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Title: Dendritic Cell/AML Fusion Vaccination following chemotherapy induced remission

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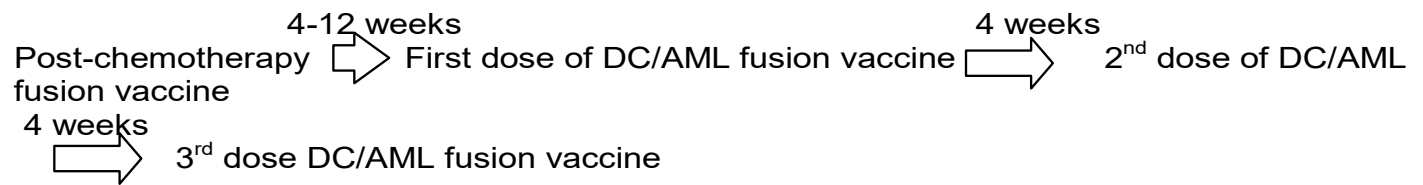
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Agent(s): DC/AML fusion vaccine (IND #8184)

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1. OBJECTIVES

The study will be conducted in two stages. In the first stage, patients will be treated with DC/AML fusion cells in conjunction with GM-CSF after having obtained a chemotherapy induced remission. The primary objective of this stage is to explore toxicity. The secondary objective is to assess immunologic responses to DC/AML fusion cells in patients who are in remission following chemotherapy, and clinical response as assessed by time to disease progression.

In the second stage, an expanded cohort of patients will receive DC/AML fusion vaccination in conjunction with GM-CSF. The primary objective is to determine anti-tumor effects, as assessed by time to disease progression.

The secondary objectives of this stage are:

- To determine if cellular immunity is induced by treatment with DC/AML fusion cells in patients who have obtained a remission following chemotherapy.
- To correlate levels of circulating activated and regulatory T cells and T cell expression of PD1 with immunologic response
- To define anti-tumor effects as assessed by time to disease progression.
- To quantify response to neo-antigens arising from patient specific mutations and shared AML antigens and correlate these findings with clinical outcome

1.1 Study Design

This is a phase II open label study. Patients will be enrolled into the first cohort until 10 patients are considered evaluable (received 2 vaccinations), up to a maximum of 20 evaluable patients. This cohort has been completed without excessive toxicity. Of the initial 16 patients who were vaccinated and assessed for clinical and immune response, 12/16 (75%) remain in remission with a median follow up of 48 months. We will therefore enroll additional patients to be treated with DC/AML fusions to assess the clinical and immune effects of vaccination in a total of 35 patients.

Patients will be enrolled to the study at presentation with newly diagnosed or first relapsed AML and will undergo tumor collection and cryopreservation as outlined in Section 5..

1.2 Primary Objectives

Patients will receive DC/AML fusion vaccination in conjunction with GM-CSF following a chemotherapy induced remission.

Primary objective: To assess the toxicity associated with treating AML patients with DC/AML fusion cells in the post-chemotherapy setting

Secondary objective:

1. To explore immunological response to DC/AML fusion vaccination in patients who have achieved a chemotherapy-induced remission.
2. To correlate levels of circulating activated and regulatory T cells with immunologic response
3. To define anti-tumor effects by determining time to disease progression.

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2. BACKGROUND

2.1 Study Agent(s)

2.1.1 Dendritic Cells (DC): Potent Antigen Presenting Cells

A promising strategy for generating tumor specific immune responses involves the use of potent antigen presenting cells known as DCs. DCs represent a complex network of cells characterized by the expression of costimulatory and adhesion molecules necessary to initiate primary immune responses(1-4). Myeloid DCs are derived from CD34+ precursors in the bone marrow and exhibit increased stimulatory capacity with maturation. DCs in patients with cancer have quantitative and functional deficiencies which contribute to tumor associated immune tolerance. Immature DCs predominate in the tumor bed and maturation is inhibited by factors released by tumor cells such as VEGF, TGF β , and IL-10. In contrast, functionally active DC can be generated in large quantities ex-vivo by culture of peripheral blood mononuclear cells in the presence of cytokines(5-8). DC based tumor vaccines are being explored as a strategy to stimulate immune responses that selectively eliminate malignant cells. DC loaded with specific tumor antigens by pulsing with peptide or proteins or by inserting tumor specific genes elicit antigen specific anti-tumor responses in preclinical experiments, animal models and clinical studies(9-16). Single antigen approaches are limited, however, by the small number of known tumor-specific antigens, their variable immunogenicity, and the potential ability of tumor cells to alter expression of individual antigens in order to evade immune recognition. Alternatively, DCs may be loaded with antigens derived from whole tumor cells(12, 17-19).

We have developed a promising DC based cancer vaccine involving the fusion of tumor cells with autologous DCs using polyethylene glycol (PEG)(20, 21). DC/tumor fusions present a broad array of antigens in the context of the potent antigen presenting machinery of the DC fusion partner. DC/tumor fusions uniquely stimulate both helper and cytotoxic T cell responses through the presentation of internalized and newly synthesized antigens, respectively(22). In animal tumor models including multiple myeloma, vaccination with DC/tumor fusions is protective from an otherwise lethal challenge of tumor cells and, most significantly, results in the eradication of disease in tumor bearing animals(23-25). In a MUC1 transgenic mouse model, fusion cells effectively induced anti-tumor immunity against a MUC1 expressing malignancy without the development of autoimmunity(26). The immunologic potency of fusion cells was enhanced by the use of cytokine adjuvants, such as IL-12(27). In pre-clinical human studies, fusion cells stimulate antigen specific CD4 and CD8+ responses and CTL responses targeting patient-derived tumor cells(28-30). In a hematological malignancy model, fusion cells more potently stimulate anti-tumor immunity than DC pulsed with apoptotic bodies or tumor lysate(31).

In clinical studies, whereas DC/tumor fusions stimulate anti-tumor immunity in a majority of patients, clinical responses are observed in only a subset(32, 33). We have completed phase I trials of fusion cell vaccination in patients with metastatic breast and renal cancer(32). 23 patients underwent vaccination with autologous DC/tumor fusions. Tumor cells were isolated from accessible lesions and fused with DCs differentiated from adherent mononuclear cells by culturing for 1 week with GM-CSF and IL-4. Fusion cells were quantified by determining the

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percentage of cells that co-expressed unique DC and tumor antigens. In the breast cancer study, successive cohorts of patients were treated with 1×10^5 , 3×10^5 , and 1×10^6 fusion cells subcutaneously at 3 week intervals. In the renal carcinoma study, dose escalation of the vaccine proceeded from 1×10^6 to 4×10^6 fusion cells. Dose escalation was completed without significant toxicity or difficulty achieving the desired dosing levels. Vaccination was well tolerated. Adverse events judged to be potentially related to vaccine included, most commonly, transient pain at sites of tumor and injection site reactions. 2 breast cancer patients demonstrated disease regression, including one patient who experienced complete resolution of 8x6 cm chest wall mass. 6 patients (5 renal, 1 breast) demonstrated stable disease with disease progression 3-9 months following completion of vaccination. To assess the effect of vaccination on tumor specific immunity, we serially measured the percentage of T cells expressing IFN γ following in vitro exposure to autologous tumor lysate. Of 18 patients undergoing assessment, vaccination resulted in at least a 2-fold increase in tumor lysate reactive CD4+ and CD8+ T cells in 10 and 7 patients, respectively. Of note, this response was most prominent in the breast cancer patient who experienced significant disease regression. This initial study demonstrated that vaccination with DC/tumor fusions is feasible, well tolerated, associated with immunologic responses in a majority of patients and clinical responses in a subset.

DCs derived from patients with malignancy demonstrate functional deficiencies that may contribute to immune tolerance. In a subsequent trial for patients with metastatic renal cancer, we examined the safety, immunologic response, and clinical efficacy of vaccination with fusions of autologous tumor cells with DCs derived from normal volunteers(33). 30 patients underwent tumor procurement of which 24 patients underwent vaccination. 21 and 20 patients were evaluable for immunologic and clinical response to vaccination, respectively. No clinical evidence of auto-immunity was observed in the vaccinated patient population. 10 patients had at least a 2-fold increase in the percentage of CD4+ and/or CD8+ T cells expressing IFN γ following tumor lysate exposure. 2 patients had partial responses following fusion cell vaccination by RECIST criteria. 1 patient experienced 72% regression of 4 target pulmonary nodules at week 10 and complete regression of these lesions at weeks 16 and 20. The patient has remained without evidence of disease progression at 103 weeks following vaccination. A second patient with metastatic disease to the liver, lung and lymph nodes had 28% and 36% regression of abdominal and hilar lesions at 20 and 24 weeks, respectively. The patient remained without evidence of disease progression at 6 months post-study, however progressed at 12 months of follow-up. 8 additional patients demonstrated stable disease for a minimum of 10 weeks, including 3 patients who remain without disease progression at 74, 79, and 82 weeks following vaccination. At 1 year, progression free survival and overall survival was 25% and 74%, respectively. The mean fold increase of IFN γ by CD8+ T cells was 3.74 and 0.97 for patients with disease response or stabilization and those with progressive disease, respectively ($p=0.03$). This study demonstrated that clinical disease regression and long term disease stabilization was feasible in patients with advanced metastatic RCC undergoing DC/RCC fusion vaccination. Immunologic response correlated with clinical response in a statistically significant manner.

DC/MM fusions are highly effective for inducing anti-tumor immunity in multiple myeloma. In a murine model, we have demonstrated that vaccination with DC/MM fusions results the eradication of established disease(27). Vaccine efficacy was augmented by the presence of IL-12. We have shown that fusions generated with patient derived myeloma cells and DCs

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exhibit phenotypic characteristics of potent antigen presenting cells including expression of costimulatory molecules, IL-12 and the chemokine receptor, CCR7, necessary for migration to sites of T cell traffic in the draining lymph nodes(34). Patient derived DC/MM fusions stimulate cytotoxic T cell mediated lysis of autologous myeloma cells in vitro.

In an acute myelogenous leukemia model, DC were fused with patient derived leukemic blasts cells. DC/leukemia fusions were documented by co-expression of leukemia (CD117) and DC derived antigens (CD80, CD86, and CD83) by immunocytochemical and immunofluorescent analysis. We have examined the capacity of DC/leukemia fusions to stimulate anti-tumor immune responses directed against AML in vitro. Interferon gamma production by T cells in response to stimulation by fusion cells was significantly higher than after stimulation by blasts.

Vaccination of patients with advanced MM with mature DC/tumor fusion vaccine in conjunction with GM-CSF

We are completing a phase I/II study in which patients with advanced MM undergo vaccination with mature DC/MM fusions in conjunction with GM-CSF. Recent studies have demonstrated that GM-CSF augments the capacity of tumor vaccines to generate tumor specific immunity. To date, 18 patients have been enrolled. Most patients had advanced refractory disease with a median number of 4 prior treatment regimens and 14 patients having undergone prior autologous stem cell transplantation. DCs were generated from adherent mononuclear cells cultured for 5 days with GM-CSF and IL-4 and matured by exposure to TNF α for 48-72 hours. The mean yield of DCs was 1.23×10^8 cells with a mean viability of 88%. Myeloma cells were isolated from bone marrow aspirates. The mean yield and viability of myeloma cells was 5.7×10^7 cells and 92%, respectively. Fusion cells were generated by coculture of DCs with myeloma cells in the presence of 50% polyethylene glycol. Fusion cells were quantified by determining the percentage of cells that co-expressed unique DC and myeloma antigens. Mean fusion efficiency and viability was 39% and 84%, respectively. Dose escalation has been successfully completed with 9 patients being treated at the maximum planned dose of 4×10^6 fusion cells. As a measure of immunologic potency, DC/MM fusions stimulated allogeneic T cell proliferation, with mean stimulation indexes of 48, 36, and 9 for the DC, fusion and myeloma cell populations, respectively. Adverse events potentially related to vaccine included injection site reactions, edema, rash, fever (infection), chills, fatigue, muscle aches, pruritis, and diarrhea. One patient with a history of prior deep venous thrombosis (DVT) developed a DVT and pulmonary embolus of uncertain relation to the vaccine. Biopsy of vaccine site reactions demonstrated a dense infiltrate of CD8 $^+$ T cells and CD1a $^+$ immature DCs indicating that GM-CSF recruited native DCs to the site of vaccination that potentially amplify vaccine response.

