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

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Abstract:
Vaccination using irradiated, adenovirus transduced autologous myeloblasts to secrete GM-CSF (GVAX) early after allogeneic hematopoietic stem cell transplantation (HSCT) can induce potent immune responses. We conducted a randomized phase II trial of GVAX after HSCT for MDS-EB or relapsed/refractory AML. Myeloblasts were harvested before HSCT to generate the vaccine. Randomization to GVAX vs. placebo (1:1) was stratified by disease, transplant center, and conditioning. GVHD prophylaxis included tacrolimus and methotrexate. GVAX or placebo started between day +30-45 if there was engraftment and no GVHD. Vaccines were administered SC/ID weekly x 3, then q2 wks x 3. Tacrolimus taper began after vaccine completion. 123 patients enrolled, 92 proceeded to HSCT, and 57 (GVAX 30, Placebo 27) received at least 1 vaccination. No CTC grade {greater than or equal to} 3 vaccine related adverse events were reported, but injection site reactions were more common after GVAX (10 vs. 1, p=0.006). With a median follow up of 39 months (range, 9-89), 18-month PFS, OS and relapse incidence were 53% vs 55% (p=0.79), 63% vs. 59% (p= 0.86), and 30% vs. 37% (p=0.51) for GVAX and placebo, respectively. NRM at 18 months was 17% vs. 7.7% (p=0.18), Grade II-IV aGVHD at 12 months 34% vs. 12% (p=0.13), and cGVHD at 3 years 49% vs. 57% for GVAX and placebo, respectively, p=0.26. Reconstitution of T, B, and NK cells were not decreased or enhanced by GVAX. There were no differences in serum MICA/B or other immune biomarkers between GVAX and placebo. GVAX does not improve survival after HSCT for MDS/AML. (Clinicaltrials.gov identifier: NCT01773395)

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GM-CSF Secreting Leukemia Cell Vaccination for MDS/AML after Allogeneic HSCT: A Randomized Double Blinded Phase 2 Trial

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Running title: GVAX/placebo Vaccination for MDS/AML after AlloHSCT

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Key Points:

- GVAX vaccination early after alloHSCT is well tolerated, but does not improve long-term disease-free survival after transplantation
- This study highlights the challenges of conducting planned early posttransplant intervention trials after alloHSCT

Abstract

Vaccination using irradiated, adenovirus transduced autologous myeloblasts to secrete GM-CSF (GVAX) early after allogeneic hematopoietic stem cell transplantation (HSCT) can induce potent immune responses. We conducted a randomized phase II trial of GVAX after HSCT for MDS-EB or relapsed/refractory AML. Myeloblasts were harvested before HSCT to generate the vaccine. Randomization to GVAX vs. placebo (1:1) was stratified by disease, transplant center, and conditioning. GVHD prophylaxis included tacrolimus and methotrexate. GVAX or placebo started between day +30-45 if there was engraftment and no GVHD. Vaccines were administered SC/ID weekly x 3, then q2 wks x 3. Tacrolimus taper began after vaccine completion. 123 patients enrolled, 92 proceeded to HSCT, and 57 (GVAX 30, Placebo 27) received at least 1 vaccination. No CTC grade ≥ 3 vaccine related adverse events were reported, but injection site reactions were more common after GVAX (10 vs. 1, p=0.006). With a median follow up of 39 months (range, 9-89), 18-month PFS, OS and relapse incidence were 53% vs 55% (p=0.79), 63% vs. 59% (p= 0.86), and 30% vs. 37% (p=0.51) for GVAX and placebo, respectively. NRM at 18 months was 17% vs. 7.7% (p=0.18), Grade II-IV aGVHD at 12 months 34% vs. 12% (p=0.13), and cGVHD at 3 years 49% vs. 57% for GVAX and placebo, respectively, p=0.26. Reconstitution of T, B, and NK cells were not decreased or enhanced by GVAX. There were no differences in serum MICA/B or other immune biomarkers between GVAX and placebo. GVAX does not improve survival after HSCT for MDS/AML. (Clinicaltrials.gov identifier: NCT01773395)
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 since it relies on the “graft-versus-leukemia” effect mediated by the new donor derived immune system. As the field of transplantation advances with improved HLA typing, less toxic conditioning regimens, superior GVHD prophylaxis strategies, supportive care, and new antimicrobial treatments/surveillance, 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 the 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 upon the lymphopenic milieu created by the preparative regimen and surge of homeostatic cytokines, such as IL-7, IL-12 and IL-15, that would be favorable toward activating immune responses.\(^2\)-\(^5\)

