Study Protocol and Statistical Analysis Plan

Title: A randomized placebo-controlled phase II trial of irradiated, adenovirus vector transfected GM-CSF secreting autologous leukemia cell vaccination (GVAX) versus placebo vaccination in patients with advanced MDS/AML after allogeneic hematopoietic stem cell transplantation

NCT number: 01773395

Date: 21-OCT-2020

Protocol Version Date: October 21, 2020

Local Protocol #:12-217

Title: A randomized placebo-controlled phase II trial of irradiated, adenovirus vector transfected GM-CSF secreting autologous leukemia cell vaccination (GVAX) versus placebo vaccination in patients with advanced MDS/AML after allogeneic hematopoietic stem cell transplantation

IND Sponsor: Robert Soiffer, MD BB-IND 17904

Principal Investigator: **Vincent T. Ho, MD**
Dana- Farber Cancer Institute

Site Responsible Investigators: Jacalyn Rosenblatt, MD
Beth Israel Deaconess Medical Center (BIDMC)

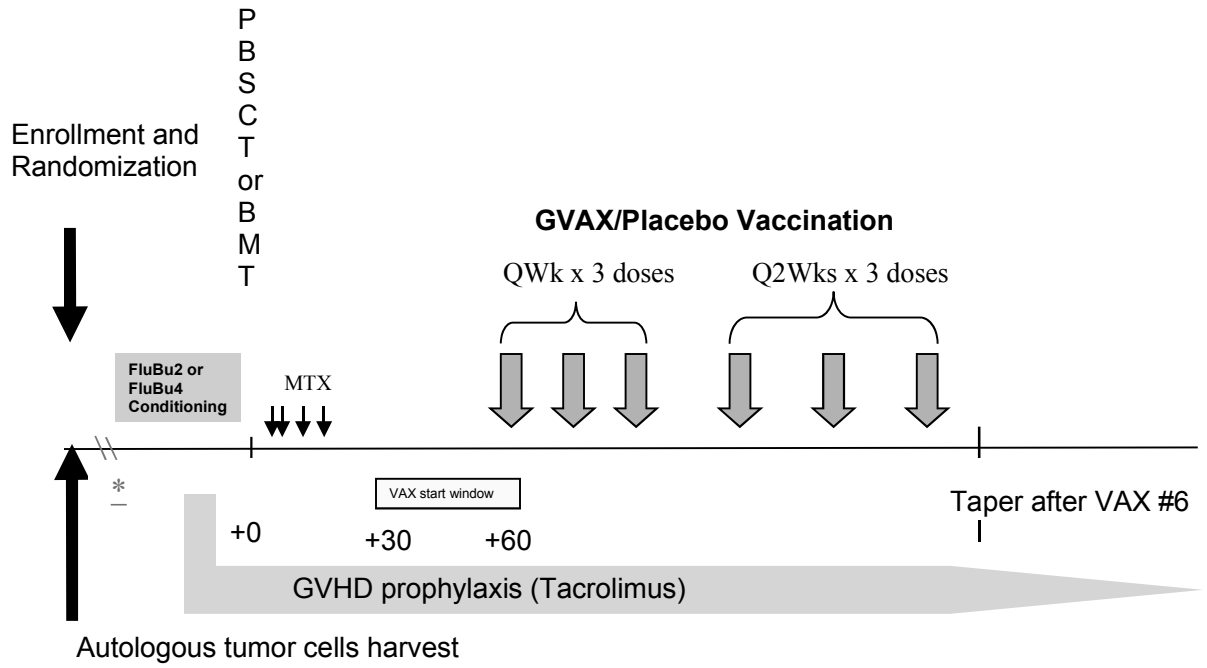
Yi-Bin Chen, MD
Massachusetts General Hospital (MGH)

Statistician: Haesook Kim, PhD/DFCI

Responsible Research Nurses:
Jennifer Brock, RN/DFCI
Susan Stephenson, RN/DFCI

Agent: GM-CSF secreting autologous leukemia cell vaccine (GVAX)

SCHEMA



* Cytoreductive therapy for MDS or AML may be given between harvest and transplant conditioning, at the discretion of the treating MD

TABLE OF CONTENTS

1.0 OBJECTIVES

This is a randomized placebo-controlled Phase II study of irradiated, adenovirus vector transfected GM-CSF secreting autologous leukemia cell vaccination (GVAX) vs. placebo given early after allogeneic HSCT for advanced MDS or AML patients with active disease going into transplantation.

1.1 Study Design

GVAX vaccines will be generated by adenoviral vector mediated GM-CSF gene transfer into myeloblasts harvested from study subjects prior to admission for hematopoietic stem cell transplant (HSCT). Patients with Myelodysplastic syndromes (MDS) with excess marrow blasts or Acute myeloid leukemia (AML) not in remission are eligible. Leukemia cells will be harvested for vaccination generation after enrollment. Patients will be randomized (1:1) to GVAX versus placebo arm prior to transplant admission. The assignment of treatment arm will be blinded to the patients, treating physicians, nurses, research staff, and data assessors. Between the leukemia cell harvest and admission for allogeneic HSCT, patients are allowed to receive additional therapy (e.g. HMA therapy, chemotherapy etc.) at the discretion of the treating physician. Between day+30 to +60* after HSCT, patients with adequate hematologic recovery and no GVHD requiring systemic therapy will receive GVAX or placebo vaccine (normal saline) injections weekly x 3 week, then starting on week 5, every 2 weeks for 6 weeks, to complete a set of six total vaccinations. Patients will remain on tacrolimus for GVHD prevention during the vaccination period. Tacrolimus taper may commence after completion of vaccination, at the discretion of the treating physician, with the goal to be off immune suppression by 6-9 months post transplant in the absence of GVHD.

* Day 60 or next business day, if day 60 occurs on a weekend or holiday

1.2 Primary Objective:

Progression-free survival (PFS) at 18 months after randomization

1.3 Secondary Objectives:

a) To assess the safety of vaccination following allogeneic (ablative or reduced intensity) stem cell transplantation

b) To assess incidence of grade 2-4 and grade 3-4 acute GVHD, and chronic GVHD after vaccination following allogeneic stem cell transplantation.

c) To assess PFS after start of vaccination

d) To assess relapse and non-relapse mortality after vaccination

e) To assess overall survival after vaccination

f) To assess biologic activity of GVAX as compared to placebo vaccination after HSCT (see Section 8.0)

2.0 BACKGROUND

2.1 Study Agent

GVAX vaccines will be generated by adenoviral vector mediated GM-CSF gene transfer into myeloblasts harvested from study subjects prior to admission for HSCT.

2.2 Study Disease: Allogeneic SCT for Advanced MDS/AML

Although allogeneic hematopoietic stem cell transplantation is a potential curative therapy for patients with advanced hematologic cancers, disease relapse remains the single most frequent cause of treatment failure. Success of allogeneic HSCT relies heavily on the development of anti-tumor activity generated by the new donor derived immune system post transplant, also known as the graft-versus-tumor effect (GVT). Attempts to enhance GVT by early withdrawal of immune suppression or administration of donor lymphocyte infusions (DLI) have been hampered by increased rates of graft-versus-host disease (GVHD). Strategies to safely inducing GVT without promoting GVHD are needed.

2.3 Rationale

The early post-transplant period after allogeneic HSCT provides an attractive platform for adoptive immunotherapy and cancer vaccination strategies that could elicit tumor specific responses from the resurging donor immune cells. Vaccination during the first 2 months after transplantation may further capitalize upon the lymphodepletion resulting from the conditioning chemotherapy, and take advantage of the homeostatic lymphoid expansion that occurs during this period. Indeed, our pilot study in patients with advanced MDS/AML (DFCI protocol 04-023), as described in section 2.4 below, demonstrated that vaccination with GVAX early after allogeneic HSCT is feasible, safe, and associated with very encouraging clinical outcomes with detectable immune responses.

To extend this effort, we propose a randomized placebo controlled trial investigating the efficacy of vaccination using GM-CSF transfected autologous leukemia cells (GVAX) in patients with advanced MDS or high risk AML early after allogeneic HSCT.

2.4 Correlative Studies Background

2.4.1 GM-CSF Based Tumor Vaccines (GVAX)

Several animal tumor models have demonstrated potent and specific anti-tumor immune responses with appropriate immune stimulation. The local release of immuno-modulatory cytokines has been shown to be a useful adjuvant to tumor cell-based vaccine strategies. This can be most effectively achieved by transfection of the cytokine gene into the tumor cell, which is then irradiated and injected into the animal as a tumor-specific vaccine. In one study in which over 10 cytokines were compared in poorly immunogenic murine tumors, GM-CSF was identified as the most potent cytokine capable of generating systemic immunity that was CD4+ and CD8+ T cell dependent.[2] This vaccine approach has been shown to protect and cure mice after challenge with various cancer cell lines, including AML,[3] and suggests that it is effective in treating minimal disease states.

The use of GM-CSF as a vaccine adjuvant has been explored in several phase I clinical trials, in diseases such as AML, melanoma, hepatocellular carcinoma, pancreatic cancer, prostate cancer and renal cell carcinoma.[4-7] These GM-CSF augmented vaccines were safe and well tolerated in patients. The vast majority of adverse events were grade 1-2 injection site reactions characterized by erythema, induration, tenderness and localized pruritis. Systemic adverse events have included generalized pruritis, rash, fatigue, fever, headache and malaise.

At DFCI, a series of vaccination studies using GM-CSF based vaccines have been performed. In one phase I clinical trial, irradiated autologous melanoma cells engineered by retroviral mediated gene transfer to secrete GM-CSF (GVAX) were used to vaccinate 21 patients with metastatic melanoma.[7] In subsequent studies, irradiated autologous tumor cells engineered to secrete GM-CSF by adenoviral mediated gene transfer were administered by intradermal and subcutaneous injection at weekly and biweekly intervals in 34 patients with metastatic melanoma[6] and 34 patients with metastatic lung cancer [5] (average GM-CSF secretion 745 ng/10^6 cells/24 hours and 513 ng/10^6 cells/24 hours, respectively). In each of these studies, a common feature was the development of intense localized skin reaction at the vaccine injection sites, associated with a dramatic influx of dendritic cells, macrophages, eosinophils and T lymphocytes which were not present prior to vaccination. These reactions were associated with subsequent immune mediated tumor rejection at distant metastatic sites. Collectively, these studies showed that local production of GM-CSF improves tumor antigen presentation by increasing the number and activity of professional antigen presenting cells in the tumor microenvironment. In the adenoviral melanoma trial, at a minimum follow up of 36 months, 10 patients are alive (29%) and 4 with NED.[6] Studies to elucidate the targets of the immune response have provided evidence of the presence of a coordinated humoral and cellular response to disease specific antigens.[8, 9]

2.4.2 GVAX after Allogeneic HSCT for MDS/AML

We have previously completed a pilot clinical trial using AML-GVAX in patients with advanced MDS or refractory AML after allogeneic stem cell transplantation (DFCI protocol 04-023), the results of which have recently been published.[10] In this study, patients with MDS-RAEB or AML not in remission who have a donor matched at HLA-A,B, and DRB1 were eligible. Leukemia blasts were collected for GVAX generation prior to start of conditioning. The preparative regimen consisted of fludarabine 30mg/m2/d IV x 4, and busulfan 0.8mg/kg IV q12H x 8 doses. G-CSF mobilized PBSC was infused on day 0. GVHD prophylaxis included tacrolimus starting day -3, and methotrexate 5 mg/m2 days 1,3,6,11. GM-CSF (Leukine) 250 mg/m2 SC QD was administered from day+1 until neutrophil engraftment. GVAX was initiated between day +30 to +45 if there was adequate hematologic count recovery and no grade II-IV acute GVHD. GVAX was administered weekly for the first three doses, and q2weeks for the last three doses. Taper of tacrolimus began after vaccine completion. Irradiated autologous tumor cell DTH were given pre and post vaccinations to assess immune responses.

