Whole tumor cell vaccines engineered to secrete GM-CSF (GVAX)

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Abstract
Generation of immunity against cancer through vaccination has long been an elusive goal for tumor immunologists. Putative candidates for vaccination targets include oncofetal antigens, viral antigens, neoantigens, and differentiation antigens. The first attempts at cancer vaccination used injections of whole autologous tumor cells. However, these unmodified tumor cells did not engender a robust immune response. Subsequent efforts were focused at enhancing the immunogenicity of whole autologous tumor cell vaccines through genetic modification, often through virally mediated transduction of genes encoding immunostimulatory molecules. Of many immunostimulatory cytokines evaluated in the context of gene-modified tumor cell vaccines, granulocyte–macrophage colony-stimulating factor (GM-CSF) emerged as the most potent in generating protective antitumor immunity. Vaccination using irradiated, GM-CSF producing tumor cells (GVAX) consistently induced antitumor immunity across several experimental tumor models. The term GVAX can connote GM-CSF secreting cell vaccines prepared with different vectors as well as vector targets including autologous tumor cells, allogeneic tumor cell lines, and bystander third party tumor cell lines. GVAX has been evaluated against solid tumors, hematologic malignancies, and in the context of hematopoietic stem cell transplantation. GVAX has been extensively studied in clinical trials, both alone and in conjunction with lymphodepleting chemotherapy, immune checkpoint inhibitors, and other vaccines.

Keywords
GM-CSF, GVAX, vaccine

1  TUMOR CELL VACCINES

The generation of an effective immune response to malignancy depends upon intricate coordination between the innate and adaptive branches of the immune system. For cancer immune surveillance to be effective, the immune system must first recognize malignant antigens, recruit and renew a population of antigen-presenting cells, and provide sufficiently strong activation of adaptive immune components to engender a sustained, anticancer response. Dendritic cells (DCs) phagocytose apoptotic malignant cells and process their peptides into immunogenic antigens and can receive antigenic peptides from necrotic cancer cells. Malignant cells often lack costimulatory factors that are necessary for strong T-cell activation. DCs, upon phagocytosing and processing malignant antigens for MHC
presentation, upregulate B7 family members, migrate to regional lymph nodes, and stimulate the priming and/or expansion of cancer-specific CD4+ and CD8+ T cells. From there, CD4+ T cells are able to foster an antitumor antibody response, provide help to CD8+ T cells to attack the malignancy through perforin-granzyme effector molecules, and directly contribute to tumor clearance through the secretion of proinflammatory cytokines.

A long studied approach to boost antitumor immune responses has been the generation of cancer vaccines, an antigenic payload that stimulates the priming and expansion of cancer-reactive T-cell clones. Putative candidates for vaccination targets include oncofetal antigens that are re-expressed in malignant tumors through epigenetic modification, viral antigens, neoantigens, and differentiation antigens, the latter which may also be expressed on normal tissues. Defined tumor antigen vaccines are derived from specific gene products and can include peptides, full-length proteins, or genetically encoded vectors. Delivery of defined antigen or whole-cell material can also be facilitated by direct loading onto autologous dendritic cells ex vivo, followed by inoculation into patients. Tumor cell cancer vaccines employ the entire tumor cell and, in the cases of autologous tumors, the associated tumor stroma that can potentiate immune activation.

The first attempts at cancer vaccination in the early 20th century used injections of whole autologous tumor cells, irradiated so that they did not cycle. However, these unmodified tumor cells did not engender a robust immune response. Subsequent efforts were focused on enhancing the immunogenicity of whole autologous tumor cell vaccines through genetic modification, often virally mediated. Of many immunostimulatory cytokines evaluated in the context of gene-modified tumor cell vaccines, granulocyte–macrophage colony-stimulating factor (GM-CSF) emerged as the most potent in generating protective antitumor immunity. Vaccination using irradiated, GM-CSF producing tumor cells (GVAX) consistently induced antitumor therapeutic potential of GVAX. In mice, priming of Treg is observed with DCs differentiated from bone marrow in the presence of GM-CSF. MFG-E8 attenuated Treg induction and potentiated GM-CSF efficacy, providing strong evidence for Treg targeting as a means to increase the therapeutic potential of GVAX. In mice, priming of Treg is observed with DCs differentiated from bone marrow in the presence of GM-CSF and linked to DC expression of OX40L and Jagged1. Human DCs generated in the presence of GM-CSF without other cytokines can be tolerogenic.

