estimation of vaccine cell dose; M.S. and F.S.H. performed the Luminex assays of immune biomarkers; S.N., C.C., J.K., E.P.A., J.H.A., M.G., R.R., R.S., Y.-B.C., Jaclyn Rosenblatt, D.A., C.J.W., and R.J.S. saw patients and contributed patients to the study; and Y.-B.C. and D.A. also served as local site Principal Investigators for the study; and all authors reviewed and assisted in the development of the final manuscript.

Conflicts-of-interest disclosure: G.D. is currently an employee of Novartis and owns stock in Novartis. F.S.H. reports grants and personal fees from Bristol Myers Squibb, Novartis, and personal fees from Merck, EMD Serono, Surface, Compass Therapeutics, Apricity, Sanofi, Pionyr, 7 Hills Pharma, Torque, Bicara, Checkpoint

Therpaeutics, Genentech/Roche, Bioentre, Gossamer, Lovance, Trillium, Catalym, Immunocore, Amgen, Rheos, and Zumutor. F.S.H. also has a patent Methods for Treating MICA Related Disorders (#20100111973) with royalties paid; a patent Tumor antigens and uses thereof (#7250291) issued; a patent Angiopoiten-2 Biomarkers Predictive of Antiimmune checkpoint response (#20170248603) pending; a patent Compositions and Methods for Identification, Assessment, Prevention, and Treatment of Melanoma using PD-L1 Isoforms (#20160340407) pending; a patent Therapeutic peptides (#20160046716) pending; a patent Therapeutic Peptides (#20140004112) pending; a patent Therapeutic Peptides (#20170022275) pending; a patent Therapeutic Peptides (#20170008962) pending; a patent Therapeutic Peptides Patent number (9402905) issued; a patent Methods of using pembrolizumab and trebananib pending; a patent Vaccine compositions and methods for restoring NKG2D pathway function against cancers Patent number (10279021) issued; a patent Antibodies that bind to MHC class I polypeptide-related sequence A Patent number (10106611) issued; and a patent Anti-galectin antibody biomarkers predictive of anti-immune checkpoint and anti-angiogenesis responses Publication number (20170343552) pending. The remaining authors declare no competing financial interests.

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