This study was designed in response to the urgent need for a less toxic and more effective treatment for MDS. Allogeneic bone marrow transplantation, the only therapy which has demonstrated long term disease free survival, is only available to a minority of patients because of its inherent toxicity and stringent requirements. Other drugs showing activity in MDS are agents such as DNA methyltransferase inhibitors, histone deacetylase inhibitors and immunomodulatory derivatives of thalidomide; which have all been implicated as possessing an ability to augment immune effector function. Of this group, 5-azacytidine, a DNA methyltransferase inhibitor, is the only approved drug treatment for MDS. A large randomized multigroup trial of 5-azacytidine versus aggressive supportive care, showed a modest but improved overall survival and quality of life in patients receiving the drug.[1] Nevertheless, the possibility that all of these treatment modalities may be functioning by interaction with the immune system, collectively suggests that MDS is an immunologically responsive disease and a reasonable setting for investigating the efficacy of a tumor cell vaccination strategy.

The overall goal of this protocol is to study whether or not vaccination with K562/GM-CSF Vaccine Cells can modulate the expression of the dysplastic MDS clone and subsequently affect patient outcome.

The specific objectives of this study are as follows:

Primary Objectives

a. Determine if vaccination with the K562/GM-CSF Vaccine is safe and can induce a hematologic or cytogenetic response in patients with Myelodysplastic Syndrome

Secondary Endpoints

a. Determine if K562/GM-CSF vaccination in patients with Myelodysplastic Syndrome can induce an immune response to common myeloid antigens such as WTI-1, survivin, or proteinase-3 as defined by a 30% increase from baseline in specific cytotoxic T cells measured by ELISPOT analysis on two analyses repeated in duplicate.

b. Determine if immune response correlates with any clinical responses such as hematologic response, resolution of cytogenetic abnormalities, or decrease in other parameters such as WT-1 mRNA levels.

Introduction

Designing efficacious treatments for MDS is complicated by 2 aspects of this disease, the characteristics of the patient population and the diversity of syndromes which are associated with it. Patients with this disorder are likely to be elderly with underlying comorbid disease and suboptimal performance status which often are limiting factors in identifying treatment options. In addition, the heterogeneity of the various hematologic disorders which characterize MDS, and the lack of a precise pathophysiologic understanding of more than a few MDS subtypes, confounded attempts to design comprehensive and targeted therapies. Current treatment modalities target abnormalities generally thought to play a role in MDS, such as under- or overactive apoptosis, failure to express silenced genes required for hematopoietic cell differentiation, or overabundant angiogenic signaling. There is no widely accepted standard of care since few, therapeutic strategies have been shown to change the natural history of this disease and improve survival.

Due to the often advanced age of patients at presentation, poor performance status, or low risk disease, supportive care in the form of transfusions, prophylactic antibiotics, and where indicated, growth factor support such as G-CSF and Erythropoetin, are the sole measures of treatment.

Allogeneic bone marrow transplant, the only curative treatment option for MDS, is limited to a minority of patients who are diagnosed before age 60, exhibit a good performance status, and have access to an HLA matched sibling or matched un-related donor (MUD). There is also significant potential for treatment related morbidity and mortality with this procedure.

Another treatment strategy which has shown activity in MDS is the use of differentiation/epigenetic modification agents such as DNA methylation and histone deacetylation inhibitors . In fact, the DNA methyltransferase inhibitor 5- azacytidine, is now approved for use in high risk MDS patients based on several clinical trials showing overall response rates of approximately 40-63% [2] with modest increases in survival. Representative results were obtained in a CALGB sponsored study of 99 elderly, high-risk MDS patients, where the duration of response was approximately 15 months and time to transformation to leukemia or death was 21 months in the treatment arm and 13 months in the supportive care arm.[1] Similarly, the combination of 5-azacytidine with the histone deacetylation inhibitor phenylbutyrate was shown to achieve complete and partial remissions in 8 out of 16 patients with MDS and AML.{Gore, 2004 GORE2004 /id} Additional studies with other histone deacetylase inhibitors such as MS-275 and Valproic acid are ongoing.