3 CLINICAL APPLICATIONS OF GM-CSF WITH AND WITHOUT VACCINATION IN CANCER THERAPY

GM-CSF has been incorporated into two clinically approved therapeutic vaccines. Sipuleucel-T is an autologous myeloid cell vaccine preparation in which dendritic cells are differentiated ex vivo in the presence of a protein conjugate of GM-CSF and the antigen prostatic acid phosphatase. In a randomized trial, Sipuleucel-T prolonged survival in patients with asymptomatic or minimally symptomatic metastatic castrate-resistant (hormone refractory) prostate cancer. Talimogene laherparepvec, or T-VEC, is a modified oncolytic herpes virus that produces human GM-CSF and is injected intra-tumorally for the treatment of discrete inoperable metastatic melanoma lesions. T-VEC improved durable response rates and overall survival (OS) compared
to GM-CSF therapy alone. T-VEC has been evaluated in combination with checkpoint inhibitors including ipilimumab in melanoma and pembrolizumab for sarcoma and has shown preliminary evidence of therapeutic synergy. A phase 2 randomized study demonstrated superiority of ipilimumab plus T-VEC to ipilimumab alone. Thirty-eight patients (39%) in the combination arm and 18 patients (18%) in the ipilimumab arm had an objective response (odds ratio, 2.9; 95% CI, 1.5–5.5; \( p = 0.002 \)). T-VEC has also been effective in patients with locoregional disease resistance to checkpoint inhibition.

GM-CSF (sargramostim) has been incorporated into immunomodulatory cancer therapeutic regimens without vaccination in hopes of stimulating antitumor inflammatory responses. A regimen combining GM-CSF with anti-GD2 antibody, IL-2, and isotretinoin has been approved for the treatment recurrent neuroblastoma. GM-CSF when combined with the checkpoint inhibitor ipilimumab improved OS with a decrease in gastrointestinal toxicity compared with ipilimumab alone in patients with melanoma.

4 GM-CSF ENFORCED WHOLE-CELL VACCINATION-GVAX

Though it can simultaneously function as a pro- and anti-inflammatory factor, GM-CSF production by whole-cell vaccines on net stimulates adaptive anticancer immune responses by inducing myeloid differentiation and DC cross-priming. In this formulation of the whole-cell vaccine, the expression of GM-CSF is enforced in autologous cancer cells or allogeneic cancer cell lines through genetic manipulation. Vaccination with irradiated tumor cells engineered to secrete GM-CSF stimulates potent, specific, and long-lasting antitumor immunity in multiple murine tumor model systems. Immunization results in improved tumor antigen presentation by dendritic cells and macrophages recruited to the vaccination site and the coordinated functions of CD4+ and CD8+ T cells, NKT cells, and antibodies against a variety of targets mediating protective immunity (Figure 1).

GVAX presents challenges for sustainable vaccine production. First and foremost, the autologous cancer cells that are injected into the patient serve as both the source of GM-CSF and the cancer antigens. As the autologous cancer cells are swarmed by immune cells due to the presence of their surface antigens, it is unclear how long the embattled vaccine survives, let alone produces systemic levels of GM-CSF to stimulate and maintain an anticancer immune response. Initially, transduction using retroviruses was employed, but for potential safety concerns, the platform was changed to employ adenoviral vectors. However, adenoviral transduction into autologous cancer cells is plagued by inconsistent efficiency, resulting in variable potency of the vaccine. Alternative formulations of GVAX are designed to minimize these issues by using a sustainable source of GM-CSF vehicle. A GM-CSF encoding plasmid can be stably transfected into K562 cells, and these GM-K562 cells then act as "bystanders" in the immune reaction rather than targets. GM-K562 cells are then admixed with irradiated, antigenic autologous cancer cells to formulate the vaccine. GM-CSF transduction of allogeneic irradiated allogeneic tumor cell lines as a source of antigen has also been evaluated in clinical trials, thus bypassing the need to harvest autologous tumors. One potential limitation of this approach is that the antigenic repertoire of the allogeneic tumor cell lines may not sufficiently reflect the antigenic profile of individual patient tumors.