To date, 6/9 evaluable patients have demonstrated immunologic response, defined by at least 2 fold increase in IFN γ expression by CD4 and/or CD8 T cells in response to ex vivo exposure to autologous tumor lysate. The mean fold increase following vaccination was 10.5 and 9.5, respectively. In a representative example, vaccination resulted in a 32 and 20 fold increase in IFN γ expression by CD4 $^+$ and CD8 $^+$ T cells, respectively. Vaccination was associated with an increase in circulating tumor specific T cells as evidenced by an increase in CD8 $^+$ cells binding the MUC1 tetramer. We have also performed SEREX analysis to assess humoral response to the DC/MM fusion vaccine and identify novel myeloma targets being recognized. A cDNA library from the CD138 $^+$ primary myeloma cells was expressed in *E. coli* using a λ ZAPII phage system. Sera was obtained from patients prior to and following vaccination and screened against the

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cDNA library. After 2-3 consecutive screenings and excluding the clones encoding immunoglobulin gene fragments, the final clones were evaluated by sequence analysis. Humoral responses directed against 2 genes, RGS 19 and Hsp90 β , were detected one month post-vaccination. Antibodies against these targets were not present prior to vaccination or in sera obtained from 5 normal volunteers. RGS 19 has been identified as tumor associated antigen found in lymphoproliferative diseases. Of 16 evaluable patients, 66% of patients experienced disease stabilization from 2-24 months post vaccination. In some patients, responses were transient and associated with a corresponding rise in regulatory T cells.

Tumor cells create an immunosuppressive environment characterized by ineffective T cell function and the increased presence of regulatory T cells that dampen immune activation and potentially limit the response to cancer vaccines(35-37). Regulatory T cells co-express CD4 and high levels of CD25, minimally proliferate in response to mitogenic stimuli, and inhibit T cell responsiveness through the expression of inhibitory ligands and cytokines such as TGF β (38). Distinctive phenotypic characteristics include co-expression of the glucocorticoid- induced tumor-necrosis factor receptor (GITR), cytotoxic T lymphocyte antigen-4 (CTLA-4), and Foxp3. An increased presence of regulatory T cells has been noted in the circulation, draining lymph nodes, and tumor beds of cancer patients at levels that correlate with disease burden(39-41). Cancer vaccines may paradoxically expand regulatory cells that subsequently inhibit response(42). Depletion of T regulatory cells has resulted in improved response to tumor vaccine in animal models and a clinical study(43, 44).

Fusion vaccination induces regulatory T cells that potentially inhibit vaccine response

Vaccination with DC/tumor fusions induces anti-tumor immunity in a majority of patients; however, responses are transient and not always associated with clinical benefit. One potential limiting factor is that regulatory T cells, which potentially blunt vaccine efficacy, are increased in patients with malignancy and may be further expanded by tumor cells in the vaccine preparation. We have thus examined the effect of the DC/tumor fusion vaccine on the expansion of activated and regulatory T cells in vitro. Coculture of fusion cells and autologous T cells resulted in a 6.3 fold increase in CD4+CD25+ T cells that expressed CD69, (4.7%- 29.5, N=5; p=0.01) consistent with an activated phenotype. However, stimulation with DC/tumor fusions also resulted in 9 and 5.2 fold increases in CD4+CD25+ T cells that expressed GITR and CTLA-4, respectively. The mean percentage of CD4+CD25+ T cells expressing IFN γ was 40% (N=14) and 68% prior to and following fusion cell stimulation (p=0.005), respectively. The percentage of CD4+CD25+ T cells expressing the inhibitory cytokine, IL-10, rose from 20% to 59% (p=0.0002). Foxp3 expression increased from 26.5% (n=9) to 63% (p=0.01). As such, fusion cells induce the expansion of both immunostimulatory and immunosuppressive T cell subsets resulting in a complex response in which regulatory T cells may prevent the development of effective anti-tumor immunity. We subsequently examined the functional characteristics of the T cell populations stimulated by DC/tumor fusions. Following fusion cell stimulation, CD4/CD25^{low} and CD4/CD25^{high} populations were isolated by FACS sorting. CD4/CD25^{high} cells uniformly expressed FOXP3 and demonstrated phenotypic characteristics consistent with regulatory T cells as manifested by inhibition of T cell responses to PHA, allogeneic DCs, and tetanus toxoid. In contrast, CD4/CD25^{low} cells did not inhibit responses by CD4/CD25- cells. This data confirms that fusion cells stimulate a mixed population of activated and regulatory T

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cells that can be segregated by the intensity of CD25 expression and highlight the importance of regulatory T cell depletion as part of the fusion vaccine strategy.

Developing strategies that promote the expansion of functionally competent tumor reactive T cells and limit the influence of regulatory T cells is necessary to augment the efficacy of the DC/MM fusion vaccine. One approach is vaccination in conjunction with autologous stem cell transplantation which facilitates vaccine response by inducing a minimal disease state and limiting the inhibitory influence of the myeloma cells. In pre-clinical models, stem cell transplantation results in the in vivo depletion of regulatory T cells, transient loss of tumor mediated tolerance, and enhanced capacity to respond to tumor vaccines (45, 46). An increase in tumor reactive clones is observed during lymphopoietic recovery following transplant. In an ongoing study, we have demonstrated that vaccination following stem cell transplantation results in the eradication of residual disease as manifested by resolution of myeloma paraprotein and marrow involvement.

Vaccination with DC/MM fusions in conjunction with autologous stem cell transplantation

Autologous stem cell transplantation results in effective tumor cytoreduction and improved disease outcomes, but patients ultimately relapse from sites of persistent disease. In animal models, transplantation is associated with transient reversal of tumor mediated tolerance and enhanced response to cancer vaccines. Transplantation is associated with regulatory T cell depletion and increased presence of tumor reactive clones during post-transplant lymphopoietic reconstitution. Based on these findings, we are conducting a study in which patients with multiple myeloma undergo stem cell transplantation followed by post-transplant vaccination with 3 doses of DC/MM fusions. To date, 20 patients have been enrolled. All patients have undergone successful vaccine generation and subsequently are undergoing pre-transplant induction, stem cell mobilization and high dose chemotherapy with stem cell rescue. Mean yield of the DC and myeloma preparations was 1.8×10^8 and 8.1×10^7 cells, respectively. Mean fusion efficiency was 41% and the mean cell dose generated was 4.2×10^6 fusion cells. Mean viability of the DC, myeloma, and fusion preparations was 88%, 87%, and 76%, respectively. As a measure of their potency as antigen presenting cells, fusion cells potently stimulated allogeneic T cell proliferation in vitro. Mean stimulation indexes were 12, 57, and 31 for T cells stimulated by myeloma cells, DCs, and fusion cell preparations at an APC: T cell ration of 1:10. Adverse events judged to be potentially vaccine related included injection site reactions, pruritis, myalgias, fever, chills, and tachycardia. 9 patients have completed vaccinations of which 6 have achieved a complete remission. 3 patients demonstrated resolution of post-transplant paraprotein levels only after undergoing vaccination. 1 patient with highly aggressive disease who experienced disease progression in the early post-transplant period, demonstrated initial response and then stabilization of disease with vaccination.

We are examining the effect of transplant and vaccination on measures of cellular immunity, anti-tumor immunity and levels of activated as compared to regulatory T cells. T cell responses to PHA mitogen and tetanus toxoid were transiently depressed post-transplant. Similarly, DTH responses to candida antigen were absent post-transplant in all but 1 patient. In contrast, a significant increase was noted post-transplant in circulating tumor reactive lymphocytes as determined by T cell expression of IFN γ by CD4 and CD8 cells following ex vivo coculture autologous myeloma cell lysate (Mean expression by CD8 cells was 0.9 and 10.1 pre and post-

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transplant, respectively $p=0.03$; mean expression of CD4 cells was 0.8 and 3.9; $p=0.02$). A further amplification of tumor reactive lymphocytes was seen with vaccination in a subset of patients. A decrease in the median levels of circulating regulatory T cells and a relative increase in the ratio of activated (CD4/CD25^{low})/regulatory (CD4/CD25^{high}) cells was observed following transplantation. This finding suggests that although nonspecific T cell responses are muted in the early post-transplant period, there is a greater capacity to recognize tumor antigens, potentially due to the depletion of regulatory T cells and the decline in tumor mediated immune suppression. In summary, fusion cell vaccination in conjunction with stem cell transplantation was well tolerated, induced anti-tumor immunity and clinical responses in patients with multiple myeloma. The post-transplant period is characterized by increased levels of activated as compared to regulatory T cells and enhanced levels of T cells with the capacity to respond to myeloma cells. The increase in tumor reactive T cells post-transplant is further amplified following exposure to the DC/MM fusion vaccine.

2.2 Study Disease

2.3 Acute Leukemia: Overview

Acute myelogenous leukemia is a hematological malignancy characterized by the proliferation and accumulation of immature hematopoietic cells in the bone marrow and peripheral blood. These leukemic blasts replace the bone marrow, and inhibit the growth and maturation of normal erythroid, myeloid, and megakaryocytic precursors. Presenting signs and symptoms are usually related to decreased production of normal hematopoietic cells. Untreated, AML is usually fatal within weeks to months from diagnosis. AML causes approximately 1.2% of all cancer deaths in the United States, and represents approximately 90% of adult acute leukemias.

Treatment for acute myelogenous leukemia

The goal of therapy for acute myelogenous leukemia is to obtain and maintain a complete remission. Most remission induction regimens consist of a combination of an anthracycline and cytosine arabinoside. 60%-70% of patients achieve a complete remission with induction chemotherapy, 25-40% requiring more than one course to achieve a complete remission(51). Optimal treatment for patients who achieve a complete remission is controversial. Chemotherapy is often not curative for patients with AML due to the development of resistance particularly by early leukemia progenitor cells that serve as a reservoir for disease recurrence. In contrast, the potential efficacy of cellular immunotherapy in this setting is highlighted by the decreased incidence of disease relapse following allogeneic transplantation due to the anti-leukemic effects of alloreactive donor effector cells. This graft versus disease effect is manifested in patients with AML by the comparatively higher risk of relapse for patients undergoing autologous or T cell depleted transplantation and the capacity of donor lymphocytes to induce disease regression post-transplant in a subset of patients. However, the lack of specificity of the alloreactive response results in graft versus host disease, which remains a major source of morbidity and mortality following allogeneic transplantation. As a result, improved disease control associated with allogeneic transplantation is counterbalanced by increased treatment related mortality. An intensive area of research involves developing immunotherapeutic strategies to more selectively target leukemia cells, resulting in improved treatment efficacy while minimizing associated morbidity.