At our institution, we have previously demonstrated that granulocyte–macrophage colony-stimulating factor (GM-CSF) production by whole cell vaccines stimulates adaptive anti-cancer immune responses by inducing myeloid differentiation and DC 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 demonstrated 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 NK cells, and B lymphocytes.\(^6,11-14\) In murine transplant models, Teshima and colleagues have shown that GVAX elicits potent tumor specific immunity when given 6 weeks after allogeneic transplantation.\(^15\)
We previously reported a pilot clinical trial testing the feasibility and safety of GVAX early after reduced intensity conditioning alloHSCT in patients with active MDS/RAEB or relapsed/refractory AML.\textsuperscript{16} In this study, GVAX vaccination was well tolerated and did not elicit severe acute or chronic GVHD. Despite undergoing a RIC transplantation with active disease, 10/15 patients who started GVAX vaccination after transplant had durable responses, and 9/10 patients who completed all 6 vaccinations within the first 100 days achieved sustained long term complete remissions. We also demonstrated that immune responses in survivors correlated with declining levels of soluble MICA and MICB levels, and antibody responses to a variety of anti-angiogenic cytokines including angiopoietin 1 and angiopoietin 2.\textsuperscript{16,17}

Given these encouraging results, we conducted a follow-up study and hereby report the results of the phase II, multi-center, randomized double blinded clinical trial testing GVAX vs. placebo vaccination early after alloHSCT in patients with MDS-EB 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 (IND #17904, ClinicalTrials.gov Identifier: NCT01773395). Informed consent was obtained from all subjects according to 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 are deemed to be an appropriate candidate for either myeloablative or reduce intensity conditioning HSCT, and meet all of the following criteria: age $\geq 18$; MDS-RAEB or relapsed or refractory AML not in remission (defined as $\geq5\%$ marrow blast or $\geq5\%$ circulating blasts); available 8/8 or better matched related or unrelated donor (by high resolution typing) at HLA-A,B, C and DRB1, and Eastern Cooperative Oncology Group (ECOG) performance status 0-2. Patients with uncontrolled infection, active CNS leukemic involvement, HIV positivity, or inadequate organ function (serum creatinine $\geq 2.0$ mg/dl; ALT or...
AST ≥ 3X ULN; total bilirubin ≥ 2.0 mg/dl) were excluded. After enrollment, study subjects undergo leukemia cell harvests for GVAX vaccine generation via marrow aspiration, or peripheral blood draw with the goal of obtaining a minimum of 2 x 10^7 total myeloblasts. For subjects randomized (1:1 randomization) to the GVAX arm, harvested blasts were subjected to GVAX manufacture as detailed below. Randomization was stratified by disease, transplant center, conditioning intensity, and the intent of RIC (Bu2/Flu) vs. MAC (Bu4/Flu) 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 prior to 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 G-CSF. Leukemic cells were transduced with a replication defective adenoviral vector encoding human GM-CSF, as previously reported. 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 approximately 24 hours. Supernatant was harvested and GM-CSF secretion was measured by ELISA. Routine sterility cultures and testing for endotoxin and mycoplasma contamination were performed prior to 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 were fixed for an individual patient and the dosage was determined by dividing the total cell yield following transduction into six aliquots. For total cell yields greater than 6x10^7, individual aliquots were capped at 1x10^7 cells per dose. For patients randomized to 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 30mg/m²/d IV x 4 (total 120 mg/m²) and busulfan 0.8mg/kg IV q12H x 8 (total 6.4 mg/kg) from day-5 to –2. The MAC preparative regimen consisted of fludarabine 30mg/m²/d IV x 4 (total 120 mg/m²) and busulfan 0.8mg/kg IV q6H x 16 (total 12.8 mg/kg) from day-5 to –2. Unmanipulated G-CSF mobilized PBSC 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/m² on days 1,3,6,11. Taper of tacrolimus was allowed starting about 4 weeks after completion of GVAX vaccinations (approximately day +120). GM-CSF (Leukine) 250 mg/m² SC QD was administered from day+12 until neutrophil engraftment. Infection prophylaxis included acyclovir for HSV/VZV and trimethoprim sulfamethoxazole or atovaquone for Pneumocystis jirovecii. Systemic antifungal prophylaxis was not routinely given. 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 was performed at approximately 30 days after HSCT, prior to initiation of GVAX or placebo vaccination. No planned post-transplant maintenance therapy was allowed on this study.

