Published in final edited form as:

Leuk Lymphoma. 2018 December ; 59(12): 2801–2811. doi:10.1080/10428194.2018.1443449.

Pilot Trial of K562/GM-CSF Whole-Cell Vaccination in MDS Patients

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Abstract

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell malignancies. Currently approved drugs are given with non-curative intent as the only known cure is allogeneic bone marrow transplantation, which relies on the donor's immune system driving an allogeneic effect. Previous efforts to harness the endogenous immune system have been less successful. We present the results of a pilot study of K562/GM-CSF (GVAX) whole-cell vaccination in MDS patients. The primary objective of safety was met as there were no serious adverse events. One patient had a decrease in transfusion requirements and another demonstrated hematologic improvement suggesting a signal for clinical activity. *In vitro* correlative studies indicated biological effects on immune cells following vaccination. Although only a pilot study, results are encouraging that an immunotherapeutic approach with a whole-cell vaccine may be feasible in MDS patients.

Keywords

cancer vaccine; immunotherapy; myelodysplastic syndrome; phase 1 clinical trial

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases in terms of genetic alterations, phenotype, and prognosis, which leads to challenges in choosing treatment paradigms for patients. Immunotherapy is a promising strategy for treating many malignancies,[1–3] and may be particularly useful in MDS as the allogeneic effect of bone marrow transplantation (BMT) is the only known cure for this disease. Whole-cell vaccination is an immunotherapeutic approach that has been tested in multiple tumor

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Disclosure of interest

The authors report no conflicts of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

types[4–7] and is designed to elicit cross-reactive anti-tumor responses. GVAX is a whole-cell vaccine based on the K562 cell line derived from a patient with chronic myeloid leukemia (CML) in blast crisis.[8] K562 expresses multiple myeloid antigens[9] including WT-1[10] which is over-expressed in many myeloid tumors,[10–12] and has been engineered to express GM-CSF, which promotes dendritic cell maturation and enhances antigen presentation.[13] GVAX has previously been studied in CML patients and showed signs of clinical activity,[14] and we hypothesized that it may also be effective in MDS since it may share many of the same tumor antigens.

There is a pressing need to develop safe and effective treatments for MDS as current options are limited. Immunotherapy may be a particularly attractive option for the many patients who are unfit for BMT or aggressive chemotherapy. Whole cell vaccines may express multiple antigens that are cross-reactive with patients' myeloid tumors, and the paracrine secretion of GM-CSF may lead to enhanced DC function and antigen presentation. Therefore, a pilot study was developed to evaluate the safety of GVAX for patients with MDS and to assess potential signals of clinical and biologic activity.

Materials and Methods

Eligibility and dosing: Adults with any MDS subtype were eligible if they were off treatment for at least 2 months prior to enrollment, not on immunosuppressants, and had not previously undergone BMT. Patient risk category was determined by the Revised International Prognostic Scoring System (IPSS-R).[15] GVAX was constructed as previously described[14] and manufactured by Cell Genesys, Inc. Five patients were enrolled and received GVAX monotherapy using the dosing schedule previously studied in CML patients.[14] Subjects received 1×10^8 irradiated GVAX cells by intradermal injection at weeks 0, 3, 6, 9, and 17. Peripheral blood, bone marrow (BM), and skin biopsies were taken pre-vaccination, 4 days following the first dose, and 4 weeks after the final vaccination. Additional blood samples were taken on the day of each vaccination and peripheral blood mononuclear cells (PBMC) were isolated using a standard Ficoll gradient and cryopreserved until use in immunologic assays. One patient (Patient B) who demonstrated clinical activity then later had progressive disease received a single booster vaccination of 1×10^8 cells at the time of disease progression via a protocol amendment to Johns Hopkins clinical trial J05115.

***In vitro* killing assay:** The ability of patient PBMCs to lyse target vaccine cells was assessed using a 4 hour JAM assay.[16] Briefly, vaccine cells (i.e. targets) were labeled by culturing in the presence of $2.5 \mu\text{Ci/mL}$ ^3H -thymidine for 18 hours. After washing, the targets were cultured in quadruplicate for 4 hours with graded doses of PBMC (constant 10,000 targets/well and 50:1, 25:1, 12.5:1 and 6.25:1 PBMC effectors: targets). After harvesting on glass filter mats the remaining radioactivity from intact target cells was measured using a Trilux 1450 scintillation counter. Specific lysis was calculated as (counts per minute in negative control – counts per minute in sample)/(counts per minute in negative control) * 100%. Error bars represent standard error of quadruplicate wells.