Immunotherapy for acute myelogenous leukemia: rationale

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A variety of tumor associated antigens have been identified in AML cells, including transcription factor WT1, the serine protease proteinase 3 (PR3), and the epithelial mucin MUC-1(52-55). WT-1 is overexpressed in blasts in most patients with AML. WT-1 specific antibodies are found in approximately a quarter of AML patients. Cytotoxic T cells specific to WT-1 have been generated which lyse AML targets(56). In animal models, vaccination with the WT1 epitope results in immunologic responses and tumor regression(52). Clinical trials utilizing WT1 peptide vaccination in patients with AML are in progress, and both immunologic and clinical responses have been observed(57). The proteinase 3 protein is expressed by myeloid leukemia cells and may be recognized by the immune repertoire. Cytotoxic T lymphocytes (CTL) targeting a peptide derived from proteinase 3 are capable of lysing AML cell in vitro(58). MUC-1 is overexpressed in many epithelial tumors, and has been shown to be expressed in approximately two thirds of AML samples(55). MUC-1 specific CTL have been shown to induce lysis of primary AML blasts, demonstrating that MUC-1 derived peptides are potential targets for immunotherapeutic strategies(55, 59).

2.4 Rationale

In the proposed study, we will examine toxicity, immunologic effect and clinical efficacy of DC/AML fusion cell vaccination in AML patients who have obtained a complete remission following chemotherapy. Patients will undergo chemotherapy to obtain a complete remission, followed by treatment with DC/AML fusion vaccination. We will quantify the presence of tumor reactive T cells targeting leukemia cells, leukemic stem cells and leukemia associated antigens at serial time points prior to and following therapy. We will quantify the presence of regulatory T cells and T cells expressing PD-1 in the circulation pre and post-therapy and assess its effect on vaccine response.

3. PARTICIPANT SELECTION

Patients will be enrolled to the study at presentation with newly diagnosed or first relapsed AML and will undergo tumor collection and cryopreservation as outlined in Section 5.

An initial cohort of 16 patients has been treated with DC/AML vaccine alone without excessive toxicity, and 12 of the initial 16 patients remain in remission with a median follow up of 53 months. We will therefore enroll additional patients to be treated with DC/AML fusions in an expansion cohort, to assess the toxicity and efficacy of the regimen in a total of 35 patients..

3.1 Eligibility Criteria

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

1. Patients with AML at initial diagnosis or at first relapse
2. Patients must be ≥ 18 years old
3. Patients must have ECOG performance status of 0-2

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4. Life expectancy of greater than 9 weeks
5. Laboratories:
 - Bilirubin ≤ 2.0 mg/dL
 - AST/ALT $< 3 \times$ ULN
 - Creatinine ≤ 2.0 mg/dl
6. The effects of DC/AML fusion cells 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
7. 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.

1. Patients must not have active or history of clinically significant autoimmune disorders/conditions, defined as requiring systemic therapy, including Type I diabetes. Type II diabetes, vitiligo, stable hypothyroidism, and thyroid disease well controlled with thyroid replacement will not be considered exclusion criteria.
2. Because of compromised cellular immunity, patients who have a known history of HIV will be excluded
2. Patients must not have significant cardiac disease characterized by symptomatic congestive heart failure, unstable angina pectoris, clinically significant cardiac arrhythmia
3. Patients must not be pregnant. All premenopausal patients will undergo pregnancy testing. Men will agree to not father a child while on protocol treatment. Men and women will practice effective birth control while receiving protocol treatment.
4. 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 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 5 years: non-invasive cancer (such as, any in situ cancers) and basal cell or squamous cell carcinoma of the skin.

3.3 Eligibility Prior to Cell Collections for Dendritic Cell Generation:

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Inclusion Criteria

1. Patients must have obtained a complete remission with chemotherapy defined by the absence of circulating blasts, and less than 5% blasts on bone marrow examination following hematopoietic recovery
2. Resolution of all chemotherapy related grade III-IV toxicity as per CTC criteria 4.0
3. Laboratories:
Absolute Neutrophil Count $\geq 1,000/\mu\text{L}$
Platelets $> 50,000/\text{uL}$
Bilirubin $\leq 2.0 \text{ mg/dL}$
Creatinine $\leq 2.0 \text{ mg/dL}$
AST/ALT $< 3.0 \times \text{ULN}$
4. For patients with evidence of minimal residual disease prior to vaccination, assessment of minimal residual disease status by cytogenetics or FISH will be followed post vaccination

Exclusion Criteria:

1. Patients must not have serious intercurrent illness such as infection requiring IV antibiotics, or significant cardiac disease characterized by significant arrhythmia, ischemic coronary disease or congestive heart failure
2. Patients who, with their treating physician, choose to proceed with an allogeneic transplant at the time of remission will not be vaccinated and will come off study

3.4 Eligibility Prior to Post-chemotherapy Immunotherapy (before first vaccine)

1. Resolution of all chemotherapy related grade III-IV toxicity as per CTC criteria 4.0
2. Laboratories:
WBC $\geq 2.0 \times 10^3/\text{uL}$
Platelets $> 50,000/\text{uL}$
Bilirubin $\leq 2.0 \text{ mg/dL}$
Creatinine $\leq 2.0 \text{ mg/dL}$
AST/ALT $< 3.0 \times \text{ULN}$
3. At least 2 doses of fusion vaccine were produced.

4. 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

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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. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at 617-632-3761 and follow the instructions for registering participants after hours.

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. Participants will be registered to the QACT three times as per section 3.1, 3.3 and 3.4 of the protocol.**

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 when applicable.
5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

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4.3. Sites external to DF/HCC: Please refer to Appendix III, DSMP Section 5.7 for registration procedures.

Following registration, participants should undergo tumor cell collections within 72 hours or as soon as possible. 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. The Study Coordinator should be notified of participant status changes as soon as possible.

Patients will be evaluated and may undergo standard induction and post-remission chemotherapy at any of the participating sites. Cell collections for vaccine generation, research blood work, vaccination, will occur only at Beth Israel Deaconess Medical Center or Rambam Medical Center.

5. TREATMENT PLAN

Treatment will be administered on an out-patient basis. Expected toxicities and potential risks for Dendritic Cell Vaccine 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 with the intent to treat the participant's malignancy.

In this clinical trial, we will examine the immunologic effect, toxicity, and clinical efficacy of immunotherapy with DC/AML fusion cells for patients with AML who obtain a remission following chemotherapy. The underlying hypothesis of this strategy is that immunotherapy will be most effective in the minimal residual disease state. Patients will receive 2 to 3 doses of DC/AML fusion cells in conjunction with GM-CSF beginning 4-12 weeks following completion of chemotherapy. The primary endpoint of the study will be to assess toxicity. The secondary endpoint will be to assess the ability DC/AML fusion cells and GM-CSF to induce anti-tumor immunity. Time to disease progression will be assessed.

Stopping rules

If 3 or more patients experience a TLT among the 10 patients enrolled in cohort 1, accrual will be suspended. TLT will be defined as grade 3 or higher toxicity as determined by CTC 4.0 possibly, probably or definitely related to the study treatment (the DC/AML fusion vaccine).

All cell manipulation and cultures will be performed according to current Good Manufacturing Practice (cGMP) standards and will take place at the Beth Israel Deaconess Medical Center (BIDMC) Stem Cell Laboratory.

5.1 Timing of testing and evaluations

Screening evaluation: Testing to be performed following signing of informed consent. If studies were done as a part of standard disease assessment within the proscribed time period, they may be used for eligibility assessment

Within 2 weeks of registration

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- 1 - Medical History, Physical Exam, Assessment of performance status
- 2 - Bone Marrow Aspirate/biopsy, including cytogenetics
- 3 - Electrocardiogram (EKG)
- 4 - Laboratory evaluation:
 - CBC with differential,
 - Liver Function Tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin*, LDH, Alkaline Phosphatase),
 - Electrolytes(Na, K, Cl, CO₂, Ca, Mg, PO₄), BUN Creat,
 - Pregnancy Test, if applicable, for women of childbearing potential
 - Erythrocyte Sedimentation Rate (ESR)
 - Antinuclear Antibody (ANA)
 - TSH

* Direct bilirubin only required if Total bilirubin is not within normal limits

Within 8 days of isolation of both tumor and dendritic cells standard infectious serologies will be collected as per BIDMC and RMC practice for storage of cellular products. (drawn in 2 red top tubes and 2 pink top tubes and sent to Dr. Avigan's lab at BIDMC or Dr. Katz's lab at Rambam Medical Center). 3-5cc of bone marrow and peripheral blood will be collected and sent to Dr. Cathy Wu's lab at DFCI.

5.1.1 Isolation of Tumor Cells

At the time of disease presentation, eligible patients will undergo collection of leukemia cells. Bone marrow aspirates will be obtained (20-30cc) under local anesthesia and mononuclear cells will be isolated by ficoll density gradient centrifugation. Patients who have a presenting WBC 20K or greater with at least 20% circulating blasts may have tumor cells collected from peripheral blood rather than from a bone marrow collection. In such cases, 50 cc of peripheral blood will be collected and mononuclear cells will be isolated by density centrifugation. If leukapheresis is required for clinical indications, the product may be used for subsequent vaccine preparation. Autologous plasma will be obtained by harvesting supernatant following centrifugation of 50-100 ml of peripheral blood. Peripheral blood or bone marrow mononuclear cells will be frozen in 10%/DMSO/90% autologous plasma stored in liquid nitrogen. A minimum of 5×10^7 PBMC will be required to go forward with freezing for subsequent vaccine preparation. An aliquot of the tumor cells will undergo immunohistochemical staining and/or FACS analysis for expression of tumor markers (including CD64, CD14, CD38, CD117, CD34, MUC-1), and co-stimulatory molecules. The ability of the leukemia cells to induce proliferation of allogeneic T cells will be measured. A repeat marrow harvest may be performed if the first marrow harvest does not yield adequate tumor cells.

Following collection of tumor cells, leukemia therapy will be administered according to current standards of practice at the discretion of the treating physician. Patients are permitted to receive hydroxyurea prior to initiation of tumor cell collection if clinically indicated. Patients who achieve remission status as defined by absence of circulating blasts, and less than 5% blasts on bone marrow exam following hematopoietic recovery will proceed with immunotherapy. When patients are in remission, leukemic blasts will be thawed, cultured and viability as well as gram stain will be assessed. An aliquot from this sample will undergo microbiological assessment. When cell yields allow, two doses of 1×10^5 - 1×10^6 cells (based upon cell availability) will be resuspended in PBS, irradiated to 6,000 rads (60 Gy) and frozen at -30°C for subsequent DTH testing. Remaining cells may be frozen for use in subsequent in vitro assays. Tumor lysate will

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be prepared by freeze/thawing of an aliquot of tumor cells for immunological analysis.

5.1.2 Chemotherapy

Patients receive 1-2 cycles of induction chemotherapy as per standard of care or on a clinical trial. Patients who receive hypomethylating agents may receive up to 4 cycles of therapy to achieve remission as per standard of care. Choice of induction therapy will be determined at the discretion of the treating physician. Patients not in remission after two cycles of induction chemotherapy will be removed from study. Patients receiving hypomethylating agents who are not in remission after four cycles of chemotherapy will be removed from the study. 0-4 cycles of post-remission chemotherapy may be given. The choice of post-remission therapy and number of cycles will be determined at the discretion of the treating physician. Post-remission therapy should begin from 1 week to 3 months after recovery of peripheral blood counts following induction therapy. Patients will undergo post chemotherapy immunotherapy once that they have recovered from grade 3-4 toxicity. Participants will begin immunotherapy with DC/AML fusion vaccination 4-12 weeks following the last cycle of chemotherapy

5.1.3 Isolation of DC

Patients who achieve complete remission and meet eligibility criteria will undergo leukapheresis to obtain adequate numbers of PBMC. Leukapheresis will be performed when patients are documented to be in complete remission, after recovery of blood counts (an ANC >1000 and PLT >50) from the first or second cycle of chemotherapy. When possible, this will be performed via peripheral access. If peripheral access is inadequate, patients will undergo placement of a temporary central venous catheter. After completion of leukapheresis, PBMC will be quantified. If an inadequate yield of PBMC is obtained for the patient's dose requirement, a repeat procedure will be performed.