**Vaccination administration**

GVAX or placebo vaccination was initiated between day +30 to +45 after HSCT if the following criteria were met: no grade II-IV acute GVHD requiring systemic steroids; no uncontrolled acute infection; adequate hematologic recovery with ANC > 500/ul off growth factors, Platelet > 10K/ul without transfusion, and no CTC (v.4.0) grade ≥ 3 non-hematologic toxicity. Patients not meeting above criteria to start vaccination by day+45 after HSCT were removed from study. Patients with persistent or progressive disease at day +30 were eligible to start vaccinations if there is 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 are to be completed before day +108 post SCT. Patient remained on therapeutic dosing of tacrolimus to maintain trough serum levels between 5-10 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 graft-versus host disease (GVHD) was graded according to Keystone criteria. Non-GVHD adverse events were reported according to the National Cancer Institute Common Toxicity Criteria v.4 guidelines. Disease responses were assessed by marrow aspiration and biopsies performed on the day of starting vaccine 1, 1 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 last vaccine, and at 6, 12, and 18 months after transplant.

*Monitoring immune reconstitution after allo-HSCT by flow cytometry*
Peripheral blood samples were obtained at all the time points listed above to monitor recovery of CD3+ T cells, CD4+ conventional T cells (Tcon), CD4+CD25+CD127low 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 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*
Since our previous pilot study had shown that long term survivors after completing GVAX vaccinations had decline in levels of circulating MICA and MICB in their blood, and
development of antibodies against a variety of angiogenic cytokines that appeared to correlate with their response to vaccinations, we employed a Luminex kits (Bio-Techne Inc, MN) to assess for MICA, MICB along with an extended panel of markers that have been correlated with angiogenic cytokines, T cell responses, NK cells status, soluble checkpoint markers, neutrophilic chemokines, including Ang-1, Ang-2, CX3CL1, INF-gamma, 4-1BB, CD25, PDL1, IL6, CXCL10, IL-8, G-CSF, IL2, Progranulin, HGF, IL-1b, Tie2, IL12p70, IL10, CCL4, CCL2, CXCL6, CXCL5, CXCL2, PDGF, VEGF. 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 using the Luminex FlexMap3D platform were Median Fluorescent Intensity values were extrapolated to Standard Curves for quantification, as previously reported.\textsuperscript{22,23}

**Statistical Analyses**

Based on historical data and our previous study result,\textsuperscript{16} we had projected sample size on the premise that the 18-months 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 for an additional 18 months. Utilizing a two-component cure rate for the null and a three-component cure rate model for the alternative hypothesis,\textsuperscript{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 DSMB. 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 DSMB recommendation, the study was terminated after 57 pts were vaccinated.

Baseline characteristics were reported descriptively and compared using Fisher’s exact test, Chi-square test or Wilcoxon-Rank-Sum test, as appropriate. The primary endpoint was progression-free survival (PFS) and other endpoints of interest included overall survival (OS), relapse, and non-relapse mortality (NRM). All time-to-event endpoints 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 enter complete remission after vaccination and scheduled immune suppression taper were not considered as a treatment failure. OS and PFS were estimated 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; Gray test was used for group comparison of cumulative incidences. Univariable and multivariable Cox regression analysis was performed to examine factors that are associated with PFS and OS. For multivariable model, high-risk features or factors that were associated with \( p<0.1 \) from univariable models were included. Risk factors considered in 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 alloHCT, patient-donor CMV sero status, HCT comorbidity score, and year of transplant. Prior to modeling, the linearity and proportional hazards assumptions and two-way interactions with the study were examined. For comparison of laboratory parameters, Wilcoxon-rank-sum test was used. Multiplicity was not considered. All \( p \)-values were two-sided and the significance level was set to 0.05. All analyses were performed using SAS 9.4 (SAS Institute Inc, Cary, NC), 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 using the classification and regression tree for survival data.\textsuperscript{25,26}

**Data Sharing Statement**

Additional data may be found in a data supplement available with the online version of this article. For original data, please contact Vincent_Ho@DFCI.Harvard.edu. Individual participant data will not be shared.