Proliferation assay: PBMCs were plated at 100,000 cells per well. Background proliferation was determined by culturing cells in media alone, and antigen-specific stimulation was achieved by co-culturing PBMCs with 3,000 irradiated vaccine cells per well for 3 days, then ^3H -thymidine was added to the co-culture for 4 hours and radioactive uptake was measured using a Trilux 1450 scintillation counter (Perkin Elmer, Shelton, CT). Proliferation is expressed as fold-increase above background, and error bars represent the standard deviation from triplicate wells.

Cytokine secretion: Cytokines were measured in supernatants generated from proliferation assay cultures described above before ^3H -thymidine was added. Cytokines and chemokines were measured using a multiplexed bead-based immunoassay (Human 17-plex Bioplex pro-panel or Bioplex 200; Biorad, Hercules CA) which was performed per the manufacturer's protocol and protein concentrations were determined by 5-parameter log curve generated using standards provided by the manufacturer. Antigen-specific production of cytokines was calculated by subtracting the amount of cytokine produced from media stimulation alone (negative control) from the amount produced following stimulation with GVAX cells. PHA was used as a positive control (data not shown).

TCRseq: PBMCs from Patient B were thawed and stained with the following antibodies purchased from BD Biosciences (San Jose, CA): CD3-APC, CD56-PE-Cy7, CD4-BV605, and CD8-FITC. CD4⁺ and CD8⁺ T cell populations were sorted using a MoFlo Legacy sorter (Beckman Coulter, Miami, FL). Dry pellets were frozen and sent to Adaptive Biotechnologies where genomic DNA was extracted, sequencing libraries were generated, and massively parallel sequencing of the TCR CDR3 V β region was performed according to published methods for survey level depth.[17] Data were analyzed using online software tools available from Adaptive Biotechnologies.

Results

Enrollment occurred between September 2007 and May 2009 and was limited to a total of 5 patients due to funding and vaccine availability. The median age was 64 (range 55–75), and multiple MDS subclasses were represented as detailed in Table 1. All doses of vaccine were administered on time and the only adverse events were localized skin reactions that were common, but did not require treatment with antihistamines or steroids. In order to analyze the correlative laboratory studies, patients were designated as having demonstrated clinical activity (CA) or no clinical activity (NCA), defined as having either hematologic improvement according to International Working Group 2006 criteria[18] or decrease in transfusion requirements. Clinical histories and outcomes are outlined in Table 1. Patient B demonstrated improvement in thrombocytopenia, with the platelet count improving from 135 K/mm³ to normal immediately post-treatment and remaining without evidence of progression for over 4 years despite his platelets returning to pre-vaccination levels during that time. Patient B did eventually progress and based on the suspicion that the initial vaccination series had stabilized the marrow function, a single-patient amendment was IRB-approved to re-vaccinate him when his disease progressed. Unfortunately, there was no evidence of CA with re-vaccination. Patient F exhibited a doubling of time between transfusions that lasted over 12 months following the completion of the planned

vaccinations. The remaining patients had no objective improvement in hematologic indices or transfusion requirements, and therefore had NCA.

Immunologic correlates of response were evaluated by several methods. The JAM killing assay was used to measure antigen-specific killing by whole PBMCs against ³H-thymidine-labeled GVAX cells that served as targets, and data from one representative NCA patient are presented along with the results from the two patients with CA (Fig. 1A). Interestingly, one of the NCA patients (Patient A) demonstrated a clear improvement in *in vitro* killing over the course of the study but did not have CA as defined by the aforementioned parameters, and similar results were seen for NCA Patient E (data not shown). Patient B entered the study with moderate levels of specific lysis but did not improve over the course of vaccination. Interestingly, he demonstrated the highest levels of killing 4 months after re-vaccination when he had clearly progressive disease. Patient F exhibited the highest level of killing pre-treatment, and the response decreased following vaccination. Overall, there was not a clear correlation between CA and *in vitro* killing as determined by the JAM assay.