PBMC will be isolated from the leukapheresis product and cultured in the presence of autologous plasma for 1-2 hours. The adherent fraction will be cultured in the presence of 1% autologous plasma/RPMI medium with 12.5 ng/ml rhIL-4 and 1000 U/ml GM-CSF for five to seven days to generate dendritic cells. 25 ng/ml of TNF α will be then be added for 48-72 hours to enhance DC maturation. Viability and gram stain will be assessed prior to fusion.

These cells will be assessed for morphologic characteristics and expression of characteristic DC markers that include CD11c, HLA DR, CD80, CD86, and CD83. In addition expression of CD117 and MUC-1 will be determined. Functional properties will be assessed using MLR assays in which DC will be co-cultured with allogeneic T cells. T cell proliferation will be measured via tritiated thymidine incorporation

5.1.4 Preparation of DC/AML Fusions

Vaccine preparation may occur upon completion of induction/re-induction therapy, or upon completion of up to 4 cycles of post-remission chemotherapy. Samples will be frozen as outlined below and thawed at the time of vaccine administration. Tumor cells and DC at ratio of 1:10-1:3 (dependent on cell yields) will be mixed and extensively washed in serum-free medium (RPMI 1640). After low speed centrifugation, the cell pellets will be re-suspended in 500 μ l of 50% solution of polyethylene glycol (PEG) in Dulbecco's phosphate buffered saline without Ca⁺⁺, Mg⁺⁺. After one to five minutes, the PEG will be progressively diluted by the slow addition of serum-free medium. The cells will be washed free of PEG and cultured in RPMI 1640 with 10%

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autologous plasma and GM-CSF in a 5% CO₂ atmosphere at 37° C. The percentage of the cell population that represent DC/tumor fusions will be determined by quantifying the cells as defined by dual expression of unique DC and leukemia markers such as: a) CD86 and CD117 or CD 34 or MUC-1 or b) CD83 and CD117 or CD34 or MUC-1; c) if the leukemia cells do not express DR, then DR and CD117 or CD34 or MUC-1 as measured by immunocytochemical staining and/or FACS analysis. Dosing will be determined by the absolute number of fusion cells identified in this manner. Fusion cells must demonstrate >10% fusion efficiency to proceed with vaccination. The fusion cells will then be separated into appropriate aliquots of fusion cells. 2-3 doses of 5 x10⁶ fusion cells, depending on cell yields, will be prepared and will be frozen in 10% DMSO/90% autologous plasma in liquid nitrogen. At that time, appropriate aliquots will be sent for microbiological analysis.

5.1.5 Evaluation prior to initiation of immunotherapy

Within 14 days of initiating post-remission immunotherapy, patients will undergo:

- 1 - Medical History, Physical Exam, Assessment of Performance Status,
- 2 - Bone Marrow Aspirate and Biopsy and an additional sample of 5-10cc may be collected for immune monitoring studies. For patients with a cytogenetic abnormality at presentation, cytogenetics/FISH will be assessed.*
- 3 - EKG,
- 4 - Laboratory evaluation:

CBC with differential,
liver function tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin**, LDH, Alkaline Phosphatase),
Electrolytes (Na, K, Cl, CO₂, Ca, Mg, PO₄) BUN, creatinine
erythrocyte sedimentation rate (ESR), and
antinuclear antibody (ANA)
TSH

Research blood work will be sent for assessment of:

DC subsets, immunologic response to tumor lysate (proliferation, IFN γ , and tetramer studies) (drawn in 5 green top tubes and sent to Dr. Avigan's lab at BIDMC or Dr. Katz's lab at Rambam Medical Center)
DTH skin testing to candida and irradiated tumor cells.
Neoantigen discovery and quantification of T cell response against identified neo-antigens (3-5 cc of bone marrow and peripheral blood will be drawn and sent to Dr. Wu's lab at DFCI)

* Bone marrow aspirate and biopsy may be done within 30 days of initiation of Immunotherapy

** Direct bilirubin only required if Total bilirubin is not within normal limits

5.1.6 Evaluation during immunotherapy period

The following evaluation will be performed prior to each vaccine (+/-2 days):

- 1-Medical History, Physical Exam, Assessment of Performance Status
- 2-Vaccine Associated Toxicity Assessment

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3–Laboratory evaluations:

CBC with differential,
liver function tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin**, LDH, Alkaline Phosphatase),
electrolytes(Na, K, Cl, CO₂, Ca, Mg, PO₄) ,BUN, Creatinine
erythrocyte sedimentation rate (ESR)*,
antinuclear antibody (ANA)*
TSH*
ECG*

Research blood work will be sent for assessment of*:

DC subsets, immunologic response to tumor lysate (proliferation, IFN γ , and tetramer studies) (drawn in 5 green top tubes and sent to Dr. Avigan's lab at BIDMC or Dr. Katz's lab at Rambam Medical Center)

* Do not have to be repeated on cycle 1 day 1 if performed within 7 days of initiating treatment.

** Direct bilirubin only required if Total bilirubin is not within normal limits

The following will be performed the week that immunotherapy is not administered +/- 2 days:

- 1 -Medical History, Physical Exam, Assessment of Performance Status
- 2 – Treatment Associated Toxicity
- 3 – Laboratory evaluations: CBC with differential, liver function tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin**, LDH, Alkaline Phosphatase), electrolytes(Na, K, Cl, CO₂, Ca, Mg, PO₄) ,BUN, Creatinine
** Direct bilirubin only required if Total bilirubin is not within normal limits

5.1.7 Post-remission Immunotherapy

4-12 weeks following the last cycle of chemotherapy, patients will be reassessed for eligibility for immunotherapy. Patients meeting eligibility criteria outlined in section 5 will begin immunotherapy between 4-12 weeks following chemotherapy. Patients who do not meet eligibility by 12 weeks following the last cycle of chemotherapy will come off study.

Patients will be vaccinated with 5×10^6 fusion cells in the upper thigh region. The site will be alternated for each vaccine administration (right and left extremity). Vaccination will be administered subcutaneously using a 25-gauge 5/8-inch needle. On the day of vaccination, the clinical research nurse/physician assistant will administer 100 ug of GM-CSF subcutaneously at the site of the vaccine. The patient will be trained to inject the remaining three GM-CSF injections (100ug dose once a day) for self-administration subcutaneously at home. Tumor vaccine will be administered first, followed by GM-CSF injection. If a patient experiences a grade 2 or higher vaccine site reaction during the days when GM-SCF is administered, subsequent doses of GM-CSF may be held until the vaccine site reaction improves to grade 1 or less. For subsequent vaccinations, patients will receive pre-medication with diphenhydramine (Benadryl) 25-50 mg and/or acetaminophen (Tylenol) 650 mg to minimize potential allergic related symptoms. A total of 3 vaccinations will be given at monthly intervals as outlined in the study schema. When cell yields allow for only two doses of vaccine to be generated, patients

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will receive two doses of vaccine at monthly intervals.

When feasible, inflammatory responses at the vaccination site will be biopsied and infiltrating lymphocytes will be quantified. Biopsy of vaccine site reactions are optional, and patients will sign a separate consent form for the procedure.

Follow-Up Period

Four weeks following the final vaccination (+/-2 days), patients will undergo:

- 1 - DTH response to Candida,
- 2 - DTH response to irradiated tumor cells,

The following tests are to be preformed at the time of the final vaccination and monthly (+/- 7 days) for 6 months:

CBC with differential
liver function tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin*, LDH, Alkaline Phosphatase),
electrolytes(Na, K, Cl, CO₂, Ca, Mg, PO₄) ,BUN, creatinine

* Direct bilirubin only required if Total bilirubin is not within normal limits

In addition, at one month, three months, and six months (+/- 7 days) after their final vaccination patients will undergo:

- 1 - A bone marrow aspirate/biopsy and an additional sample of 5-10cc may be collected for immune monitoring studies. For patients with a cytogenetic abnormality at presentation, cytogenetics/FISH will be assessed

- 2 - ANA/ESR

- 3- T-cells subset,

- 4- Research blood work for the following:

Research blood work will be sent for assessment of:

DC subsets, immunologic response to tumor lysate (proliferation, IFN γ , and tetramer studies) (drawn in 5 green top tubes and sent to Dr. Avigan's at lab BIDMC or Dr. Katz's lab at Rambam Medical Center)

Neoantigen discovery and quantification of T cell response against identified neo-antigens (3-5 cc of bone marrow and peripheral blood will be drawn and sent to Dr. Wu's lab at DFCI)

- 5- TSH

5.2 Duration of Therapy

Duration of therapy 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 2-3 vaccinations or until one of the following criteria applies:

- Disease progression,

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- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- 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.

5.3 Duration of Follow Up

Patients will be followed until the time of disease progression with phone contact or clinic visit every 3 months for the first three years. Patients who are still in remission after three years will continue to be followed yearly until progression. Participants removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

5.4 Criteria for Removal from Treatment

Patients with documented disease progression requiring other treatment or patients in whom continued participation in the study is felt to be contraindicated by the principal or treating investigator will be removed from treatment at the discretion of the investigator and can receive appropriate therapy.

Patients experiencing treatment related toxicity as outlined above will be removed from treatment. The investigator should:

- 1) Document the reason(s) for withdrawal.
- 2) Follow the patient for resolution of toxicity.

Unexpected or Life Threatening Complications (see previous comments)

In the event of unusual, life-threatening, or lethal toxicity the Study Chair will notify the FDA within in 24 hours.

5.5 Duration of Survival Follow Up

All patients enrolled to the study and who received treatment will be followed for survival with phone contact or clinic visit every 6 months after being removed from treatment due to disease progression or unacceptable adverse event.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made using the recommendations outlined below. Toxicity assessments will be done using the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 which is identified and located on the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

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

6.1 Anticipated Toxicities

Treatment-Related Effects: A complete list of expected adverse events can be found in Section C of the Investigator's Brochure for DC/Tumor Fusion Vaccines.

6.2 Toxicity Management

6.2.1 Identification of treatment limiting toxicity

All toxicities will be graded according to the NCI Common Toxicity Criteria (Version 4.0). If ≥ 3 patients experience TLT in either cohort, the study will be suspended. 35 evaluable patients will be enrolled. Patients who receive at least 2 vaccinations will be considered evaluable. Patients will be monitored for one week following administration of vaccine during the period of immunotherapy and then monthly thereafter for 6 months during which time they will be assessed for evidence of treatment related toxicities with particular attention to auto-immune disease and cytopenias. Patients experiencing treatment related grade III or higher toxicity will not receive further therapy. The primary endpoints of the study will be assessment of toxicity. The secondary objective is to assess immunologic response. To assess disease status, bone marrow biopsies will be performed prior to initiation of immunotherapy and at 3 month intervals thereafter. Time to disease progression from the onset of immunotherapy will be determined. Patients who come off of the study before receiving 2 vaccines for reasons other than toxicity, will not be considered evaluable. Inevaluable patients will be replaced.

Potentially treatment related events will be defined as:

- 1) Toxicity secondary to autoimmune events
- 2) Toxicity not attributable in incidence or intensity to known sequelae of chemotherapy or the underlying disease

Dendritic Cell Fusion Vaccine: Patients who experience a grade III or higher vaccine associated toxicity will not receive further therapy.

There are no dose reductions for vaccine treatment.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Dendritic Cell Fusion Vaccine

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Dendritic cell/tumor fusion cells will be generated for each patient and stored as described in section 5.1.4.

7.2 GM-CSF

GM-CSF is commercially available.