The anti-tumor effect of these various treatments can all be characterized by a common mechanism that involves interaction with the immune system. Specific reference is made to the graft versus leukemia effect associated with allogeneic bone marrow transplantation, as well as to the reported abilities of the DNA methyltransferase and histone deacetylation inhibitors to significantly affect several components of the immune system. Thus, these observations strongly suggest that immune regulation may be an important component of MDS therapy. Hence, the proposed treatment of MDS by immune modulation of the expression of the dysplastic clone through vaccination with the K562/GM-CSF Vaccine.

<u>GM-CSF secreting tumor cell vaccines :</u>
These vaccines have already been tested in Phase I, Phase I/II and Phase II trials, for melanoma, myeloma, acute myeloid leukemia, renal cell, pancreatic, prostate, ovarian and lung cancer [3 4-7 8 9 10 11 12 13 14-17]

Overall, these vaccines were well tolerated, with no reports of any dose limiting toxicities or manifestations of autoimmunity. Self-limited, injection site reactions, characterized by erythema, and induration were observed in most patients. Grade 1 to 2 fatigue, fever and flu-like syndromes were the most commonly observed systemic adverse events and they were only experienced by occasional patients. Evidence of anti-tumor immunity elicited by the vaccine included dose dependent DTH reactions and cytotoxic T cell activity to autologous tumor, as well as tumor reactive antibodies. Although clinical efficacy was not a designated endpoint in many of these early stage trials, there were occasional reports of tumor regression and tumor marker responses.

The strategy utilized in this protocol, namely immunization with an allogeneic, GM-CSF secreting vaccine cell line that expresses myeloid antigens which are also expressed by the dysplastic clone, rather than with the patients' own diseased cells, is based on murine studies. This work showed that the tumor specific CD 8 + T cell response is primed by processed, exogenous tumor antigens which are presented by the host's antigen presenting cells, rather than by the tumor's presentation of its endogenous antigen.[18] Thus, these observations justifies bypassing the need to harvest tumor from each patient which is not always feasible, establish primary tumor cell lines in culture, perform gene transfer, expand the number of transduced tumor cells in vitro, and characterize the amount of GM-CSF that is produced prior to irradiation and storage, in favor of the allogeneic vaccine cell strategy. This approach is currently being investigated in ongoing clinical trials in pancreatic[4] and prostate cancer (hormone naïve and refractory).[19 13]

METHODS:

Clinical trial:

This is an investigator sponsored, pilot study, designed to determine the safety and feasibility of vaccination with cell-based, GM-CSF secreting, vaccines as a treatment strategy in MDS. The patient's immune response to these vaccines will also be evaluated in this setting.

Treatment Plan:

All patients will be treated with a total of 5 doses of vaccine with each vaccine dose containing 1 x 10^8 K562/GM-CSF Vaccine Cells. The vaccine will be delivered by intradermal injection to the non-dominant upper extremity, and both lower extremities, with rotating sites for subsequent vaccination. The initial 4 vaccines are set three weeks apart, with a scheduled 8 week break which will then be followed by the final and fifth vaccine.

Blood will be obtained for immune assays to monitor vaccine responses at baseline, prior to each vaccine, 4 weeks post vaccine numbers 4 and 5, and at the 3 and 6 month follow-up exams. Assays will include ELISPOT to antigenic peptides from WT-1, survivin and proteinase 3; intracellular cytokine staining; tetramer analysis to antigens including WT-1, survivin and proteinase 3; and WT-1 mRNA levels. Bone marrow biopsies will be carried out at baseline and 4 weeks after the final vaccine to perform a cytogenetic analysis, including FISH for standard MDS abnormalities.