**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 30, Placebo 27) received at least 1 vaccination starting between day+30 to 45 according to protocol. Among transplanted patients who did not start vaccination, the primary reasons
were GVHD requiring systemic steroid therapy (n=21), relapse requiring therapy (n=3), grade 3+ non-hematologic event (n=3), graft failure (n=1), and withdrawal of consent (n=1). Six patients were transplanted 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 endpoint. Baseline characteristics of these vaccinated patients are shown on Table 1. Baseline transplant and disease characteristics were well balanced between the two arms. Median marrow blast percentage at enrollment were 13% for the GVAX arm and 11% for the placebo arm, and 93% of patients received PBSC 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.

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 were similar (p=0.2). 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 $2.1 \times 10^6$ (range 0.22-10 x$10^6$ cells/dose). The GM-CSF secretion data as measured by ELISA were available in 25 of the 30 pts who received at least 1 GVAX vaccine. The mean GM-CSF secretion per 24 hours was 421 ng/per $10^6$ cells, with a median of 213.4 ng/24hrs per $10^6$ cells (range 3.05-2430). This level of secretion was higher than in the previous phase I trial where the median GM-CSF secretion was 8.58 ng/24hrs per $10^6$ cells (range 0.4-600). 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 Graft-versus-Host Disease
GVAX vaccination was well tolerated. Only 2 grade 3 non-hematologic adverse events (hypoalbuminemia, and hyperbilirubinemia) were reported in the 30 pts who received GVAX. Both were considered possibly related to vaccination. However, mild local injection site reactions were more common in GVAX compared to placebo vaccinations. These included pruritus, skin induration, and erythema multiforme in 10 GVAX patients, while only 1 patient on the placebo arm reported pruritus, and 1 patient had redness at the injection site (p=0.006).

Grade II-IV acute GVHD at 1 year after HSCT was 34% in GVAX and 12% in the placebo, but this difference did not reach statistical difference (p=0.13). Incidence of grade III-IV GVHD was 16% in GVAX and 0% in the placebo arm (p=0.09). Cumulative incidence of chronic GVHD at 3 years was 47% in GVAX and 59% in placebo (p=0.26). Cumulative incidence of NIH moderate or severe cGVHD was 23% for GVAX, vs. 33% in placebo (p=0.49). (Table 2)

Relapse and Survival after HSCT and Vaccination
With a median follow up time of 39 months (range 9 to 89 months) after HSCT, the 18-month progression free survival (PFS primary endpoint) was 53% for GVAX and 55% for placebo (p=0.79). Overall survival at 18 months were also similar, 63% for GVAX and 59% for Placebo (p=0.86). There was also no statistical difference in cumulative incidence of relapse in the GVAX vs. placebo arms, although there was a trend toward higher non-relapse mortality in the first year after transplant with GVAX. (Figure 2 and Table 2)

When we restricted the analysis of the primary endpoint (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=0.54. When the analysis was stratified by conditioning intensity, there was also no difference between GVAX vs. placebo after RIC (p=0.38) or MAC conditioning (p=0.9) for PFS and for OS (Figure 3).

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 (MRD) status on these patients are not available as MRD 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. <5% marrow blasts, there was also no difference in PFS or OS for those who received GVAX vs. placebo.

Patients transplanted for MDS have better PFS and OS than those transplanted for AML, but their respective outcomes were similar in the GVAX and placebo groups. When GVAX and placebo arms were combined, pts with MDS had a 3-year PFS of 56%, compared to 33% for patients with AML, p=0.03. The difference in PFS was primarily driven by relapse (3-year CI of relapse: 57% in AML vs 27% in MDS, p=0.018)

**Immune recovery after HSCT and vaccination**

Post-transplant reconstitution of total WBC, absolute lymphocyte counts, CD4 and CD8+ T cells, B cells, and NK cells was not adversely impacted or enhanced by GVAX. Median absolute CD4+ counts remained consistently above 200/ul starting 1 month after GVAX vaccination. B cell recovery occurred between 5-9 months after vaccination and NK cells recovered in both arms within the first hundred 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 **(Supplement Figure S1)**.

**Plasma Biomarker correlates**

To assess whether GVAX vaccinated patients would exhibit different MICA/B or other immune biomarker profiles compared to HSCT patients who received placebo, we used a Luminex platform on 27 markers including MICA, MICB and other biomarkers of immune response. We found no distinguishable patterns after HSCT for patients who received GVAX vs. placebo in any of the markers. **(Supplement Table 1)** In regards to MICA and MICB, which appeared to correlate with disease burden and decreased after GVAX in the previous trial, MICA levels in the current study significantly increased after first vaccination in both groups and remained increased until one year post HCT (p<0.01 at all time points compared to pre HSCT), and there was no appreciable difference between GVAX vs. placebo. There was also no difference in the
MICA and MIC B profiles for patients who relapsed after vaccination vs. those who did not relapse.