A total of 24 patients were transplanted on this study (13 unrelated, 11 related donors). Vaccines were successfully produced for all subjects. The average GM-CSF secretion rate was 52 ng/10^6 cells/24 hours. The average pre-freeze viability was 98%. Diseases included: AML (16), MDS/RAEB (6), CML myeloid blast crisis (2). Median age was 62 years (range, 41-71). All patients had active disease at the time of transplant admission. Median marrow blast content prior to HCT was 20% (range, 6-91%). Median vaccine cell dose was 1.0 x 10^7 cells (range, 0.1-1.0 x10^7). Nine patients did not initiate

vaccination because of rapidly progressive disease post-HCT (4), grade II-IV acute GVHD (3), sepsis (1), pneumonitis (1). Fifteen patients initiated vaccination. Ten patients completed all six vaccinations. 5 patients did not complete all vaccinations (progressive leukemia-3, appendicitis-1, and grade II skin acute GVHD-1). Two patients developed late acute GVHD in the setting of tacrolimus taper after all vaccinations were completed. No patient develop grade 3-4 GVHD. The cumulative incidence of grade 2 acute GVHD for vaccinated patients was 20%. Seven of 15 patients vaccinated developed chronic GVHD (6 mild, 1 moderate). All responded to corticosteroid therapy. The cumulative incidence of chronic GVHD was 47%. Among the 15 patients who started vaccination after transplant, 9 are alive and in complete remission with a median follow up of 45 months (range 33-63 months). Kaplan-Meier estimate for 2-yr overall survival (OS) for all vaccinated patients was 56 ± 14% (Figure 1). Nine of 10 patients who completed all 6 vaccines remain in complete remission with an estimated Kaplan-Meier overall survival of 88 $\pm$ 12%.

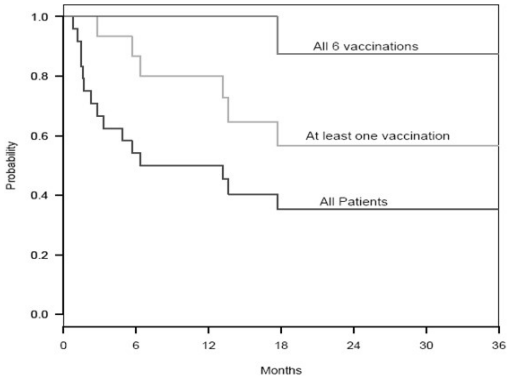


Figure 1. Survival after RIC HCT for advanced AML and MDS. OS is displayed for all pts (blue line), those that received at least 1 vaccination (green line), and those that completed all vaccinations (red line).

To obtain a historical control cohort most comparable to patients who started vaccination in this trial, we analyzed our institutional data for contemporaneous patients aged >40 years undergoing RIC SCT between 2004-2007 with similar Bu/Flu conditioning for untreated MDS RAEB or AML not in remission. We further restricted the population to those who did not develop grade II-IV GVHD before day+45, and who were alive at day +60 to render them most similar to patients on our trial who would have started GVAX. Using these criteria, we identified 34 patients (16 AML, 18 MDS RAEB) in the historical control group. The 2-year overall survival for this group of patients was 18% as compared to 56% among patients who received at least 1 vaccination in this study (DFS (18% vs. 56%, $p = 0.02$).

Injections of irradiated, autologous, GM-CSF secreting leukemia cells elicited local skin reactions in 14 assessable patients, and the intensity of these responses typically increased with subsequent inoculations. Clinically, the vaccine sites were characterized by erythema and induration that resolved within 72 hours. Punch biopsies demonstrated accumulations of dendritic cells, macrophages, neutrophils, eosinophils, and lymphocytes. Immunohistochemistry revealed abundant $CD1a^+$ dendritic cells and $CD4^+$ and $CD8^+$ T cells, with scattered $CD20^+$ B cells. Intensity of histologic response increased between the first and fifth vaccine. To assess DTH response to vaccination, we injected irradiated, autologous, non-transduced myeloblasts before and after vaccination. Initial DTH responses revealed no significant infiltrates in all thirteen patients assessed. However, when tested again at the time of the fifth vaccine while still on immune suppression or thereafter, seven of eight subjects developed significant DTH responses with brisk cellular infiltrates (Figure 2). All 7 patients with DTH reactions achieved long-term clinical remissions. In one long term surviving patient, a bone marrow specimen obtained shortly after completing immunization demonstrated the presence of abundant $CD3^+$ T cells that were associated with malignant myeloblasts and frequent eosinophils distributed through the marrow (Figure 3). Three months later, no leukemia was evident in the bone marrow, which instead displayed normal hematopoietic differentiation. These findings suggest vaccination early after HCT can provoke reactions in distant sites of disease.

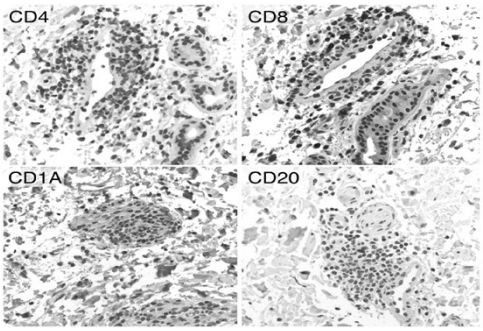


Figure 2. DTH reactions to irradiated non- transduced leukemia cells after vaccination. Depicted is infiltration of CD4, CD8, CD20, and CD1a cells.

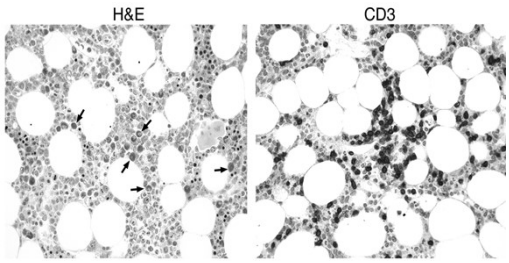


Figure 3. Vaccination after HCT is associated with anti-leukemic immune response in the bone marrow. CD3+ T cells (right) and eosinophilic infiltrates (left) are highlighted.

In solid tumor patients that responded to GM-CSF secreting tumor cell vaccines, we have identified a correlation between immune-mediated tumor destruction and decreases in the levels of circulating soluble MHC class I chain-related protein A (MICA).[11] MICA and the closely related MHC class I chain-related protein B (MICB) are ligands for NKG2D, an activating receptor expressed on NK cells and $CD8^+$ T lymphocytes that contributes to anti-tumor cytotoxicity.[12] Tumor cells may escape from NKG2D mediated immune destruction through the shedding of surface MICA and possibly other ligands. Prior to vaccination, 13/15 patients had high levels of shed MICA and/or MICB. Six of 7 long-term responding subjects with detectable ligands showed marked decreases in response to therapy. In the 3 patients who achieved complete remissions following vaccination and tacrolimus withdrawal, the levels of soluble ligands closely mirrored the disease course. In contrast, patients who did not have sustained remissions failed to display persistent decreases in shed MICA and MICB. To determine whether declines in soluble NKG2D ligands are linked to clinical response, we examined serial serum samples obtained from 35 patients who maintained a complete remission after allo-HCT alone. Only a minority showed high pre-treatment levels of soluble MICA in a range similar to the vaccinated subjects, perhaps indicative of the advanced disease-risk status of the immunized patents. Nonetheless, none of the control subjects with high levels of shed MICA exhibited the marked decrease in ligands detected in the vaccinated patients.

We also investigated whether we could identify potential targets of vaccination post-HSCT. One target, protein disulfide isomerase (PDI) has been identified through screening a murine renal cell carcinoma cDNA expression library with sera from mice that received GVAX. ERp5, a closely related disulfide isomerase involved in MICA shedding, evokes potent humoral reactions in solid tumor patients who clinically respond to GM-CSF-secreting tumor cell vaccines. High titer antibodies to human PDI were similarly induced in an AML patient who achieved a complete response after post-HSCT vaccination.[13] In addition, sera from 5 of 9 long term survivors recognize L1CAM (CD171), a surface protein overexpressed in a variety of tumors, where it promotes transformation through MAP kinase pathways.[14] Monoclonal antibodies targeting L1CAM mediate tumor destruction in xenograft models.[15] These

findings raise the possibility that vaccination-induced antibodies might mediate tumor eradication.

Given that all of the patients in this trial had active disease at the time of transplant and then received a reduced intensity conditioning regimen, we would have expected few to enter complete and sustained remission. These promising results suggest GVAX vaccination is safe and may have anticancer activity in patients with MDS/AML after allogeneic HSCT.

3.0 PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 AML, MDS-RAEB (including CMML with excess blasts and MDS/MPN overlap syndrome) not in remission (defined as ≥ 5% blast in bone marrow or peripheral blood) prior to leukemia cell harvest. Patients may receive additional cytoreductive therapy after leukemia cell harvest and before admission for transplant, at the discretion of the treating physician. For patients with MDS-EB1(<10%blasts) or CMML-1, it is recommended that they proceed directly to transplant after the harvest if donor is available. If there is an extended delay, interval therapy with HMA is allowed.

3.1.1.1 Patients may or may not have active disease at the time of transplant conditioning, but for RIC candidates, it is strongly recommended that disease is cytoreduced such that the pre-transplant admission marrow shows:

- <30% blasts in a normocellular marrow (>=50% cellularity), or
- <50% blasts in a hypocellular marrow (<50% cellularity)

3.1.2 HLA 8/8 or 7/8 matched related or unrelated donor available, as determined by antigen or allele level typing at HLA A, B,C, and allele level typing at HLA DRB1.

3.1.3 ECOG performance status 0-2.

3.1.4 Age ≥18 years. Because no dosing or adverse event data exist on the use of GVAX in participants <18 years of age, children are excluded from this study.

3.1.5 Patient deemed to be suitable candidate for myeloablative or reduced intensity conditioning allogeneic HSCT using PBSC or marrow as stem cell source.

3.1.6 Participants must have normal organ function as defined below:

Total bilirubin ≤ 2.0 mg/dL

(In patients with Gilbert's Syndrome, Total Bilirubin ≥ 2.0 is permitted)

AST (SGOT)/ALT (SGPT) ≤ 3 X institutional upper limit of normal

Serum creatinine ≤ 2.0 mg/dL

3.1.7 The effects of GVAX on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.8 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Leukemia with active CNS involvement

3.2.2 Positive HIV or HTLV-1 serology.

3.2.3 Participants may not be receiving any other Non-FDA approved study agents at the start of conditioning for stem cell transplantation. Patients may receive Non-FDA approved agents at the time of screening/enrollment as long as such agent(s) will be discontinued by the start of conditioning for transplantation.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to GM-CSF.