8. CORRELATIVE/SPECIAL STUDIES

8.1.1 Determination of Baseline Immune Function

Immunologic measurement will focus on the frequency of tumor specific T cells in the patient's peripheral blood prior to and following treatment with DC/AML fusion cells. Pre-immunotherapy level of immune competence will be determined by 1) quantifying circulating CD4/CD8 populations; 2) assessing the delayed type hypersensitivity response to recall antigen (candida); 3) in vitro proliferation of T cells to mitogenic stimulation (PHA), and 4) measuring levels of circulating regulatory T cells as defined by CD4/CD25+/foxp3+ cells.

8.1.2 Characterization of DC, Tumor, Fusion Cells

The DC, tumor and fusion cells will be assessed for expression of characteristic DC and tumor markers such as CD11c, CD117, CD34, MUC-1, HLA DR, CD80, CD86, and CD83. Functional properties will be assessed using MLR assays in which DC, tumor, or fusion cells, will be co-cultured with allogeneic T cells. T cell proliferation will be measured by tritiated thymidine incorporation. MLR assays will be performed on an aliquot of the fusion cells obtained prior to therapy. The percentage of fusion cells in the cell population will be assessed through immunocytochemical analysis by quantifying those cells that coexpress a) CD86 and CD117 or MUC-1 or b) CD83 and CD117 or MUC-1; c) CD11c and CD117 or MUC-1, as measured by immunocytochemical staining and/or FACS analysis. When cell yields allow, fusion cells will be isolated by FACS gating around cells that co-express tumor and DC antigens and analyzed for expression of CCR7 and IL-12 by multichannel flow cytometric analysis.

8.1.3 Impact of therapy on circulating tumor reactive lymphocytes

The primary endpoint will be to assess the impact of immunotherapy on circulating levels of tumor reactive T cells. As our primary immunologic endpoint in the present study, we will determine the effect of therapy on circulating levels of CD4 and CD8+ T cells that express IFN γ by intracellular FACS analysis following in vitro exposure to tumor lysate. This assay allows for the characterization of the anti-tumor response without prior identification of an individual tumor specific peptide target. As such, the assay is well suited for a whole tumor/DC vaccine that is designed to generate a polyclonal response.

Immunologic assessments will be performed prior to induction chemotherapy, following completion of chemotherapy, prior to each dose of DC/AML fusion cells, and at 1, 3, and 6 months following the last treatment. At each time point, PBMC will be isolated from peripheral blood or graft by ficoll density centrifugation and cryopreserved. Following completion of the study, PBMC will be thawed and 1×10^6 PBMC will be cultured with lysate generated by freeze thaw cycle of 1×10^5 unselected leukemia cells or leukemia stem cells. When cell yields allow, leukemia stem cells will be isolated as described above. CD34+/CD38-/lineage-/Thy1^{low} (SC) will be isolated by multichannel FACS sorting and lysate will be generated. As a control, PBMC

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will be cultured with tetanus toxoid (10 ug/ml) or media alone. Following 5 days of coculture, expression of IFN γ by CD4+ and CD8+ populations will be determined by intracellular FACS analysis. Cells will be restimulated with tumor lysate for 6 h and cultured overnight with 1 ug/ml GolgiStop. The cells will be stained with CD4 or CD8 antibodies conjugated to FITC and permeabilized with Cytofix/Cytoperm plusTM. Cells will be stained with PE-conjugated anti-human IFN γ , fixed in 2% paraformaldehyde and analyzed by flow cytometry. The cytolytic capacity of tumor reactive lymphocytes will be determined by measuring expression of granzyme B and perforin in CD8+ T cells following ex vivo exposure to autologous tumor lysate. The presence of naïve, memory effector, and central memory cells in the tumor reactive population will be determined by multichannel FACS analysis.

The fold increase in the percent of T cells expressing IFN γ will be determined by comparing the pre-therapy level to the peak level observed following immunotherapy. Similar to the EliSpot assay, identification of T cells that express IFN γ in response to ex vivo exposure to tumor lysate allows for the quantification of circulating tumor reactive clones. However the use of intracellular FACS allows for the further functional characterization of these cells. As a measure of cytolytic capacity, CD8+ T cell production of granzyme B and perforin will be quantified. Expression of granzyme B and perforin by CD4 and CD8+ T cells following ex vivo exposure to autologous tumor lysate will be determined by bidimensional FACS analysis.

When cell yields allow, we will assess the capacity of T cells to lyse autologous tumor cells in a standard chromium release assay. Autologous leukemia cells will be pulsed with ⁵¹Chromium for 1 h at 37°C followed by repeated washes to remove unincorporated radioactivity. Following ex vivo stimulation with autologous tumor lysate (as outlined above), T cells will be isolated using a anti-CD3 separation column and incubated at a ratio of 60:1, 30:1, and 10:1 with chromium labeled autologous leukemia cells (2x10⁴ cells/well). ⁵¹Cr release will be quantified following 5 hour coculture of effector and target cell populations. Percentage cytotoxicity will be calculated using mean of triplicates by a standard assay as follows: % specific cytotoxicity = [(sample counts - spontaneous counts)/(maximum counts - spontaneous counts)] x 100. Spontaneous release was less than 25% of the maximum ⁵¹Cr uptake. Lysis of an irrelevant tumor line will be assessed as a control.

In a completed study of patients with renal carcinoma undergoing vaccination, a correlation between immunologic response and disease response was noted. A 3 fold mean increase in T cells expressing IFN γ in response to tumor lysate was observed in patients with disease stabilization or regression. In contrast, patients with disease progression did not exhibit any increase in IFN expression. Similarly, in a previous study of patients undergoing vaccination with DCs pulsed with melanoma peptides, a correlation between clinical response and at least a 3 fold increase in IFN γ expression following vaccination was observed. These studies provide validation for the use of this immunologic assay as a measure of clinically meaningful immunologic responses to vaccine based immunotherapy. In our ongoing study evaluating DC/myeloma fusion vaccination in the post-transplant setting, 50% of patients had a 10 fold increase in interferon gamma secretion following vaccination compared to pre-transplant. As such, a minimum of 10 fold increase in tumor reactive T cells in response to cellular immunotherapy is considered significant.

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8.1.4 Effect of treatment on circulating T cells binding the MUC1, WT1, and PR3 tetramer in HLA-A2.1 patients

We will also examine the effect of fusion vaccination alone or in combination with Pidilizumab (MDV9300) on immunologic responses against previously identified leukemia antigens, WT1, MUC1, and PR3. In HLA-A2.1 patients, the percent of CD8+ T cells binding tetramer constructed from these antigens will be determined. Functional characteristics of the tetramer positive population will be determined by measuring IFN γ , granzyme B, IL-4, and IL-10 expression in the tetramer positive cells by intracellular FACS staining.

8.1.5 DTH Reaction to Irradiated Tumor Cells

When cell yields allow, DTH reactions against irradiated tumor cells will be assessed prior to initiation of immunotherapy and 4 weeks +/- 7 days following completion of therapy. As outlined above, tumor cells will be irradiated and frozen at the time of the preparation of tumor cells for fusion. Greater than 48 hours prior to initiation of therapy and at completion of the study, 1×10^5 - 1×10^6 irradiated cells (determined by cell availability) will be thawed and injected intradermally to assess the DTH response. When feasible, inflammatory responses will be biopsied and infiltrating lymphocytes will be quantified using pathological staining. If possible, these infiltrating lymphocytes will be separated and evaluated for the ability to lyse autologous tumor and autologous monocytes pulsed with lysate or apoptotic bodies. Biopsy specimens will be processed to generate single cell suspensions. Viable cells will then be cultured in 1000 units/ml of IL-2 for several weeks. Lysis of autologous tumor targets will be measured. Biopsy specimens will undergo immunocytochemical staining to assess for infiltration of CD4, CD8 T cells. Recruitment of native DCs to the vaccine site will be assessed by CD1a expression in the vaccine bed. A portion of the biopsy will remain unfixed and will subsequently undergo mechanical dissection to generate a single cell suspension. The nature of the T cell infiltrate will be analyzed with respect to presence of tumor specific T cells as defined above.

8.1.6 SEREX Assessment

We will also seek to identify novel antigens that are being targeted by cellular immunotherapy. Humoral responses induced by and vaccination will be identified. Patient sera prior to and following vaccination will be incubated with leukemia derived proteins expressed by cDNA libraries on IPTG-treated nitrocellulose membranes. The filters will then be incubated overnight with pre and post vaccination patient serum, and diluted at 1:500 in TBST. Specific binding of antibody to recombinant proteins expressed on the lytic plaques will be detected by incubation with alkaline phosphatase-conjugated goat anti-human IgG antibody. Visualization of the antigen-antibody complex will be accomplished by staining with S-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (BCIP/NBT). cDNA inserts from clones becoming positive following vaccination will be isolated by excision of phagemids and then sequenced with T3 and T7 primers (Dana-Farber Cancer Institute Molecular Biology Core Facility).

8.1.7 Phenotypic assessment of circulating T cell populations, quantification of regulatory T cells and activated memory effector cells

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Prior to chemotherapy, following remission from induction chemotherapy, prior to each vaccine and at 1, 3 and 6 months following completion of therapy, circulating regulatory and activated T cell populations will be quantified. Peripheral blood will be collected from patients, and the percentage of activated effector cells will be determined by isolating the CD4/CD25+ fraction by flow cytometric gating and examining expression of CD69. Regulatory T cells will be quantified by assessing expression of Foxp3, GITR, CTLA-4, CD127, and CD62L on the CD4/CD25 population. The relative prevalence of naïve versus memory cell response will be determined by staining for CD45RA, and CD45RO, CCR7, CD28, respectively. We will examine whether the ability to generate at least a 10 fold increase in the percentage of T cells expressing IFN γ in response to ex vivo exposure to autologous tumor lysate is associated with lower levels of regulatory T cells and increased levels of memory effector cells as compared to that observed in patients who do not mount a significant immunologic response.

We have previously demonstrated that following DC/tumor fusion stimulation, the CD25^{high} population manifests characteristic features of regulatory T cells including lack of response to mitogens, inability to respond to recall antigens such as tetanus, and the ability to suppress, in a dose dependent fashion, T cell responses to mitogens, tetanus and allogeneic dendritic cells. In contrast, the CD25^{low} cells did not manifest functional characteristics of regulatory T cells. As such, at serial time points identified above, we will quantify the percentage of cells that dually express CD4 and CD25 and the percentage of CD25^{high} and CD25^{low} will be determined. The percentage of activated effector cells will be further elucidated by quantifying the percentage of CD4/CD25+ /CD69+ cells. Regulatory T cells will be quantified by assessing expression of FOXP3 for the CD4/CD25 population. GITR and CTLA-4, expression will be measured as secondary regulatory markers. The cytokine profile of the CD25^{high} and CD25^{low} populations will be determined by multichannel FACS analysis. Expression of intracellular stimulatory (IL-2, IFN γ) and inhibitory (IL-4, IL-10, TGF β) cytokines will be determined following stimulation with ionomycin. Tumor reactive clones will be quantified by determining the percentage of cells in the T cells that express IFN γ following ex vivo exposure to autologous tumor lysate. When cell yields allow, functional assessment of the regulatory T cell population will be determined. CD25^{high} cells will be isolated by FACS sorting. T cell responsiveness will be assessed by coculture with PHA (2 μ g/ml) for 3 days. Alternatively, cells will be cocultured with allogeneic mature DCs for 5 days at a ratio of 10:1, 30:1, 100:1, and 1000:1. T cell proliferation will be determined by thymidine uptake after overnight pulsing. The ability of the CD25^{high} cells to suppress primary T cell responses will also be measured. CD25^{high} cells will be added at a ratio of 1:5, 1:2, and 1:1 to unselected T cells undergoing stimulation with PHA, anti-CD3/CD28, or allogeneic DCs. Proliferative responses will be compared to cocultures in the absence of CD25^{high} cells. Expression of PD-1 on CD4+ and CD8+ populations will be assessed.