**Correlation with vaccine GM-CSF secretion and outcomes**

Since 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, 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/24hr, n=10) had outcomes compared to patients who received GVAX with high GM-SCF secretion (>100 ng/ml/24hr, n=15) (Figure 5). PFS and OS were significantly improved among patients who received low secretion GM-CSF vaccines compared to those who received GVAX with high secretion (p= 0.009 for PFS; p=0.0027 for OS). The 3-year estimate for relapse also appeared to be lower: 10% (95% CI 0.5, 37) among the low GM-CSF vaccine recipients, vs. 47% (95% CI 19, 70) in the high GM-CSF secreting vaccine recipients, although this did not reach statistical significance (p=0.09).

**Discussion**

Through its immune-modulatory effects, enforced GM-CSF production via adenovirus transfected autologous tumor cells stimulates adaptive anti-tumor 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 demonstrated frequent dense infiltrates of B7-1-expressing DCs 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 appeared to induce antibodies against multiple angiogenic cytokines which correlated with improved outcomes, as well as antibodies against major histo-compatibility chain-related protein-A (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 stand-alone GVAX trials have been largely disappointing, leading investigators to combine GVAX with other therapies that could augment the vaccine response.
The addition of GVAX after allogeneic HSCT represents a logical extension of such a strategy for patients with MDS/AML since anti-leukemic 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 demonstrated that this approach is feasible, and the encouraging clinical results among patients who completed all 6 vaccinations led us to pursue the current multi-center double blinded randomized trial.\textsuperscript{16}

Unfortunately, the results of our study showed no improvement in progression free or overall survival 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 non-relapse mortality. 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.\textsuperscript{30-33} In the advanced pancreatic cancer trial where patients were randomized to GVAX plus ipilimumab versus FOLFIRINOX based chemotherapy, 42 subjects who received ipilimumab + GVAX had an overall survival of 9.38 months, compared to 42 subjects that received FOLFIRINOX who had an overall of 14.7 mo.\textsuperscript{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 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, where we would have anticipated a long term PFS of 30\% or less. This improvement may reflect better selection of patients for transplant in this trial, as well as therapeutic advances over the last decade with hypomethylating agent (HMA) as cytoeducative “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 I pilot study conducted in the early 2000’s where these therapies were not available, and
patients started their transplant conditioning with higher marrow blasts/disease burden. As such, it is possible that the any potential incremental benefit from vaccination is no longer discernable because the 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 show any difference in circulating MICA or MICB levels after vaccination compared to placebo. In our previous pilot study, there appeared 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 to 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 discernable 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 I trial led us to investigate whether the GM-CSF secretion could impact 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/24hr) had a significantly improved PFS and OS compared to 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. In this study, patients who received low GM-CSF secreting GVAX appears to have superior survival compared to placebo,
but our interpretation must be cautioned because of 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 it 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 demonstrates 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 allogeneic HSCT patients, or potentially any clinical trial which requires the study subject 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 starting 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 advanced MDS/AML patients showed that the early post allogeneic HSCT period is a feasible platform for a cancer vaccination strategy. GVAX vaccination appears to be safe, but is 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, co-administration of adjuvants, TLR-7 agonist, or agonists of the Stimulator of Interferon Genes (STING) pathways. Combination therapy with 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 allogeneic HSCT 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: RJS, GD, HTK and VTH conceived the study. VTH wrote the study protocol, oversaw the conduct of the trial as the study PI, contributed patients to the study, interpreted the results and wrote the manuscript. HTK analyzed and interpreted the study results and assisted in writing the manuscript. JB was the primary research RN for the trial, coordinated patient schedules, and collected adverse event data for the study. IG helped coordinate and performed marrow blast harvests. JR supervised the cell manipulation core facility where all vaccine manufacture, sample banking, and immune reconstitution studies were performed. HD supervised the laboratory staff in the manufacture and release of the vaccine/placebo. CR and AW performed the immune reconstitution studies. OP enumerated the number of myeloblasts after all harvests to enable the estimation of vaccine cell dose. MS and FSH performed the Luminex assays of immune biomarkers. SN, CC, JK, EPA III, JHA, MG, RR, RS, YBC, JR, DA, CJW, RJS saw patients and contributed patients to the study. YBC and DA also served as local site PIs for the study. 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 own stock in Novartis. F.S.H. has a patent Methods for Treating MICARelated 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 antiimmune checkpoint and anti-angiogenesis responses. Publication number: 20170343552 pending. All other authors have no relevant conflicts of interest related to this study.