3.2.5 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because pregnancy is an exclusion for chemotherapy and stem cell transplantation

3.2.7 Individuals with a history of a different malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 2 years. Individuals with the following cancers are eligible if diagnosed and treated within the past 2 years: cervical cancer *in situ*, and basal cell or squamous cell carcinoma of the skin.

3.2.8 Prior allogeneic transplant

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

This study will be open to all participants with AML/MDS, including women, minorities, and other underrepresented populations, as long as they meet the conditions of eligibility outlined above.

4.0 REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. QACT does not randomize after hours. Subjects must be registered between 8 A.M. and 5 P.M. Monday – Friday.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

   Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at 617-632-2295.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant.

5. The QACT Registrar will send an email confirmation of the registration to the person initiating the registration immediately following the registration. Randomization result/ study arm assignment will be emailed to Cell Manipulation Core Facility (CMCF). All other study personnel will remain blinded to the study arm assignment.

5.0 TREATMENT PLAN

5.1 Treatment can be administered on an either inpatient or outpatient basis. Expected toxicities and potential risks for irradiated, adenovirus vector transfected GM-CSF secreting autologous leukemia cell vaccination (GVAX) are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered at the start of conditioning through vaccination with the intent to treat the participant's malignancy. Pre-treatment criteria/Pre-transplant

5.1.1 **Pre-transplant evaluation**: All subjects enrolled on this study will undergo standard pre-transplant evaluation before the transplant date as required by Foundation for the Accreditation of Cellular Therapy (FACT) regulations and according the institutional standard operating procedure.

5.1.2 **Randomization**: After enrollment, patients will be randomized in a 1:1 fashion to one of 2 arms on the study: "GVAX" vs. "Placebo". Randomization will be stratified by center (DFCI, BIDMC, MGH), disease, age (≥50 vs. <50), and conditioning intensity (myeloablative Flu/Bu4 vs. reduced intensity conditioning Flu/Bu2). Leukemia cell harvest will be performed on all patients regardless of their randomization status.

5.1.3 **Leukemia cell harvest**: Blood for infectious disease marker (IDM) testing will be drawn prior to, or on the day of leukemia cell harvest. Leukemia cells for vaccine generation will be harvested via bone marrow aspiration and/or peripheral blood draw. Please note that the DFCI Cell Manipulation Core Facility can only accept collections Monday-Wednesday. The procedure for vaccine generation is detailed below:

5.1.3.1 Bone marrow collection: 40 cc of marrow aspirate will be taken to collect leukemic blasts for vaccine generation and laboratory immune studies. If the patient's most recent marrow biopsy was performed within 2 weeks of the collection, an additional aspirate and core biopsy for routine clinical staging might be performed as a part of the marrow harvest at the discretion of the treating physician, and based on standard clinical practice.

5.1.3.2 Peripheral blood draw: If the patient has blasts in peripheral blood, tumor cells may be collected by blood draw. The amount of blood required may vary depending on calculations of blood volume needed to yield a desired target of 2×10^7 leukemia cells. If the calculated volume of blood draw exceeds 50 ml, it is recommended that bone marrow aspiration be used as the method for tumor cell harvest.

5.1.3.3 Leukapheresis: In cases where peripheral blood draw and/or marrow harvest is infeasible or unsuccessful, and the patient has circulating blasts, a single leukapheresis may be

performed to harvest leukemia blasts. The leukapheresis collection will follow standard institutional leukapheresis guidelines.

5.1.3.4 Multiple harvests: If the first attempt to collect sufficient cells to generate six vaccines is insufficient, a second harvest may be performed. If the second harvest is insufficient, the participant will be taken "off-study."

5.1.3.5 All leukemia cells harvested through bone marrow aspiration and/or peripheral blood and/or leukapheresis draw will be sent to DFCI for vaccine/placebo preparation.

5.1.4 **Vaccine preparation**: For study participants randomized to the GVAX arm, autologous myeloblasts will be introduced into short-term tumor culture in the presence of G-CSF, and transduced with a replication defective adenoviral vector encoding human GM-CSF.

After transduction, the tumor cells will be washed extensively and irradiated with 10,000 cGy. A small aliquot of the transduced cells will be placed into culture and GM-CSF secretion will be determined by ELISA. The target level for GM-CSF production will be at least 40 ng/10^6 cells/24 hours, although achieving this secretion will not be required for vaccine administration. Routine sterility cultures and testing for endotoxin and mycoplasma contamination will be performed.

Tumor cells for vaccination and immunologic evaluation will be cryopreserved and stored in the vapor phase of liquid nitrogen. Six individual vaccine aliquots will be prepared for each patient. Cell dose per aliquot will be fixed for an individual patient and will range from about $1x10^6$ cells per aliquot to $1x10^7$ cells per aliquot. The exact vaccine cell dose in each patient will vary depending on the total yield of myeloblasts harvested. The dosage will be determined by dividing the total cell yield following transduction into six aliquots. For total cell yields greater than $6x10^7$, individual aliquots will remain at a maximum of $1x10^7$ cells per dose. Thus, vaccine cell dosage will vary depending on the final vaccine cell yield after processing. This variation in cell dosage is designed to maximize each patient's opportunity of receiving vaccinations and is not based on any expectation of significant differences in toxicity as a function of cell number. On the day of vaccine administration, cells will be thawed in a dedicated laminar flow biosafety cabinet in the CMCF and washed. Cells will be re-suspended in a volume of 1 ml. for administration via a masked syringe

Details of the construction of the adenoviral producer cell line and safety tests on this line are included in the Appendix (vector manufacturing process). The adenoviral vector was manufactured by Cell Genesys and approved for clinical use by the Food and Drug Administration.

5.1.5 **Placebo Vaccine Preparation**: The placebo vaccine will be composed of 1 ml sterile Normal Saline in a masked syringe. The vaccine will be thawed, and the thawed placebo/vaccine will be drawn into a masked syringe at DFCI to protect blinding. The syringe will then be released for administration.

5.1.6 **Cytoreductive therapy between harvest and HSCT**: Patients may receive additional cytoreductive therapy after leukemia cell harvest and before admission for transplant, at the discretion of the treating physician. For patients with MDS-RAEB1, it is recommended that they proceed directly to transplant after the harvest if donor is available. If there is an extended delay,

interval therapy with HMA is allowed.

5.1.6.1 Patients may or may not have active disease at the time of transplant conditioning, but for RIC candidates, it is strongly recommended that disease is cytoreduced such that the pre-transplant admission marrow shows:

- <30% blasts in a normocellular marrow (>=50% cellularity), or
- <50% blasts in a hypocellular marrow (<50% cellularity)

5.2 Allogeneic Hematopoietic Stem Cell Transplantation

5.2.1 **Flu/Bu2 RIC Transplant**: The reduced intensity conditioning regimen will consist of fludarabine (120 mg/m^2 total) and low dose intravenous busulfan (6.4 mg/kg total). Fludarabine 30 mg/m^2/d will be administered as a bolus intravenous infusion once a day for 4 days on days -5, -4, -3,-2 (based on actual body weight). Busulfan 0.8 mg/kg (based on actual body weight) will be administered intravenously twice daily (approximately 12 hours apart) on day-5,-4,-3,-2 for a total of 8 doses. Since it is dose twice daily, it is suggested that the busulfan be administered first, with the fludarabine dose sandwiched in between. Fludarabine and busulfan can be diluted per institutional practice. The rate of the fludarabine and busulfan infusions can be done according to institutional practice. In conjunction with chemotherapy, patients will receive pre-hydration and intravenous fluids and/or diuretics as needed, and antiemetics as per institutional guidelines. Day 0 is defined as the first day of infusion of donor PBSC (or bone marrow). In instances where the donor product arrival is delayed, the day 0 of transplant may be pushed back a day. Donor PBSC or marrow cells will be administered according to standard practice. For PBSC products, the stem cell dose will be a minimum of $2x10^6$, with a recommended target of $5x10^6$ CD34+ cells/kg. For marrow products, the recommended target is 2 x 10^8 TNC/kg.Following stem cell infusion, RIC SCT patients will receive GM-CSF 500 mcg SC qd starting on day +1, until absolute neutrophil count is>1000 on 2 consecutive values over 2 or more days. GM-CSF may be held if patient has circulating blasts, develops severe bone pain, or at the discretion of the transplant MD based on clinical judgment. Levofloxacin 500mg PO QD (or 250mg PO QD if CrCl < 50) or another antibiotic is recommended as bacterial prophylaxis until ANC is >500 µL. Prophylactic antibiotics for *Pneumocystis carinii* and HSV/VZV will be administered as per standard institutional guideline for RIC SCT. CMV PCR surveillance and preemptive therapy for reactivation should follow standard institutional guideline.

5.2.2 **Flu/Bu4 Myeloablative transplantation**- The myeloablative conditioning regimen will consist of Fludarabine and high dose intravenous Busulfan approximately q6hrs for 4 days, as follows:

5.2.2.1 **Fludarabine** 40 mg/m^2 per day will be administered as a bolus infusion administered by IV infusion over approximately 1 hour for 4 days on days -5, -4, -3, -2 (based on actual body weight; or adjusted ideal body weight for those >125% of ideal body weight). Adjusted ideal body weight will be defined as ideal weight plus 25% of the difference between ideal and actual weight). Fludarabine can be diluted per institutional practice.

AIBW = IBW + (0.25)(ABW-IBW)

AIBW – adjusted ideal body weight

IBW – ideal body weight

ABW – actual body weight

5.2.2.2 **Busulfan** 0.8mg/kg will be administered intravenously 4 times daily (approximately q6hrs apart) for 5 days on day -5,-4,-3,-2, -1 for a total of 16 doses, as per institutional guidelines. The rate of the busulfan infusions can be done according to institutional practice. Dosing of busulfan will be based on actual body weight, or adjusted ideal body weight for patients >125% of ideal body weight. Adjusted ideal body weight will be defined as ideal weight plus 25% of the difference between ideal and actual weight.

AIBW = IBW + (0.25)(ABW-IBW)

AIBW – adjusted ideal body weight

IBW – ideal body weight

ABW – actual body weight

In conjunction with chemotherapy, patients will receive seizure prophylaxis with Keppra from day -5 until day-1. Pre-hydration and intravenous fluids and/or diuretics will be as per the HSCT standard practice. Anti-emetics will be as per institutional guidelines.

The donor PBSC (or bone marrow) product will be infused on day 0. In instances where the donor product arrival is delayed, the day 0 of transplant may be pushed back a day. Stem cells will be administered according to standard practice. Stem cell dose will be a minimum of 2 $x10^6$, with a recommended target of $5x10^6$ CD34+ cells/kg. GM-CSF will be given to hasten white blood count recovery starting on day+12, until absolute neutrophil count (ANC) is >1000/ul on 2 consecutive values over 2 or more days. GM-CSF may be held if ANC is already > 500 on day +12 or at the discretion of the treating physician based on clinical judgment. Infection prophylaxis for herpes viruses and *Pneumocystis carinii* will follow standard institutional practice. CMV PCR surveillance and preemptive therapy for reactivation should follow standard institutional guideline.