8.1.8 Humoral response induced by fusion vaccines

Aliquots of plasma and bone marrow aspirate will be sent to Cell Signaling Inc. for processing. The following analyses will be conducted at their facility. Humoral responses induced by vaccinations against antigens of interest will be identified. Antigen specific

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antibody sequences against known tumor antigens (e.g. MUC1, wt1, PRAME, telomerase, NY-ESO, survivin, ciclyn-D1, PR1) will be identified directly from circulation polyclonal antibodies using CST NG-XMT proteomics approach based on nano-flow chromatography coupled to mass spectrometry.

8.1.9 Neoantigen discovery, and quantification of T cell response against identified neo-antigens: 3-5 cc of bone marrow and peripheral blood will be obtained at baseline, at the time of documented remission, 1, 3, 6 months after vaccination, and at the time of relapse, and will be sent to the laboratory of Dr. Cathy Wu, DFCI. Leukemia cell DNA and RNA will be extracted from AML cells isolated from ficolled 3-5 cc of bone marrow aspirates collected at baseline, 1, 3, and 6 months after vaccination and at relapse (when relevant). Matched source of germline DNA will be obtained from cultured autologous marrow fibroblasts obtained after patients achieve complete remission. Genomic analysis and neo-antigen identification will be performed in the Wu laboratory. Once mutations for each tumor sample are defined, established analysis pipelines that the Wu lab has generated to predict immunogenic peptides that arise from tumor mutations and bind to personal HLA alleles will be applied. Candidate immunogenic peptides will be synthesized and used as antigens for pulsing autologous antigen presenting cells and testing their immunogenic potential in vitro using standard T cell assays. Peptides will be pulsed onto PBMCs collected at the time points outlined above. The prevalence of antigen specific T cells will be quantified by measuring the percentage of CD4 and CD8 T cells that express IFN γ by intracellular FACS analysis. The potential role of antigenic drift in tumor immune escape will be assessed with respect to presence of the identified neo-antigens in samples obtained from patients at time of disease progression. Immune response to neo-antigens and shared antigens will be compared between patients who progress and those remaining in remission at 2 years to assess correlation of response with clinical outcome.

9. STUDY CALENDAR

	Screening Within 2 weeks of registration	Prior to initial dose of immunotherapy (within 2 weeks)	Prior to each vaccine (+/- 2 days)	One week following vaccine (+/- 2 days)	4 weeks following final vaccine (+/-2 days)	Monthly x 6 months following final vaccine (+/- 7 days)	1,3 and 6 months After final vaccine (+/- 7 days)
Informed consent	X						
History, concurrent meds	X	X	X	X	X	X	X
EKG ²	X	X	X*				
Physical exam (Ht, Wt, VS ²)	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X
CBC w/diff, plts	X	X	X	X	X	X	X

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Serum chemistry: Na, K, CL, Ca, Mg, PO4, BUN, creat, ALT, AST, Alk phos, LDH, Bili	X	X	X	X	X	X	X
Adverse event evaluation	X		X	X	X	X	X
Bone marrow aspirate and biopsy	X	X@			X		X
ANA, ESR, TSH	X	X	X*		X		X
B-HCG (for women of childbearing potential)	X						
Research blood work ¹		X	X*		X		X
Blood for neoantigen ²	X	X					X
DTH skin testing to candida and tumor		X			X		

***do not have to be repeated on cycle 1 day 1 if performed within 7 days of treatment**

¹ 5 green top tubes to be sent to the laboratory of Dr. Avigan at Beth Israel Deaconess Medical Center or Dr. Katz's lab at Rambam Medical Center² 3-5cc of bone marrow and peripheral blood to be sent to Dr. Cathy Wu at Dana Farber Cancer Institute. Samples are to be obtained at baseline, at the time of documented remission, 1, 3, 6 months after vaccination, and at the time of relapse

@ Bone marrow aspiration and biopsy may be done within 30 days of initiating immunotherapy

10. MEASUREMENT OF EFFECT

Patients will undergo pertinent blood work and a bone marrow aspiration and biopsy within 1 week of initiation of immunotherapy and 4 weeks, three months and six months after completion of therapy. Evaluation will include measurement of relevant parameters of the patient's disease, including CBC with differential, cytogenetic evaluation on bone marrow specimens in patients who have abnormal cytogenetics at presentation.

Complete Remission (CR): Requires all of the following:

Neutrophil count $>1.0 \times 10^9/L$

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Platelet count $\geq 100 \times 10^9/L$

Absence of leukemic blasts in the peripheral blood

Bone marrow examination: maturation of all cell lines must be present with $\leq 5\%$ blasts

Extramedullary leukemia, such as CNS or soft tissue involvement must not be present

Complete Remission with Incomplete Count Recovery

For the purposes of this protocol, CRi is defined as:

- Meeting all criteria for CR except for either residual neutropenia ($ANC < 1.0 \times 10^9/L$) or thrombocytopenia (platelet count $< 100 \times 10^9/L$)

Complete Remission with Incomplete Platelet Recovery

For the purposes of this protocol, CRp is defined as a subset of CRi wherein patients fulfill all criteria for CR except that platelet counts are $< 100 \times 10^9/L$

Partial Remission (PR):

Requires that all of the above criteria for complete remission be satisfied except that the bone marrow contains $> 5\%$ blasts but $< 25\%$ blasts

Relapse: relapse following complete remission is defined as:

Reappearance of blasts in the blood

Bone marrow aspirate and biopsy: $> 5\%$ blasts, not attributable to another cause

If there are no circulating blasts and the bone marrow contains 5% to 20% blasts, a repeat bone marrow performed $\geq$ week later documenting more than 5% blasts is necessary to meet criteria for relapse.

Stable Disease: Patients not meeting criteria for relapse

For patients with evidence of minimal residual disease prior to vaccination, assessment of minimal residual disease status by cytogenetics or FISH will be followed post-vaccination

Relapse free survival: defined from the time of the confirmation of a complete remission via bone marrow biopsy to the relapse of disease

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

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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.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission

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- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert 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 the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 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.

11.2 Procedures for AE and SAE Recording and Reporting

Reporting R Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

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The descriptions and grading scales found in the CTEP Active Version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) events that are unexpected and at least possibly related/associated with the intervention.
- All Grade 4 (life-threatening or disabling) events that are unexpected or not specifically listed in the protocol as not requiring reporting.
- All Grade 5 (fatal) events while the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

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Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Jacalyn Rosenblatt, MD
Telephone: 617-667-9920
Pager #: 617-632-7243 pager 36123
Email: jrosenbl1@bidmc.harvard.edu
Fax: 617-667-9922

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

11.6 Reporting to the Food and Drug Administration (FDA)

The DF/HCC Overall Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. The DF/HCC Overall Principal Investigator will report to the FDA, regardless of the site of occurrence, any adverse event that is serious, unexpected and reasonably related (i.e., possible, probable, definite) to the study treatment.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

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All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to the FDA by overnight letter sent to the designated reviewer via UPS.

11.7 Monitoring of Adverse Events and Period of Observation

All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study.

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will collect, manage, and monitor data for this study.

Note: All adverse events that have occurred on the study, including those reported through AdeERS, must be reported via CTMS or CDUS.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

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Form	Submission Timeline
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of the last day of the cycle
Adverse Event Report Form	Within 10 days of the last day of the cycle
Response Assessment Form	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 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 Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related

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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.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

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- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki
 - Title 21 Part 11 – Electronic Records; Electronic Signatures
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html
 - Title 21 Part 50 – Protection of Human Subjects
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html
 - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html
 - Title 21 Part 56 – Institutional Review Boards
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html
 - Title 21 Part 312 – Investigational New Drug Application
www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html
- State laws
- DF/HCC research policies and procedures
<http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/>

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

14. STATISTICAL CONSIDERATIONS

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In the first cohort from the original protocol, a minimum of 10 patients were to undergo serial vaccination with DC/AML fusions (5×10^6 fusion cells) beginning 4-12 weeks following their last cycle of chemotherapy. A total of 2 to 3 doses were to be given at 1 month intervals. Treatment limiting toxicity (TLT) will be defined as vaccine associated grade III or higher toxicity.

Evidence of autoimmunity will be evaluated by clinical exam and serologic assessment including ANA. Vaccination with DC/AML fusions will take place at a fixed dose of 5×10^6 fusion cells.

If 3 or more patients experience a TLT, accrual will be suspended. Cohort 1 offers the DC/AML fusion vaccine, with which we have extensive experience in other types of malignancies, to AML patients in CR1 and CR2 who are not candidates for allogeneic transplant, and this is a new malignancy for this strategy. Should the treatment prove too toxic in either cohort of 10 patients, the trial will close.

	True but Unknown Rate of DLT				
	0.10	0.20	0.25	0.30	0.40
Probability of escalation to next cohort (≤ 2 TLT in 10 pts)	0.930	0.678	0.526	0.383	0.167

If the true rate of TLT is 25%, the probability of observing 2 or fewer TLTs within a cohort of 10 patients is 0.526.

This cohort has been completed without excessive toxicity. We therefore had permission to enroll additional patients to be treated with DC/AML fusions in an expansion cohort, to assess the toxicity, immune, and clinical effects of the regimen in a total of 25 patients.

With 15 patients in an expansion cohort, the probability of observing at least one TLT is 0.99 if the true rate of TLT is 25%, but 0.54 if the true rate of TLT is 5%. Similar calculations suggest that the probability of observing 3 or more TLT events in 15 patients is 0.76 if the true rate is 25%, but 0.04 if the true rate is 5%.

In order to better understand the immunotherapy intervention, we plan to enroll another 30 patients with the expectation that 15 of them will be vaccinated to summarize the impact of neoantigen load to PFS and also characterize particular neoantigens at presence of diseases.

We note that the total of 40 patients (10 from cohort 1, 15 from first expansion cohort and additional 15 patients) refers to patients who actually initiated vaccine treatment and extra patients are enrolled in order to have 40 patients being vaccinated.

The presence of neoantigens and immune response directed at identified neoantigens will be assessed in the 15 additional patients. With 15 of the vaccinated patients evaluable for the presence of neoantigens, and assuming 10 patients are relapse free at 2 years, there is at least 80% power to detect an effect size of 1.5 at a 2-sided alpha of 0.1 using Wilcoxon's rank sum tests.

Disease-free survival will be estimated for all patients receiving at least two doses of DC/AML fusion vaccination, using the method of Kaplan and Meier, and confidence intervals will be calculated using the method of Greenwood.

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Immune response measured by percentage of T cells that express IFN γ as determined by the ratio of the maximum IFN γ expression to the baseline level pre-therapy. A threshold of 3 fold increase over baseline will define achievement of an immune response, and will serve as a categorical outcome variable. In addition, we will examine the ratio of maximum IFN γ post immunotherapy to baseline, and treat this as a continuous variable.

The number of AML driver mutations will be summarized as median and range at the time of diagnosis and at the time of disease relapse in the vaccinated patients. The association of clinical outcome at 2 years and mutation burden at diagnosis will be summarized and analyzed in exploratory studies.

15.0 PUBLICATION PLAN

The intention is to publish the results from this study will be published in a peer review journal.

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17. APPENDICES:

Appendix 1: protocol summary sheet

Appendix 2: performance status criteria

Appendix 3: DF/HCC Multi-Center Data and Safety Monitoring Plan

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APPENDIX 1

Protocol Summary Sheet

Phase: II

Drug: 1) DC/AML fusion vaccine given in conjunction with GM-CSF

Objective: Patients will receive DC/AML fusion vaccination in conjunction with GM-CSF following a chemotherapy induced remission.