The ability of host T cells to proliferate following stimulation with vaccine cells was measured by ³H-thymidine incorporation. Patients A and B both demonstrated proliferation only at the end of the vaccination series (Fig. 1B), although all patients had robust proliferation to mitogenic stimulation with PHA indicating that the cells were healthy and had proliferative capacity (data not shown). The duration of proliferation for Patient B is unknown since we do not have timepoints between the end of the primary vaccination series and the time of progression but at progression, antigen-specific proliferation was low and did not increase following the booster vaccination (Fig. 1B). In contrast, Patient F never demonstrated proliferation to K562 cells despite experiencing CA. These data demonstrate variation in proliferative capacity, and there was no clear correlation of proliferation with CA. Other facets of T cell function were characterized by measuring expression of 17 cytokines and chemokines following *in vitro* stimulation with vaccine cells. Selected results are shown in Figure 2, and complete results can be found in Table 2. All patients expressed very high levels of the pro-inflammatory cytokine IL-6 at multiple timepoints during the study without any stimulation (Table 2). Patient B was noted to produce high levels of IL-8 at two timepoints during the study (Fig. 2), which is important in neutrophil chemotaxis and phagocytosis. The other patients, even with antigenic or mitogenic stimulation, failed to produce IL-8 at any timepoint. In contrast, the chemokine MIP-1 β , which is involved in chemotaxis of innate immune cells such as monocytes as well as NK cells to sites of inflammation,[19] was produced by the NCA patient, and not in patients with CA.

Interestingly, patient B (CA) did not produce IFN- γ following stimulation, which is perhaps surprising as this cytokine is typically associated with effective T cell responses in MDS as demonstrated in trials using the immunomodulatory agent lenalidomide[20,21] as well as in a phase 1 trials of a WT-1 and PR-1 peptide based vaccine.[22] Patient B also produced lower levels of IL-4 and IL-17, which are associated with Th2 and Th17 responses, respectively, as compared to the patients with NCA (Fig. 2). Overall, cells from the patients with CA produced more pro-inflammatory cytokines than other patients, but less cytokines associated with traditional T helper (both Th1 and Th2) or T effector responses.

We performed next-generation sequencing of the CDR3 region of the TCR beta chain (TCRseq) using PBMCs from Patient B in order to understand the dynamic changes in the T cell repertoire throughout the course of vaccination, disease progression, and booster vaccination. CD4⁺ and CD8⁺ T cell populations were sorted before DNA extraction and sequencing so that the changes in the individual compartments could be analyzed. As shown in Figure 3, there was an increase in the number of unique productive TCR gene rearrangements in both the CD4 (Fig. 3A, left) and CD8 (Fig. 3A, right) compartments following primary vaccination. The number of unique sequences in each T cell compartment was decreased at progression, and did not change significantly following the booster vaccination. When the fraction of the TCR V β CDR3 sequences representing the top 20 most frequent clones was calculated (Fig. 3B), we saw a decrease in this fraction following primary vaccination, especially in the CD4 compartment. Interestingly, the number of unique TCR sequences had contracted at the time of progression and did not change with booster vaccination. This indicates that most of the contraction in the repertoire at the time of progression was in low-frequency clones. Throughout the course of the study, the CD8 compartment had less diversity than the CD4 compartment, as has been previously demonstrated in elderly subjects.[23]

Discussion

Significant research efforts have been dedicated to developing cancer vaccines because conceptually, it is very appealing to deliver a therapeutic agent which results in a host-driven antigen-specific response, ideally without the toxicities of chemotherapy or the non-specific autoimmune toxicity seen with checkpoint inhibitors.[24–26] The ideal tumor vaccine is one that expresses multiple “public” epitopes that are shared between the vaccine and patients’ tumors. One strategy is to generate individualized vaccines using a patient’s own cancer cells, which has been tested previously in a small cohort of MDS/AML patients post-BMT.[27,28] However, having a universal product that can be used for patients of any HLA type, such as GVAX, would be advantageous. In contrast with vaccines derived from autologous cells, GVAX has the advantage of being an “off the shelf” product for any patient without lead-time required for reagent generation and is less costly than generating an individualized vaccine for each patient. It expresses more tumor antigens than DNA or peptide-based vaccines, and the paracrine release of GM-CSF enhances dendritic cell function and downstream antigen presentation.[29,30] Likewise, MDS is an ideal platform for testing this vaccine because of the myeloid origin of the tumor that likely shares common antigens with GVAX, and the well-established supportive care paradigm allows for evaluation of the vaccine’s clinical impact as a single agent.