5.2.3 **GVHD prophylaxis:** GVHD prophylaxis for both myeloablative or RIC HSCT will include oral tacrolimus starting on day –3, and methotrexate on Days +1,3,6,11, as follows:

5.2.3.1 **Tacrolimus (FK506)** will be given orally at a dose of 0.05 mg/kg PO bid starting day –3. Subsequent dosing will be based on clinical toxicity, and trough blood levels with recommended target of 5-10 ng/ml. Doses may be rounded to the nearest 0.5 mg dose and adjusted as needed to maintain therapeutic serum tacrolimus levels. The dose may be replaced if the patient vomits within 15 minutes of taking a dose, at the discretion of the transplant physician. Tacrolimus may be switched to intravenous dosing to maintain the same therapeutic goal level if necessary.

5.2.3.2 **Methotrexate**

For the RIC cohort (FluBu2)- The methotrexate dose will be 5 mg/m^2 IV on day+1,3,6,11, infused as per standard institutional guidelines. In cases where stem cell infusion (day 0) spans more than 1 day, the day +1 dose of methotrexate

should start 1 day after the end of the SC infusion.

For the Myeloablative cohort (FluBu4)- The methotrexate dose will be 15 mg/m^2 IV on day +1, and 10mg/m2 on days +3,6, and 11. Methotrexate should be infused as per standard institutional guidelines. In cases where stem cell infusion (day 0) spans more than 1 day, the day +1 dose of methotrexate should start 1 day after the end of the stem cell (SC) infusion. Leucovorin rescue is allowed per standard practice guidelines if necessary, at the discretion of the transplant physician. The day 11 dose of MTX may be reduced 50% or omitted if deemed necessary based on the clinical situation, and at the discretion of the transplant physician. In cases where clinical toxicities, e.g. renal failure, pleural effusion, etc. would render completing at least 3 doses of methotrexate unsafe, substitution with mycophenolate mofetil (cellcept) 1000mg PO/IV BID or other agent such as sirolimus is allowed at the discretion of the treating physician. In instances where MTX is substituted by cellcept, it is recommended that the cellcept be discontinued around day +28 in the absence of GVHD.

5.2.4 **Tacrolimus Taper:**

5.2.4.1 Tacrolimus taper is NOT allowed during the vaccination period. Taper may commence 1 week after the last GVAX/Placebo vaccine, at the discretion of the transplant physician, with the goal to be off immune suppression by 6-9 months in the absence of GVHD.

5.2.4.2 **Tacrolimus taper for patients with progressive disease before completion of vaccinations**: Patients with evidence of progressive disease AND requiring additional therapy or taper of immune suppression will receive no further vaccinations. In these cases, the tacrolimus taper will be performed at the discretion of the treating physician.

5.3 GVAX/Placebo Vaccination

Vaccination with GVAX or placebo vaccine will commence after hematologic recovery has occurred and the patient is between day +30 to day +60* post transplant. Vaccine administration can occur on an either inpatient or outpatient basis. At any time between day+30 to day +60*, the patient may undergo a bone marrow aspirate/biopsy and GVAX/placebo vaccination may commence provided the following criteria are satisfied:

* Day 60 or next business day, if day 60 occurs on a weekend or holiday

5.3.1 **Criteria for starting GVAX/Placebo administration:**

- No grade II-IV acute GVHD
- No systemic corticosteroid therapy*
- No uncontrolled acute infection
- No CTC grade ≥ 3 non-hematologic toxicity
- Absolute neutrophil count ≥ 500/ul off growth factor

- Platelet count ≥ 10K/ul without transfusion.
- Absolute eosinophil count ≤ 5000/ul

* Except for physiologic doses of steroids for adrenal insufficiency

Participants who do not meet the above criteria to start vaccination by day +60 will be considered "off treatment", and followed for survival.

5.3.2 GVAX/Placebo Administration:

5.3.2.1 Vaccinations will be administered weekly x 3 weeks, then q2weeks x 6 weeks for a total of 6 vaccines. The weekly scheduling of vaccination can be +/- 2 days for scheduling flexibility. A physician, PA, NP, RN, or research RN can administer the injections in either an outpatient or inpatient clinical setting. Injections will be administered according to standard nursing procedure in the patient's arms and thighs on a rotating basis. Injections must be performed without the prior application of EMLA or other topical or local anesthetics, as such compounds both could obscure local reactions to the vaccination and could cause reactions that might be interpreted as vaccine reactions. Approximately half of the vaccine dose should be administered subcutaneously, and remaining half the vaccine dose intradermally. It is suggested that about half of the vaccine be given first intradermally, and the rest subcutaneously by changing that angle of the needle.

5.3.2.2 Information regarding the number and location of the injections, the date of vaccination, route of administration, total volume of vaccine administered and vaccine site injection reactions will be collected for each vaccination and recorded in the patient medical record.

5.3.2.3 A skin punch biopsy of the vaccine site is strongly encouraged (but not required) for histologic and laboratory correlative examination 2-3 days after the 1st and 5th vaccination. Skin punch biopsies will be sent to DFCI.

5.3.2.4 The Vaccine Specific Toxicity Form (Appendix I) must be completed by a study investigator at weekly during vaccination period, and at 1 month after the last vaccination.

5.3.3 Early discontinuation of GVAX/Placebo vaccination

5.3.3.1 Disease relapse or progression requiring taper or withdrawal of tacrolimus, or additional systemic therapy.

5.3.3.2 Acute GVHD or chronic GVHD requiring systemic steroid therapy.

5.3.3.3 CTC grade ≥ 4 non-hematologic toxicity possibly, probably or definitely related to vaccination.

5.3.3.4 Delay of vaccination over 14 days (see below)

5.3.4 **Delay of GVAX/Placebo vaccination**: If severe hematologic toxicity (defined as ANC <500/ul, platelet < 10K/ul, or absolute eosinophil count > 5000/ul), CTC grade ≥ 3 non-hematologic toxicity develops during the vaccination period, vaccination may be delayed up to 14 days until the event has improved by at least 1 CTC grade. If condition is not improved after 14 days in the opinion of the treating physician and/or study PI, or if the toxicity recurs upon re-initiation of vaccination, no further vaccination will be given. In an event where the toxicity is grade < 3, vaccination may also be delayed at the physician's discretion.

5.3.5 **Off Treatment Criteria with continued study visits**: Patients who receive at least 1 vaccination, but do not complete 6 vaccines for any reason other than disease relapse requiring additional therapy or hospice will be considered "off treatment". These patients will continue "on study" for study visits, tests/procedures, and assessment of toxicity and survival end points. It is preferred that study specific blood samples be drawn and bone marrow biopsies are performed according to the study calendar, but these may be foregone for clinical reasons or if the patient declines to have one performed.

5.3.6 **Off Treatment Criteria with survival follow-up only**: Patients meeting the following criteria will be considered "off treatment" and no further study visits, or study related testing will be performed. Survival information will be recorded.

5.3.6.1 Patients who have disease relapse after starting vaccination and require additional therapy (chemotherapy or immune therapy) or hospice placement.

5.4 General Concomitant Medication and Supportive Care Guidelines

Guidelines for use of concomitant medications, and supportive care medications should follow standard practice for patients after allogeneic stem cell transplantation.

- Growth factor (G-SCF or GM-CSF) use to stimulate white blood cells during the vaccination period is not allowed during weeks where patients are scheduled to receive vaccinations. This may be given on weeks during the vaccination period where no vaccinations are given.
- As described above, patients should remain on tacrolimus without active taper during the vaccination period. Routine adjustment of tacrolimus dosing to maintain trough level in therapeutic range should be performed as per standard of practice.
- Addition of other systemic immune suppressive medications, including steroids, for the treatment graft-versus-host disease will lead to discontinuation of vaccination.
- Short term use of systemic steroids, such as for blood or platelet transfusion reactions, hives, or other acute allergic reaction is allowed.

5.5 Duration of Vaccination

Duration of vaccination will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse events, treatment may continue for administration of all 6 vaccines or until one of the following criteria applies:

General:

- Intercurrent illness that prevents further administration of the vaccine
- Unacceptable adverse event(s),
- Participant demonstrates an inability or unwillingness to comply with the regimen and/or documentation requirements
- Participant decides to withdraw from the study, or
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator.
- GVAX discontinuation based on criteria as set previously.

5.6 Duration of Follow Up

Because this is a gene transfer study, all participants who received at least one vaccination will be followed for safety for up to 15 years after removal from study, or until death, whichever occurs first. Participants removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.7 Criteria for Removal from Study

Participants will be removed from study if they withdraw consent or if they do not start vaccination by day+60* after HSCT as specified in the protocol. The reason for study removal and the date the participant was removed will be documented in the patient's medical record

* Day 60 or next business day, if day 60 occurs on a weekend or holiday

6.0 EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays will be made using the following recommendations. Toxicity assessments will be done using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 which is identified and located on the CTEP website at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

All adverse events experienced by participants will be collected from the time of the first vaccination, through the study and until the final study visit (18 months from SCT). Participants continuing to experience toxicity at the off study visit will be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

A list of the adverse events and potential risks associated with the GVAX vaccination administered in this study appear below and will determine whether dose delays and modifications will be made or whether the event requires expedited reporting **in addition** to routine reporting.

6.1.1 Anticipated toxicities of GVAX vaccine:

6.1.1.1 **Localized skin reaction**: In previous studies using GM-CSF secreting tumor cell vaccines, little toxicity other than injection site discomfort was observed. The most common adverse events were injection site reaction with erythema, induration, and local pruritis that was easily controlled with topical emollients. Grade I fatigue and nasal congestion were occasionally noted. No organ toxicities or autoimmune events were noted. GVAX is an investigational product and may have other side effects that are unknown at this time.

6.1.1.2 **Potential toxicities of GM-CSF**: GM-CSF is widely used for accelerating hematopoietic recovery and its spectrum of toxicity well established. It is likely that the highest level of GM-CSF secreted by the cells in the vaccine will be far lower than the usual starting dose for systemic application (350 mcg/day). While we cannot predict the exact toxicities attributable to local secretion of GM-CSF delivered by adenoviral mediated gene transfer, toxicities observed from the previous trial were restricted to erythema, pruritus, and swelling. Local toxicities of GM-CSF protein injection have included pustular eruption, necrotizing vasculitis, erythema, pruritus, recall erythema at previous injection sites, general papular rash, and phlebitis. Systemic toxicities have included fever, bone pain, malaise, diarrhea, transient liver function test (LFT) abnormalities, leukopenia, leukocytosis, eosinophilia, arthralgia, dyspnea, fluid retention, serous effusions, and recrudescence of various auto-immune diseases.

6.1.1.3 **Potential for prolonged survival of leukemia vaccine cells**: All GM-CSF transfected autologous vaccine cells used in this trial will be irradiated with 10,000 rads to ensure they will die after several days. It is thus unlikely, but theoretically possible, that some tumor cells can survive the radiation, secrete GM-CSF long-term, or even cause leukemia relapse. These events have not been observed in any of the prior studies using retroviral or adenoviral transfected autologous tumor cell GVAX. However, in another study using a different type of GVAX vaccine composed of K562 leukemia cells transfected with GM-CSF and mixed with autologous tumor cells (DFCI Protocol 08-160), there was one case where the GM-CSF secreting K562 cells persisted at the vaccine site, and resulted in severe eosinophilia that proved to be ultimately fatal. It should be noted that K562 cells are not being used in this trial.