Rationale for vaccine dosing and scheduling:
The formulation of the vaccine with 1 x 10^8 allogeneic, irradiated K562/GM-CSF Vaccine Cells, is very similar to the dosing used in an earlier vaccine trial with an admixture of GM-CSF secreting K562 cells as a "bystander" source of GM-CSF and autologous myeloma or AML. The key parameters which govern the selection of these cell numbers are tumor antigen dosing and rates of GM-CSF delivery[16]

Dose escalation trials of allogeneic tumor cell vaccines which were engineered by gene transfer to secrete GM-CSF, demonstrated in prostate [6] and pancreatic [20] cancer, consistent DTH conversions and in vitro evidence of immunity with doses of 1 x 10^8 and 5 x 10^8 cells per immunization cycle. Immune responses were less consistently seen at lower doses. DTH responses are used as surrogate endpoints to document tumor specific responses elicited by vaccination.

The total daily amount of GM-CSF estimated to be delivered by these vaccine doses has never exceeded the recommended clinical dose of recombinant GM-CSF protein (250 micrograms per meter square per day), and measurements of serum GM-CSF levels 24, 48, and 72 hours after vaccination have consistently yielded peaks that are well below those associated with GM-CSF toxicity. These data are derived from, five separate allogeneic cell lines that have been used in the formulation of GM-CSF producing tumor cell-based vaccines. Two for prostate cancer, two for pancreatic cancer, and one for myeloma and AML. The quantity of GM-CSF produced by these different cell lines has varied from as little as 100 nanograms per million cells per 24 hours, to over 2 micrograms per million cells per 24 hours.

The strategy to distribute the vaccine over several sites derives from observations in murine models. At a given vaccine dose, improved systemic immunity was observed when the vaccine was distributed over three injection sites (i.e. over three different draining lymph node regions), than when administered in a single site[21] .

Rationale for Vaccine Timing

Overall there is less information available on the benefits of using a serial vaccine strategy versus a single immunization, and the scheduling of each vaccine cycle. Murine studies have either not been informative or consistent, for a variety of reasons. The clinical experience has shown some patients exhibit a DTH response to the vaccine after a single immunization. Others show a progressive increase in the magnitude of the response, with each successive vaccination.

Previous clinical trials used similar serial vaccine strategies. In an earlier vaccine trial using an admixture of K562/GM-CSF cells as a "bystander" source of GM-CSF and autologous myeloma or Acute Myelogenous Leukemia (AML) cells, a total of 9 vaccine cycles were administered 3 weeks apart, beginning 6 weeks post transplant. In the nontransplant setting, patients were immunized with an admixture of K562/GM-CSF Bystander cells and autologous lung cancer cells, at 3 week intervals.

Rationale for the Use of an Allogeneic Vaccine Cell Line

The ultimate goal of these studies is to enhance anti-tumor activity by inducing specific immune responses to known or cryptic tumor antigens. Harvesting autologous tumor undoubtedly provides the richest source of tumor specific and tumor associated antigens from which to produce a vaccine. However, since that opportunity is not always feasible or practical, considerable interest has developed in using allogeneic or generic cell lines that express/overexpress lineage-specific and shared tumor antigens to deliver the immunotherapy. K562, the original cell line used to develop the K562/GM-CSF Vaccine Cells, expresses several common myeloid antigens including WT-1, survivin and proteinase 3. These antigens are all up regulated in several hematologic malignancies including AML, ALL, CML in blast crisis and MDS and are implicated in playing a potential role in the expression of the malignant phenotype. They are also currently being tested as therapeutic targets in ongoing vaccine trials in AML and CML in blast crisis. [22 23-26]

Correlative Laboratory Studies:

The generation of vaccine-specific immune responses and other immunologic parameters will be monitored by assays which may include, ELISPOT, intracellular cytokine staining and tetramer analysis.