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Data Sharing Statement

Additional data may be found in a data supplement available with the online version of this article. For original data, please contact Vincent_Ho@DFCI.Harvard.edu. Individual participant data will not be shared.
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## Table 1. Baseline Characteristics

|                                      | GVAX (N=30) | Placebo (N=27) | All (N=57) | p-value |
|--------------------------------------|-------------|----------------|------------|---------|
| Age, median (range)                  | 64 (27, 75) | 63 (35, 74)    | 63 (27, 75)| 0.38    |
| Pt Sex                               |             |                |            | 0.6     |
| Female                               | 11 (36.7)   | 12 (44.4)      | 23 (40.4)  |         |
| Male                                 | 19 (63.3)   | 15 (55.6)      | 34 (59.6)  |         |
| Donor Age, median (range)            | 28 (19, 67) | 30 (19, 68)    | 28 (19, 68)| 0.17    |
| Donor Sex                            |             |                |            | 0.15    |
| 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)       |                |            | 0.41    |
| ECOG Performance Status              |             |                |            | 0.51    |
| 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                 |             |                |            | 0.97    |
| AML                                  | 11 (36.7)   | 10 (37)        | 21 (36.8)  |         |
| 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                |             |                |            |         |
| 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)   |         |
| R-IPSS 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    |             |                |            |         |
| No                                   | 5 (16.7)    | 5 (18.5)       | 10 (17.5)  |         |
| Yes                                  | 25 (83.3)   | 22 (81.5)      | 47 (82.5)  |         |
| Marrow blasts at enrollment (%)      |             |                |            | 0.28    |
| median (range)                       | 13 (4, 60)  | 11 (4, 58)     | 12 (4, 60) |         |
### Table 2. Study Outcomes Among Patients Receiving GVAX/Placebo vaccinations after HSCT

| Outcome                        | GVAX          | Placebo        | P-value |
|--------------------------------|---------------|----------------|---------|
| Grade II-IV aGVHD @1yr         | 34% (4,31)    | 12% (2.8,27)   | 0.13    |
| Grade III-IV aGVHD @1yr        | 16% (4.7, 33)| 0%             | 0.09    |
| Chronic GVHD @3yrs             | 47% (27,64)   | 59% (37,76)    | 0.26    |
| Mod-severe cGVHD @3yrs         | 23% (10,40)   | 33% (16,52)    | 0.42    |
| 18-month PFS*                  | 53% (34,69)   | 55% (35,72)    | 0.79    |
| 18-month OS                    | 63% (43, 77)  | 59% (38, 75)   | 0.86    |
| 18-month NRM                   | 17% (6, 32)   | 7.7% (12, 22)  | 0.18    |
| 18-month Relapse               | 30% (15, 47)  | 37% (19, 55)   | 0.51    |

*Primary endpoint. (): 95% confidence interval
Figure Legends

**Figure 1.** Study flow diagram

**Figure 2.** PFS, OS, NRM and Relapse after HSCT with GVAX vs. placebo

**Figure 3.** Transplant and vaccination outcomes stratified by conditioning intensity

**Figure 4.** Reconstitution of immune cell subsets after HSCT and GVAX vs. placebo vaccinations
Figure 1. Study flow diagram

Randomized n = 123

Excluded (n = 31)
- Insufficient blasts for vaccine generation (n=13)
- Withdrawal of consent (n=7)
- Death before HSCT (n=5)
- No longer meeting inclusion criteria for HSCT (n=5)
- Transplanted off study during protocol hold (n=1)

AlloHSCT n = 92

Not vaccinated (N=35)
- GVHD requiring systemic steroid therapy (n=21)
- relapse requiring therapy (n=3)
- grade 3+ non-hematologic event (n=3)
- graft failure (n=1)
- withdrawal of consent (n=1)
- Other (N=6)

GVAX n = 30

Placebo n = 27
Figure 2. PFS, OS, NRM and Relapse after HSCT with GVAX vs. Placebo
Figure 3. Transplant and vaccination outcomes stratified by conditioning intensity
Figure 4. Reconstitution of Immune cell subsets after HSCT and GVAX vs. Placebo Vaccinations