6.1.1.4 **Auto-immune diseases**: This is a theoretic possibility for which patients will be monitored. In principle, the mechanisms that allow the immune system to recognize tumor antigens could also lead to breakdown of tolerance to normal or self-antigens, generating an autoimmune reaction. In the B16 murine melanoma model, vaccination with GM-CSF expressing tumor has been associated with depigmented patches of skin, an indication of tissue-specific autoimmunity. No examples of autoimmunity were seen in previous GVAX trials in AML, melanoma, renal cell carcinoma, prostate cancer, pancreatic cancer and lung cancer.

6.1.1.5 **DMSO toxicity**. This toxicity is unlikely since the vaccine will be washed prior to administration. The cells are cryopreserved in 10% DMSO. DMSO is a hydrophilic

molecule, which diffuses through tissue rapidly. Although toxicities associated with intradermal administration of 10% DMSO in humans have not been published, toxicities associated with topical administration of 50% or more DMSO include skin irritation (vesiculation, urticaria, erythema, induration, pruritus, pain, bleeding, scaling, heat generation), garlic-like taste and odor on the breath and skin, transient disturbances of color vision, photophobia and diarrhea. The FDA has authorized the use of 50% DMSO for the treatment of interstitial cystitis in humans. Numerous studies in humans have demonstrated the relative safety of intravenous infusion of cells cryopreserved in 10% DMSO solutions, as this has become standard medical practice for infusion of cryopreserved peripheral blood and bone marrow stem cell collections in the setting of stem cell transplantation.

6.1.1.6 **Potential toxicity from Adenovirus Vector**. Wild type adenovirus serotype 5 (from which the vector used in this study is derived) is associated with an upper respiratory infection and conjunctivitis. There is controversy regarding a role in infantile intussusception. Other adenovirus serotypes are associated with cystitis, gastroenteritis, and pneumonia in military recruits during basic training. The adenoviral vector used in this study will be certified to contain less than one replication competent serotype 5 viral particles per vaccine inoculum. In the event that recombination between a latent wild type adenovirus and the introduced adenoviral vector occurs in the tumor cell, this will not lead to production of a replication competent GM-CSF expressing virus, as the GM-CSF cDNA is inserted in the E1 deleted region of the vector. Transduced cells will be injected into the skin; thus in the absence of an open wound, there is no significant risk of transmitting the virus to close contacts. If skin breakdown does occur, the skin should be covered with an adherent bandage until healing has occurred.

6.1.1.7 **Acute and chronic GVHD**. While we hypothesize that this vaccine may elicit a leukemia specific response by the donor immune system, the possibility that it may elicit or aggravate GVHD also exists. In our previous trial using this GVAX platform after RIC SCT (04-023), we did not observe any increase in acute or chronic GVHD in excess of what is expected at baseline. Differences in grade 3-4 acute GVHD between the vaccine and no vaccine arms will be assessed in planned interim analyses, see section 13.7.

6.1.1.8 **Unexpected vaccine related toxicity**: Any severe adverse event (CTC grade ≥ 3) that is not readily explained as a transplant or disease associated complication will be assessed as a possible vaccine related toxicity.

6.1.1.9 **Anticipated Toxicities of Allogeneic transplantation**

Fludarabine- The most common adverse events include myelosuppression (neutropenia, thrombocytopenia and anemia), fever and chills, infection, and nausea and vomiting. Other commonly reported events include malaise, fatigue, anorexia, and weakness. Serious opportunistic infections have occurred in CLL patients treated with fludarabine.

Busulfan- The most frequent, serious, toxic effect of busulfan is myelosuppression resulting in leukopenia, thrombocytopenia, and anemia. Hepatic veno-occlusive disease, which may be life-threatening, has been reported following the use of very high doses of busulfan in combination with cyclophosphamide or other chemotherapeutic agents prior to bone marrow transplantation. Possible risk factors for the development of hepatic veno-occlusive disease include: total busulfan dose exceeding 16 mg/kg based on ideal

body weight, high busulfan levels, and concurrent use of multiple alkylating agents. A clear cause-and-effect relationship with busulfan has not been demonstrated. At high doses, busulfan has been shown to induce clinical seizures, and as such, all patients will receive seizure prophylaxis during conditioning with high dose busulfan. Interstitial pulmonary fibrosis has been reported rarely. Busulfan is capable of inducing cataracts in rats and there have been several reports indicating that this is a rare complication in humans. In the few cases reported in humans, cataracts have occurred only after prolonged administration of busulfan. Hyperpigmentation is the most common adverse skin reaction and occurs in 5% to 10% of patients, particularly those with a dark complexion.

Tacrolimus - Primary toxicities include renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, and neurologic toxicity, including seizure and visual blindness. Rarely, tacrolimus may thrombotic microangiopathy, a severe condition characterized by acute renal failure, neurologic symptoms (confusion, seizures), anemia and thrombocytopenia.

Methotrexate- The primary toxicities expected are myelosuppression and mucositis. These effects are expected to be mild since dosing in mini-methotrexate is reduced. At high doses, MTX is also known to cause liver and/or renal damage. MTX can collect in extra-cellular fluid compartments, leading to delayed clearance and increased side effects. Therefore, in patients with significant third space fluid collections (e.g. ascites, pleural, pericardial effusions), leucovorin rescue may be administered at the discretion of the transplant physician.

GM-CSF- Short term administration of GM-CSF to hasten neutrophil recovery may lead to bone pain, back pain, headaches, and an elevated white blood cell count in the blood.

Pancytopenia- Pancytopenia is common and expected early after HSCT. If pancytopenia is prolonged, this may lead to infections, bleeding, or death. For patients with graft failure/prolonged pancytopenia, infusion of additional allogeneic stem cells or re-infusion of stored autologous stem cells (if available) will be considered. Patients who have not engrafted by day +45 will be removed from study and not receive vaccination.

Graft versus Host Disease (GVHD)- The principal target organs of acute GVHD are skin, gastrointestinal tract, and liver. Treatment in most cases will consist of increased doses of prednisone with or without the addition of other immune suppressive agents. Acute GVHD will be graded according to the modified Glucksberg score.[16] Chronic GVHD can also affect skin, gut, and liver, but in addition, can affect mucosal membranes (dry eyes, dry mouth, vaginal dryness), joints (stiffness), and lungs (dry cough, dyspnea). Chronic GVHD will be graded as limited or extensive based on the original Seattle criteria.[17]

6.2 Toxicity Management

6.2.1 Vaccine site reaction- Local vaccine site reactions are generally transient and self limiting. Local management may include cold compress, moisturizing lotions. Topical steroids and/or topical antihistamines should be avoided if possible so as to not potentially blunt the

immunologic response to the vaccine. If the reaction persists more than 1 week, or the area of erythema and induration persists, a skin biopsy of the area will be considered.

6.2.2 Severe Eosinophilia- If increased absolute eosinophil count to > 5.0 x10^9/L (5000/ul) occurs during the vaccination period, further vaccines will be delayed (see section 5.3.4). In the event that the absolute eosinophil count remains >5.0 x10^9/L for more than 2 weeks despite being off vaccination, no further vaccinations will be given. Since mild eosinophilia is common after allogeneic HSCT, and is also a well recognized and expected response to vaccine, a transient increase in absolute eosinophil count is not an automatic cause for halting all future vaccinations.

6.3 Dose Modifications/Delays

There will be no vaccine dose adjustments for this study.

Guidelines for delays or discontinuation of vaccination are described in sections 5.3.3 and 5.3.4, respectively.

6.4 Unblinding

There are no plans for unblinding while patient are on treatment.. Should an event occur where unblinding is believed to be necessary for the care of the patient, the case will be discussed with the study PI and Robert Soiffer, M.D., the IND holder. After the study is closed to accrual and the last patient has completed the GVAX/placebo vaccinations, the study may be unblinded for data analysis.

7.0 DRUG FORMULATION AND ADMINISTRATION

7.1 GVAX/Placebo

7.1.1 Description

GVAX is a GM-CSF secreting autologous leukemia cell vaccination.
The vaccine is produced through adenoviral vector mediated GM-CSF gene transfer into myeloblasts harvested from study subjects prior to admission for HSCT. The placebo vaccine will consist of a sterile normal saline solution.

7.1.2 Form

Cells for vaccine will be thawed in a dedicated laminar flow biosafety cabinet in the CMCF and washed. Cells will be resuspended in a volume of 1 ml. for administration.

7.1.3 Storage and Stability

Tumor cells for vaccination and immunologic evaluation will be cryopreserved and stored in liquid nitrogen. Six individual vaccine aliquots will be prepared for each patient. Cell dose per aliquot will be fixed for an individual patient and will range from a minimum of $1x10^6$ cells per aliquot to $1x10^7$ cells per aliquot. The dosage will be determined by dividing the total cell yield following transduction into six aliquots. Thus, a minimum of $6x10^6$ cells will be required to

prepare six aliquots of $1x10^6$ cells. For total cell yields greater than $6x10^7$, individual aliquots will remain at $1x10^7$ cells per dose.

7.1.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.5 Availability

GVAX/Placebo is an investigational agent and will be supplied free-of-charge from CMCF.

7.1.6 Preparation

7.1.6.1 GVAX

Harvested autologous myeloblasts will be transferred to the Cell Manipulation Core Facility (CMCF) at the Dana Farber Cancer Institute. The autologous myeloblasts will be introduced into short-term tumor culture in the presence of G-CSF, and transduced with a replication defective adenoviral vector encoding human GM-CSF.

After transduction, the tumor cells will be washed extensively and irradiated with 10,000 cGy. A radiation indicator tag that changes color from red to black after radiation will be appended to the bag containing the cells to assure that radiation is completed. In addition, the completion of radiation will recorded by 2 qualified members of the laboratory, who will also verify that the appropriate radiation dose of 10,000 cGy is delivered.

A small aliquot of the transduced cells will be placed into culture and GM-CSF secretion will be determined by ELISA. The target level for GM-CSF production will be at least 40 ng/10^6 cells/24 hours, although achieving this secretion will not be required for vaccine administration. Routine sterility cultures and testing for endotoxin and mycoplasma contamination will be performed.

Tumor cells for vaccination and immunologic evaluation will be cryopreserved and stored in liquid nitrogen. Six individual vaccine aliquots will be prepared for each patient. Cell dose per aliquot will be fixed for an individual patient and will range from about $1x10^6$ cells per aliquot to $1x10^7$ cells per aliquot. The exact vaccine cell dose in each patient will vary depending on the total yield of myeloblasts harvested. Vaccine dose will be calculated based on the myeloblast count performed on a post-ficoll sample assessed in the Hematology Laboratory at DFCI. Following transduction, the final product will be divided equally into six aliquots to yield 6 vaccines of the same dose. For total cell yields greater than $6x10^7$, individual aliquots will remain at a maximum of $1x10^7$ cells per dose.

On the day of vaccination, cells will be thawed in a dedicated laminar flow biosafety cabinet in the CMCF and washed. Cells will be re-suspended in a volume of 1 ml. in a syringe for administration. The syringe will be masked with special tape to prevent administrating personnel from distinguishing cellular vaccines from placebo.