An adapted IFN-gamma ELISPOT assay will be carried out on pre- and post -treatment peripheral blood mononuclear cells (PBMC) to quantify cellular cytotoxic T cell responses to known antigenic peptides the common myeloid antigens WT-1, survivin, and proteinase 3 in the K562/GM-CSF Vaccine. A positive response will be defined as a 30 % increase in the value obtained when compared with pre-vaccine results. Assays will be carried out in parallel with control peptides.

Flow cytometric analysis of intracellular cytokines will identify changes in vaccine-specific T-cells pre- and post -treatment.

Tetramer analysis will be used to characterize the specific cellular immune responses to common myeloid antigens contained in the K562/GM-CSF Vaccine such as WT-1, survivin, and proteinase-3. Should a diminished IFN-gamma response be detected in the intracellular cytokine staining or ELISPOT assays, this analysis can distinguish between the 2 likely causes; either an absence or defect of the cognate T-cells.

Finally WT-1 mRNA levels will be measured at baseline and again after each vaccination as another means of assessing response to the vaccine and correlating any changes directly to clinical parameters.

Statistical Considerations:

The primary endpoint will be evidence of either a hematologic or cytogenetic response as compared to pre-vaccination counts and transfusion requirements. Hematologic response will be defined by the International Working Group Criteria where a positive response will be defined as achieving a major response in at least one lineage as described by an erythroid increase by > 2gm.dl, platelet increase of 30K, or neutrophil increase by 100%. Cytogenetic analysis will be perfomed via standard cytogenetics as well as FISH for MDS profiles on bone marrow samples. Cytogentic remission will be defined as normalization of pretreatment cytogenetic abnormalities. A patient will be classified as a responder if he/she has either a hematologic or cytogenetic.

The proportion of responders will be reported and summarized with an exact binomial 95% confidence interval. The responders will also be further summarized into 3 categories: hematologic and cytogenetic response, a hematologic but no cytogenetic response, and a cytogenetic but no hematologic response.

The selection of a sample size of 15 patients is based on the following reasoning. We hypothesize that patients treated without the vaccine would have a null response rate, p0, of 0.10. Addition of the K562/GM-CSF Vaccine to the treatment regimen should increase this response rate by 30% to a p1 of 0.40. With a one-sided type I error of 10% and a sample size of 15 patients, there is 91% power to detect a response rate of 40% v. 10% using an exact one-sample binomial test.

Regarding the analysis of secondary endpoints, immune response to common myeloid antigens such as WT-1, survivin, or proteinase-3 will be defined as a 30% increase from baseline in specific cytotoxic T cells measured by Elispot analysis on two analyses repeated in duplicate. The percent of patients with an immune response will be reported and summarized with an exact binomial 95% confidence interval. An odds ratio for the association between immune response and the primary endpoint of overall response will also be reported with a 95% confidence interval. Fisher's exact test will be used to test the significance of this association.

An odds ratio and Fisher's exact test will be used to summarize and test the association between immune response and hematologic response only.

Among those who had cytogenetic abnormalities at baseline, the frequency and percent of patients who then experienced a resolution in these abnormalities at post-vaccine follow up will be reported. For those who have a resolution in abnormalities, the frequency and percent of patients with an immune response will also be reported.

WT-1 mRNA levels will be assessed pre- and post-vaccination series. The percentage decrease in mRNA levels will be calculated for each patient. The percent of patients with at least a 30% decrease in mRNA levels from baseline will be reported and summarized with an exact 95% confidence interval. An odds ratio and Fisher's exact test will be used to correlate a decrease in mRNA with immune response.

Anticipated Outcome

The study represents a pilot trial in an effort to develop potential alternative therapies to supportive care or treatment with the approved DNA methyltransferase inhibitor, 5-azacytidine. Based on previous trials with the K562/GM-CSF Vaccine as well as other GM-CSF secreting, cellular vaccine platforms, the toxicities of this vaccine should largely be limited to injection site reactions. Cellular vaccine responses will be evaluated by functional assays such as tetramer based flow cytometry, and ELISPOT. WT-1 mRNA levels will be used to track the any potential effect of vaccine response on clinical parameters.