The data from this pilot study of GVAX in MDS patients support prior studies noting that it is safe and well-tolerated with the only adverse events being local grade 1 skin reactions.[6,14,31,32] While this trial was not designed to evaluate efficacy, it was encouraging that two low-risk MDS patients demonstrated evidence of CA. Patient B, who had CMML, did well off-treatment for 4 years following vaccination. While it is difficult to quantify the contribution of the vaccine to his survival, we note that his clinical course was longer than the median survival of 13.3 months for patients with CMML.[33] The ability to analyze clinical activity was limited by small sample size and heterogeneity of MDS classes. The

original trial was written to include more patients, but enrollment was closed early when vaccine production was interrupted.

Results from the killing and proliferation assays demonstrated that although clear adaptive immune responses were seen in several patients at various timepoints throughout the study, there was no clear correlation with these measurements of immune activity with CA. These transient immune responses indicate that the vaccine did indeed elicit a biological response, but it was insufficient to lead to CA in all patients. The cytokine data are interesting as rather than cytokines such as IL-2 and IFN- γ , which are more traditionally associated with T cell responses to vaccines. However, studies of the tumor biology of MDS have demonstrated that production of IFN- γ and TNF- α in the bone marrow is associated with disease progression.[34,35] The mechanism proposed by Kondo et al is that IFN- γ and TNF- α lead to increased expression of NF- κ B and PD-L1 on MDS blasts, thus creating an immunosuppressive environment.[35] While the lack of IFN- γ production following vaccination in patient B could indicate a less than robust immune response in the periphery, alternatively it may be a positive indicator of a minimally immunosuppressive environment. Future studies would be needed to elucidate the role of IFN- γ in MDS patients receiving immunotherapy. We did not have sufficient clinical material to determine the cell populations that were producing each cytokine, but this would be important to assess in future trials to further refine understanding of the mechanism of action.

We hypothesized that vaccination would lead to local T cell activation in the skin, and in turn the primed T cells would traffic to the bone marrow to cross-prime a response to the shared antigens of the patient's tumor. We sought to understand how the vaccine modulated the TCR repertoire by analyzing TCRseq data for Patient B since he demonstrated a clear improvement in thrombocytopenia and did well off treatment for a prolonged period. In melanoma patients, use of the checkpoint inhibitor anti-CTLA-4 has been shown to broaden TCR diversity, and a small study demonstrated a positive correlation between baseline TCR diversity and clinical response,[36,37] suggesting that broad TCR diversity is correlated with response, either as a cause or an effect. TCRseq results for Patient B (Fig. 3) showed a diversification in the TCR repertoire following the primary vaccination series, particularly in the CD4 compartment. At present, it is impossible to identify the epitopes to which the T cells were responding solely based on TCRseq, which makes it difficult to interpret the biological significance of changes to the TCR repertoire. Future studies could utilize stimulation of T cells with overlapping peptides of common myeloid antigens in order to map the important epitopes, which may help to optimize second-generation vaccines and to develop a deeper understanding of the biological significance of changes in the TCR repertoire. Identification of the dominant epitopes would be helpful to develop an understanding of how the vaccine cells modulated antigen presentation and T cell activation.

Since we were able to see some degree of CA in 2 out of 5 patients that was not clearly associated with proliferation or killing in our assays, it is possible that the patients were responding to private epitopes not cross-reactive with vaccine cells which would not be measured in our assays since GVAX cells were used for *in vitro* stimulation. One possible explanation is that the main driver of immune responses in this situation is the local secretion of GM-CSF from the vaccine cells, as opposed to the cells being a source of cross-

reactive antigen. GM-CSF attract dendritic cells (DCs) to the site of vaccination, and increases costimulatory signals for antigen presentation.[29,38] Secretion of cytokines by activated DCs may have enhanced innate immune responses by cell populations such as macrophages that would not be captured in our assays but may have ultimately contributed to the clinical activity of the vaccine.

These results illuminate the challenge of determining the best parameters to assess the efficacy of immunotherapeutic strategies. Polyfunctional T cells appear to be important for anti-tumor immunity,[39,40] but other cell types may play an important role. Scarcity of clinical material did not permit sorting NK cells from T cells prior to the assay, but NK cells have demonstrated robust killing of K562 targets due to their lack of MHC class I,[41] and likely were responsible for lysis in the JAM assay. B cells may also play a role, as a study of a similar GM-CSF-secreting whole-cell vaccine in colon cancer patients demonstrated that high titers of anti-MUC1 antibody (an antigen included in their vaccine) were correlated with clinical response.[42] Future studies should be done in order to assess immune responses in multiple immune compartments.