7.1.6.2 Placebo

The placebo vaccine will be prepared by the CMCF staff on the day of vaccination. The vaccine will consist of 1 ml. sterile normal saline. The syringe will be masked with special tape to prevent administrating personnel from distinguishing cellular vaccines from placebo.

7.1.7 Administration

GVAX/Placebo vaccinations will be administered weekly x 3 weeks, then q2weeks x 6 weeks for a total of 6 vaccines. The weekly scheduling of vaccination can be +/- 2 days for scheduling flexibility.

A study team physician, physician assistant, nurse practitioner, registered nurse, or research registered nurse can administer the injections in either an inpatient or outpatient clinic. Injections will be administered according to standard nursing procedure in the patient's arms and thighs on a rotating basis. Injections must be performed without the prior application of EMLA or other topical or local anesthetics, as such compounds both could obscure local reactions to the vaccination and could cause reactions that might be interpreted as vaccine reactions. Approximately 1/2 of the vaccine dose should be administered subcutaneously, and remaining half the vaccine dose intradermally. It is recommended that about half of the vaccine be given first intradermally, and the rest subcutaneously by changing the angle of the needle.

Information regarding the number and location of the injections, the date and time of vaccination, route of administration, total volume of vaccine administered and vaccine site injection reactions will be collected for each vaccination and recorded in the patient's chart.

7.1.8 Ordering

Orders for the collection of cells, manufacturing of vaccine/placebo, and release of the vaccine/placebo will be done through the electronic Biologic Order Entry system, as is standard for all cellular therapy order entry at the DFCI. The GVAX/placebo vaccine will be manufactured at the Cell Manipulation Core Facility(CMCF). Once an order for processing has been received by CMCF, GVAX cells will be thawed or the placebo vaccine will be prepared for administration accordingly.

7.1.9 Accountability

GVAX/placebo accountability is maintained by the Cell Manipulation Core Facility (CMCF).

7.1.10 Destruction and Return

CMCF will receive reagents, if they are not used the reagents will be held for future use until they are out of date at which point they will be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.0 CORRELATIVE/SPECIAL STUDIES

Biologic responses to vaccination following allogeneic stem cell transplant will be assessed by laboratory and histologic analyses of blood before and after vaccination, and skin biopsy samples at vaccination. Bone marrow aspirates will also be cryopreserved, if sufficient material is available. We anticipate that vaccination will elicit robust infiltrates composed of dendritic cells, macrophages, granulocytes, and lymphocytes. The numbers and proportions of CD4+ and CD8+ T effectors and FoxP3+ Tregs will be evaluated. The local production of relevant cytokines and chemokines will also be examined using real-time PCR and commercially available RNase protection assays.

Serum and circulating mononuclear cells will be frozen prior to beginning vaccination and at regular intervals thereafter. These longitudinal samples will be analyzed for immune cell numbers and subsets. We will use multi-color flow cytometry methods (5 or 9 color), already established in the Cancer Vaccine Center Immune Assessment Core, to investigate immune cell subsets. These phenotypic measurements will be complemented with functional studies. An evaluation of T cell proliferation, cytokine production, and cytotoxicity will be undertaken, employing autologous tumors as targets, when available. The samples will also be used in research studies aimed at identifying the target antigens of vaccination, characterizing NK, T and B cell responses to these targets, and delineating the mechanisms involved in protective immunity. These research tests may include evaluation of reactivity to targets identified in earlier vaccine studies; these may include ATP6S1, melanoma-inhibitor of apoptosis protein (ML-IAP), opioid growth factor receptor (OGFr), CML28, CML66, PDI, ERp5, L1CAM, angiopoietin-1/2, MIF, progranulin, and MICA.

9.0 STUDY CALENDAR

Tests/Evaluations	Enrollment Pre-SCT	Leukemia Cell Harvest	Day 20-30 post SCT	Vaccine 1 Day30-60[9] post SCT	During vaccination	1 month* after last vaccine	6 months* post SCT	9months* post SCT	12 & 18 month* post SCT ▶
Informed Consent	X								
Physical Exam	X		X	X	Qwk	X	X	X	X
Adverse Event assessment		X	X	X	Qwk	X	X	X	X
GVHD assessment			X	X	Qwk	X	X	X	X
Pre-transplant testing[1]	X								
CBC with differential	X		X	X	Qwk	X	X	X	X
Bun/Cr, LFTs, LDH	X		X	X	Qwk	X	X	X	X
CMCF/Immune Bloods[2]	X		X	X	Qmonth	X	X	X	X
Marrow Asp/BX	X[4]	X[5,6]		X[5]		X[5]			X[5]
Peripheral blood leukemia cell harvest		X[7]							
Vaccine specific toxicity Assessment (Appendix I)				X	Qwk	X			
Skin bx of vaccination [3]				X	Vaccine 5				
cGVHD Form (Appendix II)						X[8]	X[8]	X[8]	X[8]

1 Standard pre-transplant testing per institution guidelines
2 Approximately 60cc blood
3 Skin biopsies of vaccine site(2-3 days after injection) are strongly encouraged, but not required for study participation.
4 Should be performed ≤ 28 prior to enrollment if the patient has received intervening therapy
5 Also sent for flow cytometry, FISH/cytogenetics, chimerism (if post SCT), molecular studies (if applicable). An additional marrow aspirate sample is encouraged, but not required to be drawn during protocol bone marrows, and should only be collected if there is excess marrow aspirate available and a sufficient number of cells for banking by the CMCF.
6 Optional if patient qualifies for peripheral blood leukemia cell harvest or at treating physician's discretion if previous restaging marrow aspirate/biopsy was within two weeks of collection.
7 If the patient has blasts in peripheral blood, tumor cells may be collected by blood draw. The amount of blood required may vary depending on calculations of blood volume needed to yield a desired target of 2×10^7 leukemia cells. If the calculated volume of blood draw exceeds 50 ml, it is recommended that bone marrow aspiration be used as the method for tumor cell harvest.
8 Participants will have cGVHD assessed with the cGVHD form at every protocol visit once the participant has developed cGHVD.
9 Day 60 or next business day, if day 60 occurs on a weekend or holiday
* +/- 2 weeks to allow for scheduling flexibility. Please refer to Section 5.3.5 and 5.3.6 to determine off treatment criteria with continued study visits and those with survival follow-up only.
► Because this is a gene transfer study, all participants who received at least one vaccination will be followed for safety for up to 15 years after removal from study, or until death, whichever occurs first (see Section 5.6 for additional information).

10.0 ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Definitions

10.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

10.1.2 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'
Expected adverse events are those that have been previously identified as resulting from administration of the GVAX/placebo vaccine. For the purposes of this study, an adverse event is considered expected when it is a known risk associated with the GVAX/placebo vaccination, or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with GVAX/placebo vaccination.
For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from side effects that are expected after the GVAX/placebo vaccination. Refer to section 6.1 for a listing of expected adverse events associated with GVAX/placebo vaccination.

Adverse events occurring during vaccination or within 1 month of the last GVAX/placebo vaccination will be reported to the DFCI IRB according to DF/HCC policy.

10.1.3 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

10.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will record and assess the occurrence of AEs at all participant evaluation time points during the study. Refer to section 9.0 for the evaluation time points.

As with all transplant patients, regardless of being on study, all grade ≥ 3 non-hematologic AEs and grade ≥ 4 hematologic AEs will be recorded in the participant's medical record and DFCI BMT data repository.

In addition, during and at 1 month after the last vaccination, adverse effects commonly associated with vaccination, as specified on the Vaccine Specific Toxicity Form (See Appendix I), will be recorded regularly regardless of grade.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0.
A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

10.3 AE Reporting Period and Requirements

Because transplant is standard of care for this patient population, adverse events and deaths occurring before GVAX/placebo administration will not be reported. The first day of adverse event reporting will coincide with the day the first vaccination is administered. Adverse event reporting for this study ends at the final study visit (18 months post SCT). Adverse events occurring in a participant after the study period should be reported to the Overall PI if the participating investigator becomes aware of them.

It is the responsibility of each participating investigator to report adverse events to the Overall PI as described below. The Overall PI or representative personnel will ensure the report is forwarded to the proper parties.

Notification Requirement

Toxicity	Known correlation	Attribution to study drug	Robert Soiffer, MD (IND Sponsor) Clinical Trials Office Vincent Ho, MD (Principal Investigator)	DFCI IRB
Grade ≥ 3 non-hematologic, Grade ≥ 4 hematologic, and Grade 5.	Any (Expected or Unexpected)	Any	Within 2 working days from notification[a] Via Email[b] Use Medwatch 3500A[c]	Submit directly if required per DFCI IRB AE reporting policy. Submit within IRB established reporting timelines. Dr. Ho must prospectively approve all submissions.. Ho must prospectively approve all submissions.

a. In the event that the prticipating investigator does not become aware of the adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 2 working days after learning of it and document the time of his or her first awareness of the adverse event.
b. Refer to the 12-217 Manual of Procedures.
c. Medwatch 3500A downloadable form at http://www.fda.gov/medwatch/getforms.htm

10.3.1 Reporting to the IND-Holder and the FDA

- Site Responsible Investigators (or their designee) will forward all reportable events to both the Overall PI and the IND-Holder (or IND-Holder's designee) within 2 business days of knowledge of the event.

- The Overall Principal Investigator (or his designee) will forward all events occurring at the lead site to the IND-Holder, Dr. Robert Soiffer, or the IND-Holder's designee, within 2 business days of knowledge of the event. Additionally, the Overall Principal Investigator will ensure that all reportable events that are received from participating sites, which have not already been forwarded to the IND-Holder are sent to Dr. Soiffer (or his designee) within 2 business days.

- Robert Soiffer, MD, as IND sponsor, will be responsible for all communication with the FDA. He will report to the FDA any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the GVAX/placebo vaccination according to the FDA requirements.

10.4 Reporting to the NIH Office of Science Policy (OSP)

The IND Sponsor, Robert Soiffer, MD, will be responsible for all communication with OSP. Dr. Soiffer will report to OSP, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the GVAX/placebo vaccination according to the OBA requirements.

10.5 Reporting to the Institutional Biosafety Committee (IBC)

Any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the GVAX/placebo vaccination GVAX/placebo vaccination according will be reported to the IBC according to each IBC's requirements.

10.6 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.0 DATA AND SAFETY MONITORING

11.1 Data Reporting

11.1.1 Method

The Clinical Research Coordinator will work together with the QACT to collect, manage, and monitor data for this study.

11.1.2 Data Submission

Allogeneic transplantation data will be collected for all patients enrolled on this study. These will include the allogeneic transplant data entered into the BMT repository via EDC-Inform. In addition to the transplant-specific data, the following research specific forms are required for this study:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
Vaccine Specific Toxicity Form	Within 45 days of the last vaccination
Off Treatment/Off Study Form	Within 14 days of being taken off treatment or study.

11.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this trial. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed with the Principal Investigator, statistician and study team members. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

12.0 STATISTICAL CONSIDERATIONS

12.1 Endpoints

12.1.1 **Primary Endpoint**: The primary endpoint of this study is progression-free survival (PFS) at 18 months post-randomization. Progression-free survival is defined as time from randomization to progression of disease or death whichever occurs first. Patients with relapse/progressive disease who re-enter remission after vaccination or upon withdrawal of immune suppression alone will not be considered as having disease progression. The primary analysis will be performed using the modified intention-to-treat (ITT) principle, i.e., all transplanted and eligible patients who receive at least one vaccination will be included in the analysis according to the treatment arm they are randomized to.