Primary Objective: To assess the toxicity associated with treating AML patients with DC/AML fusion cells in the post-chemotherapy setting

Secondary objective: To explore immunological response to DC/AML fusion vaccination in patients who have achieved a chemotherapy induced remission.]

Secondary Objectives

1. To correlate levels of circulating activated and regulatory T cells with immunologic response
2. To define anti-tumor effects by determining time to disease progression.

Design: Phase II open label

Population: Patients with AML who have obtained a complete remission following chemotherapy.

Dose and Route: Vaccination with DC/AML fusion cells 4-12 weeks following completion of chemotherapy. Vaccination with DC/AML fusion cells will be given in conjunction with GM-CSF 100 mcg on the day of vaccination and for three days thereafter. Patients will receive two to three doses of vaccine given at 4 week intervals.

Treatment Period: Participants will receive 2-3 vaccinations with DC/AML fusion cells in conjunction with GM-CSF at 4 week intervals. Treatment will begin 4-12 weeks following completion of chemotherapy

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Investigator/site Principal Investigator: Jacalyn Rosenblatt, MD, Beth Israel Deaconess Medical Center.

Co-Investigators: David Avigan, MD; Robin Joyce, MD; James Levine, MD; Vassiliki Boussiotis, MD; Jeffrey Zwicker, MD; Jon Arnason, MD; Lynne Uhl MD—Beth Israel Deaconess Medical Center

Richard Stone, MD, MD, Baldev Vasir, PhD, MD Daniel Deangelo MD PhD, Gregory Abel MD, David Steensma MD, Martha Wadleigh MD, and Donald Kufe, MD, Dana- Farber Cancer Institute

Noam Benyamini, MD; Tami Katz, PhD; Jacob Rowe, MD: Rambam Medical Center

Blinding: Not blinded.

Method of Patient Assignment: Sequential

Estimated Sample Size: A minimum of 35 evaluable patients will be treated with DC/AML fusion vaccine

Efficacy Variables: Immune response, Time to disease progression, tumor regression

Appendix 2: Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.

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2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

DFCI IRB Protocol #: 09-412

APPENDIX C

Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan

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1.0 INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP should serve as a reference for any sites external to DF/HCC that will be participating in the research protocol.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

1.2 Multi-Center Data and Safety Monitoring Plan Definitions

DF/HCC Multi-center Protocol: A research protocol in which one or more outside institutions are collaborating with Dana-Farber/Harvard Cancer Center where a DF/HCC investigator is the sponsor. DF/HCC includes Dana-Farber/Partners Cancer Care (DF/PCC) Network Clinical Trial Affiliates.

Lead Institution: One of the Dana-Farber/Harvard Cancer Center consortium members (Dana-Farber Cancer Institute (DFCI), Massachusetts General Hospital (MGH), Beth Israel Deaconess Medical Center (BIDMC), Children's Hospital Boston (CHB), Brigham and Women's Hospital (BWH)) responsible for the coordination, development, submission, and approval of a protocol as well as its subsequent amendments per the DFCI IRB and applicable regulatory guidelines (Food and Drug Administration (FDA), Office of Biotechnology Activities (OBA) etc.). The Lead Institution is typically the home of the DF/HCC Sponsor. The Lead Institution also typically serves as the Coordinating Center for the DF/HCC Multi-Center Protocol.

DF/HCC Sponsor: The person sponsoring the submitted Multi-Center protocol. Within DF/HCC, this person is the Overall Principal Investigator who takes responsibility for initiation, management and conduct of the protocol at all research locations. In applicable protocols, the DF/HCC Sponsor will serve as the single liaison with any regulatory agencies (i.e. FDA, OBA etc.). The DF/HCC Sponsor has ultimate authority over the protocol and is responsible for the conduct of the study at DF/HCC and all Participating Institutions. In most cases the DF/HCC Sponsor is the same person as the DF/HCC Principal Investigator; however, both roles can be filled by two different people.

Participating Institution: An institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC Investigator. The Participating Institution acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

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Coordinating Center: The entity (i.e. Lead Institution, Medical Monitor, Contract Research Organization (CRO), etc) that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines. In general, the Lead Institution is the Coordinating Center for the DF/HCC Multi-Center Protocol. Should the DF/HCC Sponsor decide to use a CRO, the CRO will be deemed the Coordinating Center.

DF/HCC Quality Assurance Office for Clinical Trials: A unit within DF/HCC developed to computerize and manage data, and to provide a Quality Control and Quality Assurance function for DF/HCC trials.

2.0 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the DF/HCC Sponsor, the Coordinating Center, and the Participating Institutions are expected to adhere to the following general responsibilities:

2.1 DF/HCC Sponsor

The Protocol Chair, **Jacalyn Rosenblatt, MD**, and the DF/HCC Sponsor, **David Avigan, MD** will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Submit the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Assure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team receives adequate protocol training and/or a Site Initiation Visit prior to enrolling participants and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and FDA reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone other than the DF/HCC Sponsor.

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- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.

2.2 Coordinating Center

The Coordinating Center will assume the following general responsibilities:

- Assist in protocol development
- Maintain copies of Federal Wide Assurance and Institutional Review Board (IRB) approvals from all Participating Institutions.
- Maintain FDA correspondence, as applicable.
- Maintain updated roster of participants.
- Verify eligibility.
- Verify response.
- Oversee the data collection process from Participating Institutions.
- Maintain documentation of Serious Adverse Event (SAE) reports submitted by Participating Institutions and submit to DF/HCC Sponsor for timely review.
- Distribute adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all participating investigators.
- Provide Participating Institutions with information regarding DF/HCC requirements that they will be expected to comply with.
- Monitor Participating Institutions either by on-site or virtual monitoring.
- Maintain Regulatory documents of all Participating Institutions.
- Conduct regular communications with all Participating Institutions (conference calls, emails, etc).
- Maintain documentation of all communications.
- Ensure that each Participating Institution has the appropriate assurance on file with the Office of Human Research Protection (OHRP).

2.3 DF/HCC Quality Assurance Office for Clinical Trials (QACT)

In addition to the Coordinating Center, the DF/HCC QACT provides the following support services to assist the DF/HCC Sponsor:

- Develop protocol specific case report forms (CRF/eCRFS).
- QA/QC data of protocol specific CRFs.
- Provide a central participant registration, which includes review of consent and eligibility.
- Provide auditing services (funding and QACT approval required).

2.4 Participating Institution

Each Participating Institution is expected to comply with all applicable Federal Regulations and DF/HCC requirements, the protocol and HIPAA requirements. All Participating Institutions will provide a list of personnel assigned to the role for oversight of data management at their site to the Coordinating Center.

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The general responsibilities for each Participating Institution are as follows:

- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain a regulatory binder in accordance with DF/HCC requirements.
- Provide the Coordinating Center with regulatory documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as needed (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center.
- Submit source documents, research records, and CRFs per protocol specific submission guidelines to the Coordinating Center.
- Submit Serious Adverse Event (SAE) reports to local IRB per local requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per local requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Secure and store investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.

3.0 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

The following section will clarify DF/HCC Requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Distribution

The Coordinating Center will distribute the final DFCI IRB approved protocol and any subsequent amended protocols to all Participating Institutions.

3.2 Protocol Revisions and Closures

The Participating Institutions will receive notification of protocol revisions and closures from the Coordinating Center. It is the individual Participating Institution's responsibility to notify its IRB of these revisions.

- **Non life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.
- **Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for

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approval.

- **Protocol closures and temporary holds:** Participating Institutions will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the Participating Institutions on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.3 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for Participating Institutions. The Participating Institution consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for PI-Initiated Multi-Center Protocols. This document will be provided separately to each Participating Institution.

Participating Institutions are to send their version of the informed consent document and HIPAA authorization, if a separate document, to the Coordinating Center for review and approval prior to submission to their local IRB. The approved consent form must also be submitted to the Coordinating Center after approval by the local IRB.

The Principal Investigator (PI) at each Participating Institution will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. Participating institutions must follow the DF/HCC requirement that only attending physicians obtain informed consent and re-consent to interventional trials (i.e. drug and/or device trials).

3.4 IRB Documentation

The following must be on file with the Coordinating Center:

- Approval letter of the Participating Institution's IRB
- Copy of the Informed Consent Form approved by the Participating Institution's IRB
- Participating IRB's approval for all amendments

It is the Participating Institution's responsibility to notify its IRB of protocol amendments. Participating Institutions will have 90 days from receipt to provide the Coordinating Center their IRB approval for amendments to a protocol.

3.5 IRB Re-Approval

Verification of IRB re-approval from the Participating Institutions is required in order to continue research activities. There is no grace period for continuing approvals.

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The Coordinating Center will not register participants if a re-approval letter is not received from the Participating Institution on or before the anniversary of the previous approval date.

3.6 Participant Confidentiality and Authorization Statement

In 1996, congress passed the first federal law covering the privacy of health information known as the Health Insurance Portability and Accountability Act (HIPAA). Any information, related to the physical or mental health of an individual is called Protected Health Information (PHI). HIPAA outlines how and under what circumstances PHI can be used or disclosed.

In order for covered entities to use or disclose protected health information during the course of a study, the study participant must sign an Authorization. This Authorization may or may not be separate from the informed consent document. The Coordinating Center, with the approval from the DFCI IRB, will provide a consent template, which covered entities (Participating Institutions) must use.

The DF/HCC Sponsor will use all efforts to limit its use of protected health information in its trials. However, because of the nature of these trials, certain protected health information must be collected per NCI requirements. These are the primary reasons why DF/HCC has chosen to use Authorizations, signed by the participant in the trial, rather than limited data sets with data use agreements.

3.6.1 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center must have the participant's full name & social security number "blacked out" and the assigned DF/HCC QACT case number (as described below) and DF/HCC protocol number written in (with the exception of the signed informed consent document). Participant initials may only be included or retained for cross verification of identification

3.7 DF/HCC Multi-Center Protocol Registration Policy

3.7.1 Participant Registration and Randomization

To register a participant, the following documents should be completed by the Participating Institution and faxed or e-mailed to the Coordinating Center (Emma Logan, Clinical Trials Specialist; Phone: 617-667-5984, Fax: 617-667-9922, Email: eklogan@bidmc.harvard.edu)

- Copy of required tests including:
 - Bone Marrow Aspirate/biopsy, including cytogenetics (if available.) If not

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available, MD note should confirm pathologic diagnosis and that results are pending.

- Electrocardiogram (EKG)
- Laboratory Tests:
 - CBC with differential
 - Liver Function Tests (LFTs) (including; ALT, AST, total bilirubin, direct bilirubin, LDH, alkaline phosphatase)
 - Electrolytes (Na, K, Cl, CO₂, Ca, Mg, PO₄, glucose), BUN, creatinine
 - Pregnancy Test, if applicable, for women of childbearing potential
 - Erythrocyte Sedimentation Rate (ESR)
 - Antinuclear Antibody (ANA)
 - TSH
- Signed informed consent document
- HIPAA authorization form (if separate from the informed consent document)
- Other appropriate forms (e.g., Eligibility Checklist)

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

- Register the participant on the study with the DF/HCC QACT
- Upon receiving confirmation of registration by the QACT, the Coordinating Center will inform the Participating Institution and provide the study specific participant case number, and if applicable the dose treatment level.

Treatment may not begin without confirmation from the Coordinating Center that the participant has been registered.