Immunotherapy represents an important treatment approach for patients with hematologic malignancies and we just now are beginning to understand how to harness patients' innate and adaptive immune systems. Hypomethylating agents such as azacitidine are the current standard of care for MDS and function by increasing gene transcription and antigen expression,[43] so using these in combination with immunotherapy such as GVAX may be a very powerful approach. It will be important to design future clinical trials to rationally combine immunotherapy along with more traditional chemotherapy and targeted agents in order to maximize clinical benefit.

Acknowledgements

We kindly thank the research support staff at SKCCC and the patients who enrolled in the study. We also thank Dr. Hao Zhang at the Johns Hopkins School of Public Health Flow Cytometry Core Facility for assistance with cell sorting and Dr. Ian Kaplan at Adaptive Biotechnologies for assistance with TCRseq analysis. Experiments done though the human immunology core facility were supported by National Institutes of Health Grant P30 CA006973.

Trial Registration

This clinical trial predated the requirement for registration with [ClinicalTrials.gov](https://clinicaltrials.gov).

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Figure 1. Antigen-specific T cell responses are demonstrated in patients with varying levels of clinical activity.

A) PBMCs were isolated from patients at indicated timepoints and used as effector cells in a JAM assay to measure specific lysis as outlined in Methods. GVAX vaccine cells were radiolabeled and used as targets in the assay. B) PBMC were stimulated with GVAX vaccine cells for 3 days before addition of ^3H thymidine to the culture. Incorporation of radioactivity was measured and stimulation index was calculated as fold increase over stimulation with media alone. Error bars represent standard deviation of triplicates.

Figure 2. PBMC from responder have increased production of inflammatory cytokines, and less production of Th cytokines.

PBMC were co-cultured with GVAX cells and secreted cytokines were measured in the supernatants. Background production of cytokines from media stimulation has been subtracted. Data for selected cytokines are presented, and complete data for all cytokines measured are presented in Table 2.

Figure 3. TCR diversity is increased following primary K562 vaccination.

A) CD4 and CD8 T cells were isolated from patient B PBMCs at indicated timepoints and deep sequencing of TCR V β CDR3 regions was performed as described in methods. Each colored oval represents the results from the indicated timepoint, and the numbers inside indicate the number of unique TCR sequences. Numbers on the periphery contained only in one oval represent the number of sequences that were unique to that particular timepoint, and numbers in intersections of ovals indicate the number of sequences that were found in both timepoints. CD4 data are presented on the left, and CD8 on the right. B) The fraction of

the total TCR repertoire occupied by the top 20 most frequent clones is indicated as colored bars. A larger colored bar therefore indicates a more clonal T cell response, and conversely a larger grey bar indicates a greater amount of diversity. CD4 data are presented at the top, and CD8 data are presented at the bottom. As a control, one healthy, age-matched donor was sequenced and is presented at the far right.

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Patient characteristics.

Patient and disease characteristics are outlined. MDS classification was made by Revised IPSS scoring. Patients were considered to have clinical activity if they had a decrease in transfusion requirement or improvement in hematologic indices per IWG 2006 criteria.

Table 1.

Patient ID, Responder status	Age	MDS classification	R-IPSS score and category	Disease course
A No clinical activity	75	RAEB-1 10% blasts del5q, del7	8 Very high	Transfusion-dependent pre-study Decrease in transfusion frequency from q3 weeks to q4 weeks during vaccination series Progression to RAEB-2, 17% blasts soon after completing vaccinations
B Clinical activity	65	CMML 0% blasts Normal karyotype	1 Very low	No transfusion requirements prior to or following vaccination Thrombocytopenic prior to study, improved to normal range during vaccination period Progressed 4 years after vaccination Revaccinated at time of progression Progression to AML 1 year after booster vaccination
C No clinical activity	59	Secondary MDS 0% blasts del7	4 Intermediate	No transfusion requirements pre-study Transfusion-dependent 1 month after vaccination MDS progression by cytogenetics 1 month after vaccination Progression to AML 2 years after vaccination
E No clinical activity	55	RARS 0% blasts Normal karyotype	1 Very low	Transfusion-dependent, on Exjade pre-study No change in transfusion requirement during study No evidence of disease progression
F Clinical activity	64	RA 0% blasts Normal karyotype	2 Low	Progressive transfusion needs, on Exjade pre-study Decreased transfusion requirements from q3-4 weeks to q8-10 weeks for 5 years after vaccination