12.1.2 **Secondary Endpoints**

12.1.2.1 Safety and toxicity of vaccination, as measured by the development of:

- Grade II-IV and III-IV acute GVHD
- CTC grade ≥ 3 non-hematologic toxicity, or

- Grade ≥ 4 hematologic toxicity attributable to vaccination

12.1.2.2 Biologic responses to vaccination following allogeneic stem cell transplant, as assessed by laboratory and histologic analyses of blood before and after vaccination, and skin biopsy samples at vaccination sites (Section 8.0). Serum and circulating mononuclear cells will be frozen prior to beginning vaccination and at regular intervals thereafter. Bone marrow aspirates will also be cryopreserved, if sufficient material is available.

12.2 Study Design and Objectives

This is a randomized placebo controlled Phase II study of GVAX versus placebo vaccination given early after allogeneic HSCT in high risk AML/MDS patients. The premise is that GVAX vaccination early after HSCT will increase progression-free survival (PFS) in this patient population. The target accrual goal is 106 eligible patients for vaccination, 53 per each arm, over a 3.8 year period and followed for an additional 18 months. This design will give approximately 80% power to detect a 75% increase in 18 month PFS in the GVAX arm (Arm A) compared to the placebo arm (Arm B) as graphically illustrated in Figure 1.

12.3 Accrual

Based on the accrual of the DFCI transplant group between 2006 and 2011, the projected accrual rate will be approximately 40 high risk AML/MDS patients with active disease who meet the eligibility criteria per year. Of these 40 eligible patients, we anticipate that about 30% will not meet the criteria for starting GVAX/Placebo administration outlined in Section 5.3.1. Based on this projection, it is anticipated that approximately 3.8 years of accrual will be necessary to enroll the targeted sample size of 106 eligible patients for vaccination. Accounting for the 30% attrition, we anticipate that about 152 patients will be enrolled on the study to obtain 106 patients eligible to start vaccination.

12.4 Randomization

After enrollment, patients will be randomized at a ratio of 1:1 between two arms using permuted block algorithm within strata. Randomization will be stratified by center (DFCI, BIDMC, MGH), disease (AML vs. MDS), age (≥50 vs. <50), and conditioning intensity (myeloablative Fly/Bu4 vs. RIC Flu/Bu2).

12.5 Sample Size and Power Calculations

In our previous study[10], the 2-year progression-free survival for 15 patients who received at least one vaccination was 46%. This estimate compared to 18% 2-year PFS from our contemporaneous control cohort that was most similar to those patients in the GVAX trial. Extrapolating this information, we hypothesize that the PFS will be increased by 75% at 18 months post randomization in Arm A (GVAX) compared to Arm B (placebo). A retrospective analysis of high risk AML/MDS patients who underwent myeloablative or RIC HSCT between 2004 and 2008 at DFCI shows that a small portion of patients are cured from the underlying disease after allogeneic HSCT and the distribution of PFS follows a two-component exponential cure rate model (Figure 1). Basing the fitted curve in Figure 1 as the null hypothesis, we hypothesize that the PFS will be increased by 75% from 26% to 46% by 18 months post HCT in Arm A. More specifically, the proposed alternative hypothesis is H_A: $S_A > S_B$,

where S_A and S_B denote the distributions of PFS in Arms A and B, as shown in (1) and (2), respectively

$$S_A = 0.45+0.45*\exp(-t*(\log(2)/2.5))+ 0.1*\exp(-t*(\log(2)/4.5)) \quad (1)$$
$$S_B = 0.24+ 0.76*\exp(-t*(\log(2)/3.5)) \quad (2)$$

This projection incorporates approximately 30-45 days of wait time from randomization to the start of GVAX/Placebo vaccination and additional ~40 days for the administration of a serial of 6 vaccinations. During this period, we expect little difference in PFS between two arms. Figure 4 depicts (1) and (2) graphically. With this design, 106 eligible patients with 69 events will be required to achieve approximately 80% power with one-sided significance level of 0.15. Since this is a direct but nondefinitive randomized comparison to a standard treatment control, we follow recommendations made by Rubinstein et al. [18, 19] and use a one-sided type I error rate of 0.15 with 80% power [18-20]. The power calculation is calculated using the R program, *Powlgrnk* (developed by Dr. Robert Gray at DFCI), which computes power of the two-group log-rank test for arbitrary failure time distributions and has been used for non-proportional hazards alternative.[21]

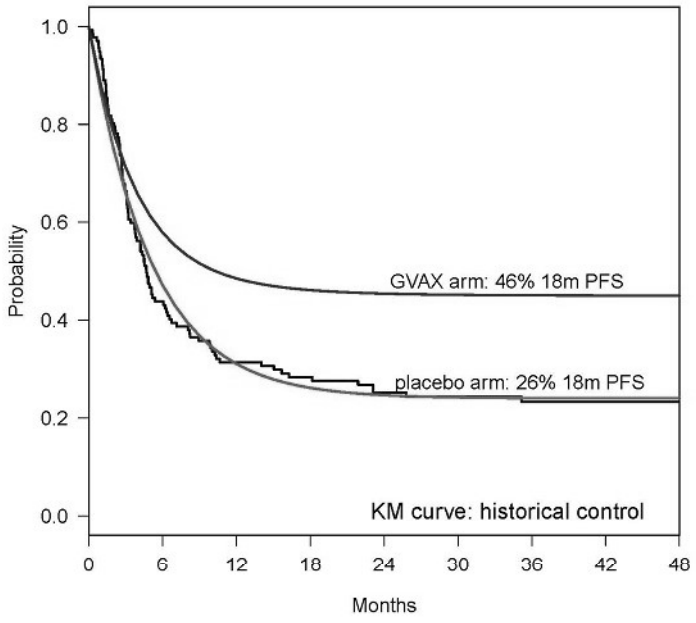


Figure 4. Target PFS. Blue line represents the projected PFS distribution in Arm A (GVAX) and red line represents the projected PFS distribution in Arm B (Placebo). The solid black line is the Kaplan-Meier curve from the historical control

12.6 Interim Analyses

The study will be monitored using standard procedures and processes by an independent Data and Safety Monitoring Committee (DSMC) of the Dana-Farber Harvard Cancer Center (DF/HCC). Interim analysis for efficacy will occur annually starting at 33% information time, which is anticipated to occur approximately 2.8 years after the start of the trial. The interim results will be reported to the DF/HCC DSMB annually. However, these interim inspections will not have an effect in terms of

stopping accrual to the study and terminate the study early in favor of alternative (superiority) or null hypothesis (futility). This is because i) due to a small sample size at each interim look, stopping early for futility would result in substantially wide confidence intervals, larger Type II error, and thus leading to greater uncertainty about the magnitude of the PFS difference, ii) even if the PFS difference is not as large as targeted, investigators are interested in learning the magnitude of the PFS difference for further investigation, iii) stopping early for superiority would provide less safety data than planned and potential difficulty with the efficacy analysis and its interpretation.

12.7 Guidelines for Grade III-IV acute GVHD and serious toxicity

Monitoring of key safety endpoints (grade 3 or higher treatment related toxicity, grade III-IV acute GVHD) will be conducted in accordance with the DF/HCC DSMB meeting. GVAX was well tolerated in the previous trial (DFCI#04023). For the entire cohort of 24 patients that included 9 patients without vaccination, the incidence rate of grade III-IV acute GVHD was 4%, which is comparable to 9% in a historical control cohort of patients whose patients and transplant characteristics were most similar to those in the GVAX trial. Based on this information, we do not anticipate that GVAX will be associated with an increased risk of grade III-IV aGVHD nor severe toxicity. However, we will compare the grade III-IV aGVHD rate and grade 3 or higher treatment related toxicity rate between two arms at each interim look. If, at any interim look, this difference is significant at the two-sided level of 0.05, this would trigger a consultation with the DF/HCC DSMB for additional review.

12.8 Statistical Analysis Plan

The primary analysis will be the modified ITT analysis for the primary endpoint of PFS at 18 months after randomization. The primary analysis will be performed using the Kaplan-Meier estimates for PFS for each arm at 18 months along with 95% confidence intervals. Several secondary analyses of PFS will be conducted, depending on an assessment of the original assumption of non-proportional hazards assumed in the study design. First of all, graphical and analytical diagnostics will be used to assess the presence of non-proportional hazards between treatment arms. If there is no evidence of non-proportional hazards, a relative risk will be estimated from the Cox model both unadjusted and adjusted for other covariates. To assess the effect of GVAX, several approaches will be taken: 1) inclusion of the treatment arm as a fixed covariate in a model, 2) inclusion of the total number of GVAX vaccinations (0-6) as a fixed covariate in a model, 3) treating the number of GVAX/placebo vaccinations as a time dependent repeated measures and an interaction between the number of vaccinations and the treatment arm in a model, 4) exploring a joint model for survival and longitudinal data.[26] If there appears to be non-proportional hazards, several analyses will be conducted: 1) a comparison of PFS at 18 months post randomization will be conducted using the linear combination test proposed by Logan et al.[24, 28.29] This method directly compares the PFS curves starting at 18 months, and accounts for patients enrolled early in the study having additional follow-up past 18 months. 2) a pseudo value regression approach[30], 3) PFS from the start of vaccination will be analyzed, 4) multivariable cure rate model will be performed as described in Ibrahim et al.[27]

Secondary objectives include relapse and non-relapse mortality (NRM), acute and chronic GVHD, overall survival. For the comparison of GVHD, NRM and relapse, we will perform competing risks data analysis using Gray test and competing risks regression analysis.[25] All laboratory correlative studies will be analyzed using appropriate statistical methods. In particular, the effect of vaccination on infiltration of dendritic cells, macrophages, granulocytes, and lymphocytes as well as T cell reconstitution will be examined by comparing pre to post vaccination data and the GVAX to the

placebo arm.

12.9 Analysis of Laboratory Correlative Studies

For the laboratory correlative studies, patients who receive at least one GVAX/placebo vaccination will be included. To identify and determine relevance of vaccine induced immune responses, we will compare the laboratory parameters described in Section 8.0 between the GVAX and placebo arm. The general overview of the analysis is that all longitudinal measurements will be graphically assessed and simple comparison will be made at each time point using either Fisher's exact test for categorical variables or Wilcoxon-Rank-Sum test for continuous variables without considering multiple testing. For long term responders, repeated measures analysis will be performed to characterize the pattern of each immunologic/biologic response. If there appears to be association between these measurements and PFS, we will incorporate these measurements as time dependent repeated covariates in time-to-event model and/or explore a joint model of longitudinal and survival data[26]. When data are available, we will first validate the previous finding of elevated shed MICA, a NKG2D ligand. In the previous report of GVAX (DFCI#04023), the median soluble MICA level (sMICA) among 7 long-term responders was 1,947 pg/ml before HCT, but fell to 383 pg/ml within 1 year post HCT. In contrast, no marked decrease was seen among 34 control patients. Conservatively extrapolating this result, if the average percent reduction in sMICA level from pre to 1 year post randomization in the GVAX arm is 70% and 10% in the placebo arm, we will have 85% power to detect a 60% difference, assuming the standard deviation of the difference is 60% (i.e., effect size=1). This power calculation is based on asymptotic power of the Wilcoxon-Rank-Sum test and assumes a sample size of 25 in the GVAX arm and 16 in the placebo arm at 1-year post randomization.