3.7.2 Registration to Multiple Time points

There are three registration time points for each participant: Initial Registration, Eligibility Prior to Cell Collections for Dendritic Cell Generation, and Eligibility Prior to Post-chemotherapy Immunotherapy. After the initial registration, the appropriate section on the eligibility checklist must be completed and the patient must be re-registered with the DF/HCC QACT prior to moving forward to the second and third time points on study.

3.7.2.1 Initial Registration

Eligibility Criteria

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

1. Patients with AML at initial diagnosis or at first relapse
2. Patients must be ≥ 18 years old
3. Patients must have ECOG performance status of 0-2

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4. Life expectancy of greater than 9 weeks
5. Laboratories:
 - Bilirubin ≤ 2.0 mg/dL
 - AST/ALT $< 3 \times$ ULN
 - Creatinine ≤ 2.0 mg/dl
6. The effects of DC/AML fusion cells 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
7. Ability to understand and the willingness to sign a written informed consent document

Exclusion Criteria

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

1. Patients must not have active or history of autoimmune disorders/conditions including Type I diabetes. Type II diabetes, vitiligo or stable hypothyroidism will not be considered exclusion criteria.
2. Because of compromised cellular immunity, patients who have a known history of HIV will be excluded
3. Patients must not have significant cardiac disease characterized by symptomatic congestive heart failure, unstable angina pectoris, clinically significant cardiac arrhythmia
4. Patients must not be pregnant. All premenopausal patients will undergo pregnancy testing. Men will agree to not father a child while on protocol treatment. Men and women will practice effective birth control while receiving protocol treatment.
5. 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 5 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 5 years: non-invasive cancer (such as, any in situ cancers) and basal cell or squamous cell carcinoma of the skin.

3.7.2.2 Eligibility Prior to Cell Collections for Dendritic Cell Generation:

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Inclusion Criteria

1. Patients must have obtained a complete remission with chemotherapy defined by the absence of circulating blasts, and less than 5% blasts on bone marrow examination following hematopoietic recovery
2. Resolution of all chemotherapy related grade III-IV toxicity as per CTC criteria 4.0
3. Laboratories:
Absolute Neutrophil Count $\geq 1,000/\mu\text{L}$
Platelets $> 50,000/\text{uL}$
Bilirubin $\leq 2.0 \text{ mg/dL}$
Creatinine $\leq 2.0 \text{ mg/dL}$
AST/ALT $< 3.0 \times \text{ULN}$
4. For patients with evidence of minimal residual disease prior to vaccination, assessment of minimal residual disease status by cytogenetics or FISH will be followed post vaccination

Exclusion Criteria:

1. Patients must not have serious intercurrent illness such as infection requiring IV antibiotics, or significant cardiac disease characterized by significant arrhythmia, ischemic coronary disease or congestive heart failure
2. Patients who, with their treating physician, choose to proceed with an allogeneic transplant at the time of remission will not be vaccinated and will come off study

3.7.2.3 Eligibility Prior to Post-chemotherapy Immunotherapy (before first vaccine)

1. Resolution of all chemotherapy related grade III-IV toxicity as per CTC criteria 4.0
2. Laboratories:
WBC $\geq 2.0 \times 10^3/\text{uL}$
Platelets $> 50,000/\text{uL}$
Bilirubin $\leq 2.0 \text{ mg/dL}$
Creatinine $\leq 2.0 \text{ mg/dL}$
AST/ALT $< 3.0 \times \text{ULN}$
3. At least 2 doses of fusion vaccine were produced.

3.7.3 Initiation of Therapy

Participants must be registered with the DF/HCC QACT before receiving treatment. Treatment may not be initiated until the Participating Institution receives a faxed or e-

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mailed copy of the participant's registration confirmation memo from the Coordinating Center. Therapy must be initiated per protocol guidelines. The DF/HCC Sponsor and DFCI IRB must be notified of any exceptions to this policy.

3.7.4 Eligibility Exceptions

The DF/HCC QACT will make no exceptions to the eligibility requirements for a protocol without DFCI IRB approval. The DF/HCC QACT requires each institution to fully comply with this requirement.

3.7.5 Verification of Registration, Dose Levels, and Arm Designation

A registration confirmation memo for participants registered to DF/HCC Multi-Center Protocol will be faxed or emailed to the registering institution within one business day of the registration. Treatment may not be initiated until the site receives a faxed or e-mailed copy of the registration confirmation memo.

3.8 DF/HCC Protocol Case Number

Once eligibility has been established and the participant successfully registered, the participant is assigned a five digit protocol case number. This number is unique to the participant on this trial and must be used for QACT CRF/eCRF completion and correspondence, and correspondence with the Coordinating Center.

3.9 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor, who in turn is responsible for reporting to the DFCI IRB.

For reporting purposes, DF/HCC uses the terms “violation”, “deviation” and “exception” to describe derivations from a protocol. All Participating Institutions must adhere to these requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

3.9.1 Definitions

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.

Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.

Protocol Violation: Any protocol deviation that was not *prospectively approved* by the IRB prior to its initiation or implementation.

3.9.2 Reporting Procedures

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission.

All protocol violations must be sent to the Coordinating Center in a timely manner.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

3.10 Safety Assessments and Toxicity Monitoring

The study teams at all participating institutions are responsible for protecting the safety, rights and well-being of study participants. Recording and reporting of adverse events that occur during the course of a study help ensure the continuing safety of study participants.

All participants receiving investigational agents and/or other protocol mandated treatment will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical examination findings, and spontaneous reports of adverse events reported by participants. All toxicities encountered during the study will be evaluated according to the NCI criteria specified in the protocol. Life-threatening toxicities must be reported immediately to the DF/HCC Sponsor via the Coordinating Center.

Additional safety assessments and toxicity monitoring will be outlined in the protocol.

3.10.1 Guidelines for Reporting Serious Adverse Events

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Guidelines for reporting Adverse Events (AEs) and Serious Adverse Events (SAEs) are detailed in protocol Section 11.

Participating Institutions must report the AEs to the DF/HCC Sponsor and the Coordinating Center following the DFCI IRB SAE Reporting Requirements. The DF/HCC Sponsor and Coordinating Center will be responsible for reporting any SAEs to the FDA in accordance with FDA regulations.

The Coordinating Center will maintain documentation of all Participating Institution Adverse Event reports and be responsible for communicating to all participating investigators, any observations reportable under the DFCI IRB Reporting Requirements. Participating Investigators will review any distributed AE reports, send a copy to their IRB according to their local IRB's policies and procedures, and file a copy with their regulatory documents.

3.10.2 Guidelines for Processing IND Safety Reports

FDA regulations require sponsors of clinical studies to notify the FDA and all participating investigators of any adverse experience associated with the use of the investigational agent that is both serious and unexpected. The DF/HCC Sponsor will review all IND Safety Reports and ensure that all IND Safety Reports are distributed to the Participating Institutions. The Participating Institutions will review and submit to their IRB according to their institutional policies and procedures.

3.11 Data Management

The DF/HCC QACT develops a set of either paper or electronic case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. The DF/HCC QACT provides a web based training for eCRF users.

3.11.1 Data Forms Review

When data forms arrive at the DF/HCC QACT, they are reviewed for completeness, protocol treatment compliance, adverse events (toxicities) and response. Data submissions are monitored for timeliness and completeness of submission. Participating Institutions are notified of their data submission delinquencies in accordance with the following:

Incomplete or Questionable Data

If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC QACT Data Analyst or study monitor. Responses to all queries should be completed and submitted within 14 calendar days. Responses may be returned on the written query or on an amended paper case report form, or in the case of electronic queries, within the electronic data capture (eDC) system. In the case of a written query for data submitted on a paper case report form, the query must be attached to the specific data being re-submitted in response.

Missing Forms

If study forms are not submitted on schedule, the Participating Institution will receive a Missing Form Report from the Coordinating Center noting the missing forms. These reports are compiled by the DF/HCC QACT and distributed a minimum of four times a year.

4.0 Requisitioning Investigational Drug

The ordering of investigational agent is specified in the protocol.

Participating Institutions should order their own agent regardless of the supplier.

Ensure that the pharmacy will be able to receive and store the agent according to state and federal requirements. The local IRB should be kept informed of who will supply the agent so that any regulatory responsibilities can be met in a timely fashion.

5.0 MONITORING: QUALITY CONTROL

The quality control process for a clinical trial requires verification of protocol compliance and data accuracy. The Coordinating Center, with the aid of the QACT provides quality control oversight for the protocol.

5.1 Ongoing Monitoring of Protocol Compliance

The Participating Institutions will be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring will occur before the clinical phase of the protocol begins, continue during protocol performance and through study completion. Additional monitoring practices may include but are not limited to; source verification, review and analysis of the following: eligibility requirements of all participants, informed consent procedures, adverse events and all associated documentation, study drug administration/treatment, regulatory files, protocol deviations, pharmacy records, response assessments, and data management.

Participating institutions will be required to participate in bi-monthly Coordinating Center initiated teleconferences. Emails highlighting overall protocol progress and important announcements will be distributed regularly.

Immediately following enrollment of the first patient, Rambam Medical Center will be responsible for submitting all primary source documentation to Beth Israel Deaconess Medical Center for monitoring. Rambam Medical Center is required to forward de-

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identified copies of participants' medical record and source documents to the Coordinating Center to aid in SDV. Documents may be sent electronically through the BIDMC secure portal. Documents will be compared with the CRFs to ensure both protocol compliance and accuracy of data collection. Following the initial monitoring, the site will undergo routine monitoring every three - six months until the study is completed.

All data submitted to the DF/HCC QACT will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. The Coordinating Center and if applicable, QACT Data Analysts assigned to the protocol, will perform the ongoing protocol data compliance monitoring.

5.2 Evaluation of Participating Institution Performance

5.2.1 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports for on-site and virtual monitoring of Participating Institutions to ensure protocol compliance and ability to fulfill responsibilities of participating in the study. The DF/HCC Sponsor may increase the monitoring activities at Participating Institutions that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations. Participating Institutions may also be subject to an audit as determined by the DF/HCC Sponsor.

6.0 AUDITING: QUALITY ASSURANCE

Auditing is a method of Quality Assurance. Its main focus is to measure whether standards and procedures were followed. Auditing is the systematic and independent examination of all trial related activities and documents. Audits determine if evaluated activities were appropriately conducted and whether data was generated, recorded and analyzed, and accurately reported per the protocol, Standard Operating Procedures (SOPs), and the Code of Federal Regulations (CFR).

6.1 DF/HCC Sponsored Trials

One on-site audit will be scheduled by the QACT, assuming at least three participants have been treated on protocol at the site. Approximately 3-4 participants would be audited at the site over a 2 day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

6.2 Participating Institution

It is the Participating Institution's responsibility to notify the Coordinating Center of all scheduled audit dates and re-audit dates (if applicable), which involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

6.3 DF/HCC Sponsor and Coordinating Center

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The DF/HCC Sponsor will review all final audit reports and corrective action plans if applicable. The Coordinating Center, must forward these reports to the DF/HCC QACT per DF/HCC policy for review by the DF/HCC Audit Committee. Based upon the audit assessments the DF/HCC Audit Committee could accept or conditionally accept the audit rating and final report. Conditional approval could require the DF/HCC Sponsor to implement recommendations or require further follow-up. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

6.4 Sub-Standard Performance

The DF/HCC Sponsor and DFCI IRB is charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

6.4.1 Corrective Actions

Participating Institutions that fail to meet the performance goals of accrual, submission of timely accurate data, adherence to protocol requirements, and compliance with state and federal regulations, will be recommended for a six-month probation period. Such institutions must respond with a corrective action plan and must demonstrate during the probation period that deficiencies have been corrected, as evidenced by the improved performance measures. Participating Institutions that fail to demonstrate significant improvement will be considered by the DF/HCC Sponsor for revocation of participation.

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