13.0 PUBLICATION PLAN

This study is intended for publication, even if terminated prematurely. Publication may include any or all of the following: posting of a synopsis online, abstract and/or presentation at a scientific conference, or publication of a full manuscript. The Principal Investigator and authors may submit a manuscript describing study results within 24 months after the last data become available; which for vaccine trials, may take up to several months after the last patient visit.

14.0 REFERENCES

1. Collins RH, Jr., Shpilberg O, Drobyski WR, Porter DL, Giralt S, Champlin R *et al.* Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation [see comments]. *J Clin Oncol* 1997; **15**(2)**:** 433-44.

2. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K *et al.* Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* 1993; **90**(8)**:** 3539-43.

3. Dunussi-Joannopoulos K, Dranoff G, Weinstein HJ, Ferrara JL, Bierer BE, Croop JM. Gene immunotherapy in murine acute myeloid leukemia: granulocyte-macrophage colony-stimulating factor tumor cell vaccines elicit more potent antitumor immunity compared with B7 family and other cytokine vaccines. *Blood* 1998; **91**(1)**:** 222-30.

4. Tani K, Azuma M, Nakazaki Y, Oyaizu N, Hase H, Ohata J *et al.* Phase I study of autologous tumor vaccines transduced with the GM-CSF gene in four patients with stage IV renal cell cancer in Japan: clinical and immunological findings. *Mol Ther* 2004; **10**(4)**:** 799-816.

5. Salgia R, Lynch T, Skarin A, Lucca J, Lynch C, Jung K *et al.* Vaccination with irradiated autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor augments antitumor immunity in some patients with metastatic non-small-cell lung carcinoma. *J Clin Oncol* 2003; **21**(4)**:** 624-30.

6. Soiffer R, Hodi FS, Haluska F, Jung K, Gillessen S, Singer S *et al.* Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. *J Clin Oncol* 2003; **21**(17)**:** 3343-50.

7. Soiffer R, Lynch T, Mihm M, Jung K, Rhuda C, Schmollinger JC *et al.* Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 1998; **95**(22)**:** 13141-6.

8. Hodi FS, Schmollinger JC, Soiffer RJ, Salgia R, Lynch T, Ritz J *et al.* ATP6S1 elicits potent humoral responses associated with immune-mediated tumor destruction. *Proc Natl Acad Sci U S A* 2002; **99**(10)**:** 6919-24.

9. Schmollinger JC, Vonderheide RH, Hoar KM, Maecker B, Schultze JL, Hodi FS *et al.* Melanoma inhibitor of apoptosis protein (ML-IAP) is a target for immune-mediated tumor destruction. *Proc Natl Acad Sci U S A* 2003; **100**(6)**:** 3398-403.

10. Ho VT, Vanneman M, Kim H, Sasada T, Kang YJ, Pasek M *et al.* Biologic activity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic stem cell transplantation. *Proc Natl Acad Sci U S A* 2009; **106**(37)**:** 15825-30.

11. Jinushi M, Hodi FS, Dranoff G. Therapy-induced antibodies to MHC class I chain-related protein A antagonize immune suppression and stimulate antitumor cytotoxicity. *Proc Natl Acad Sci U S A* 2006; **103**(24)**:** 9190-5.

12. Gonzalez S, Groh V, Spies T. Immunobiology of human NKG2D and its ligands. *Curr Top Microbiol Immunol* 2006; **298:** 121-38.

13. Fonseca C, Soiffer R, Ho V, Vanneman M, Jinushi M, Ritz J *et al.* Protein disulfide isomerases are antibody targets during immune-mediated tumor destruction. *Blood* 2009; **113**(8)**:** 1681-8.

14. Raveh S, Gavert N, Spiegel I, Ben-Ze'ev A. The cell adhesion nectin-like molecules (Necl) 1 and 4 suppress the growth and tumorigenic ability of colon cancer cells. *J Cell Biochem* 2009; **108**(1)**:** 326-36.

15. Arlt MJ, Novak-Hofer I, Gast D, Gschwend V, Moldenhauer G, Grunberg J *et al.* Efficient inhibition of intra-peritoneal tumor growth and dissemination of human ovarian carcinoma cells in nude mice by anti-L1-cell adhesion molecule monoclonal antibody treatment. *Cancer Res* 2006; **66**(2)**:** 936-43.

16. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J *et al.* Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1994; **15**(6)**:** 825-8.

17. Sullivan KM, Shulman HM, Storb R, al e. Chronic graft versus host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. *Blood* 1981; **57:** 267.

18. Rubinstein L, Crowley J, Ivy P, Leblanc M, Sargent D. Randomized phase II designs. *Clin Cancer Res* 2009; **15**(6)**:** 1883-90.

19. Rubinstein LV, Korn EL, Freidlin B, Hunsberger S, Ivy SP, Smith MA. Design issues of randomized phase II trials and a proposal for phase II screening trials. *J Clin Oncol* 2005; **23**(28)**:** 7199-206.

20. Seymour L, Ivy SP, Sargent D, Spriggs D, Baker L, Rubinstein L *et al.* The design of phase II clinical trials testing cancer therapeutics: consensus recommendations from the clinical trial design task force of the national cancer institute investigational drug steering committee. *Clin Cancer Res* 2010; **16**(6)**:** 1764-9.

21. Kim HT, Gray R. Three-component cure rate model for nonproportional hazards alternative in the design of randomized clinical trials. *Clin Trials.* 2012;9(2):155-63. Epub 2012 Feb 21.

22. Crowley J, Hu M. Covariance Analysis of Heart Transplant Survival Data.
. *JASA* 1977; **72:** 27-36.

23. Anderson JR, Cain KC, Gelber RD. Analysis of survival by tumor response. *Journal of Clinical Oncology* 1983; **1:** 710-719.

24. Logan BR, Klein JP, Zhang MJ. Comparing treatments in the presence of crossing survival curves: an application to bone marrow transplantation. *Biometrics* 2008; **64**(3)**:** 733-40.

25. Kim HT. Cumulative incidence in competing risks data and competing risks regression analysis. *Clin Cancer Res* 2007; **13**(2 Pt 1)**:** 559-65.

26. Wulfsohn MS, Tsiatis, Anastasios A. A joint model for survival and longitudinal data measured with error. *Biometrics* 1997; **53**:330-339

27. Ibrahim, Joseph George, Chen, Ming-Hui and Sinha, Debajyoti (2001) Bayesian survival analysis *Springer-Verlag Inc (Berlin; New York)*

28. Zhang X, Loberiza FR, Klein JP, Zhang M-J. A SAS macro for estimation of direct adjusted survival curves based on a stratified Cox regression model. Computer methods and programs in biomedicine 2007; 88: 95-101.

29. Klein, J. P., Logan, B., Harhoff, M., and Andersen, P. K.(2007). Analyzing survival curves at a fixed point in time.*Statistics in Medicine* 26**,** 4505–4519.

30. Klein, J. P. and Andersen, P. K. (2005). Regression modeling of competing risks data based on pseudo-values of the cumulative incidence function. *Biometrics* 61**,** 223–229.

15.0 APPENDIX

I. Vaccine Specific Toxicity Form

Fill out weekly during vaccination period, and 1 month after last vaccination.

Patient Name: **MRN:**

Appointment Date: **Clinician:**

Sign/Symptom	**Check if present**	Grade	Length and Width (If Applicable)	Attribution to vaccine 0 = No relation 1 = Unlikely 2 = Possible 3 = Probable 4 = Definite	Comments
Vaccine Site specific					
induration					
erythema					
pruritus					
Generalized Symptoms					
fever					
fatigue					
nausea					
rash					
pruritus					
Other (fill-in below)					

II. cGVHD Survey

12-217 Chronic GVHD Provider Survey

Instructions:
Please score a symptom only if you know or suspect it be *related to chronic GVHD*. Subjective symptoms are acceptable. For example, joint tightness can be scored based on subjective findings despite the absence of objective limitations.

Please score symptoms present in the *last week*. Even if they may have resolved with treatment in the past week, if they were present recently and may possibly return, please score them.

Date of Visit: _____

Patient: _____

Study ID: _____

Your Name: _____

	0	1	2	3
Skin Score	❑ No Symptoms	❑ <18% BSA with disease signs but **NO** sclerotic features	❑ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	❑ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
Mouth Score	❑ No symptoms	❑ Mild symptoms with disease signs but not limiting oral intake significantly	❑ Moderate symptoms with signs with **partial** limitation of oral intake	❑ Severe symptoms with disease signs on examination with **major** limitation of oral intake
GI Tract Score	❑ No symptoms	❑ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	❑ Symptoms associated with mild to moderate weight loss (5-15%)	❑ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
Eye Score	❑ No symptoms	❑ Mild dry eye symptoms not affecting ADL (requiring eye drops <3x per day) **OR** asymptomatic signs of kerato-conjunctivitis sicca	❑ Moderate dry eye symptoms partially affecting ADL (requiring eye drops >3x per day or punctual plugs) **WITHOUT** vision impairment	❑ Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by kerato-conjunctivitis sicca
Joint and Fascia Score	❑ No symptoms	❑ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	❑ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	❑ Contracture WITH significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
Genital Tract Score **(score even if no GYN exam, required for men too)** ❑ **No GYN Exam**	❑ No symptoms	❑ Symptomatic with mild distinct signs on exam AND no effect on coitus and minimal discomfort with GYN exam	❑ Symptomatic with distinct signs on exam AND with mild dyspareunia or discomfort with GYN exam	❑ Symptomatic WITH advanced signs (stricture, labia agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal spectrum
Lung Score	❑ No symptoms	❑ Mild symptoms (shortness of breath after climbing one flight of steps)	❑ Moderate symptoms (shortness of breath after walking on flat ground)	❑ Severe symptoms (shortness of breath at rest; requiring O_2)

Please rate the severity of this person's chronic GVHD											
on this scale ☙	❑ None (1)		❑ Mild (2)			❑ Moderate (3)			❑ Severe (4)		
and on this scale ☙ *(circle one)*	cGVHD symptoms are not at all severe 0	1	2	3	4	5	6	7	8	9	cGVHD symptoms are most severe possible 10

Is an erythematous or maculopapular rash present? ☐ Yes ☐ No

Does the patient have nausea, vomiting or diarrhea? ☐ Yes ☐ No

Liver Score	**0**	**1**	**2**	**3**
	❑ Normal LFTs	❑ Elevated bilirubin, alkaline phosphatase, AST or ALT <2x ULN	❑ Bilirubin > 3 mg/dl or bilirubin, AST or ALT 2-5x ULN	❑ Bilirubin, AST or ALT > 5x ULN

Liver score to be completed using most recent LFTs from within +/- 2 weeks of the assessment

Date LFT sample obtained _____

PFT values from within one month of the assessment

% FEV1 _____% Date of FEV1 _____ ☐ Not done

% DLCOc _____% Date of DLCOc _____ ☐ Not done