Phase 1/2 clinical trials of GVAX have been conducted for a variety of solid tumors and hematologic malignancies. In many of these studies, vaccination has induced dense infiltrates of DCs, T cells, plasma cells, and CD1d-restricted natural killer-T cells invading distant metastatic deposits and their vasculature leading to histologic evidence of tumor destruction (Figure 1). Although the clinical significance of specific immune responses remains uncertain, there are data suggesting that single-cell immune competency signatures associate with survival after GVAX therapy. Therapy-induced antibodies against major histo-compatibility chain-related protein-A (MICA), a ligand for the activating NK cell receptor NKG2D, were shown to overcome immune suppression mediated through soluble MICA and promote cytotoxicity
Induction of antibodies against multiple proangiogenic factors was correlated with improved outcomes in some vaccinated solid and hematologic cancer patients.\textsuperscript{37,41}

5 | GVAX IN SOLID TUMORS

A number of clinical trials have been conducted with GVAX, predominantly in solid tumors (Table 1). While vaccination appears safe and generation of both local and distant immune reactivity has been amply documented, evidence of tumor regression and prolonged survival has been limited. The first study on 18 patients with metastatic renal cancer vaccinated with GVAX generated with a retroviral vector reported one partial remission in a patient who demonstrated a large hypersensitivity response.\textsuperscript{47} A trial in eight patients with metastatic prostate cancer using retrovirally mediated gene transfer into prostate cancer cells reported that three of eight subjects developed new antibodies against prostate cancer cells but no objective response.\textsuperscript{48} In a trial of 21 patients with metastatic melanoma receiving a series of irradiated autologous melanoma retrovirally transduced with GM-CSF, active inflammation was noted at distant metastatic deposits. One partial response, one mixed response, and three minor responses were observed. Three patients remained free of disease for over 3 years.\textsuperscript{46}

The perceived risks and cumbersome nature of retroviral transfer led investigators to explore alternative means of transduction employing replication defective adenovirus constructs. A trial in metastatic melanoma with 34 patients demonstrated dense dendritic cell, macrophage, granulocyte, and lymphocyte infiltrates at injection sites in 19 of 26 assessable patients with the development of delayed type hypersensitivity reactions to irradiated, dissociated, autologous, nontransduced tumor cells in 17 of 25 patients.\textsuperscript{46} Metastatic lesions that were resected after vaccination showed brisk or focal T-lymphocyte and plasma cell infiltrates with tumor necrosis in 10 of 16 patients. One complete, one partial, and one mixed response were noted. Ten patients were alive, with a minimum follow-up of 36 months, four who had no evidence of disease post resection. A trial in non-small cell lung cancer (NSCLC) involving 25 patients induced no objective responses with five stable disease and two patients with extended progression-free survival (PFS).\textsuperscript{49} A multicenter Phase I/II study in NSCLC patients harvested tumor from 83 patients and successfully produced vaccine in 67 patients, of whom 43 received vaccination. Three of 33 advanced stage patients had durable complete responses lasting beyond 6 months. Longer survival was observed in patients receiving vaccines secreting GM-CSF at more than 40 ng/24 h per \(10^6\) cells (median survival = 17 vs. 7 months, \(p = 0.028\)).\textsuperscript{45} In 11 patients with alveolar small parts or clear cell sarcoma treated with GVAX, antibody responses to tissue-type plasminogen activator (tTPA) and angiopoietins-1/2 were detected. Tumor biopsies showed programmed death-1 (PD-1)-positive CD8\(^+\) T cells in association with PD ligand-1 (PD-L1)-expressing sarcoma cells. However, no tumor regressions were observed.\textsuperscript{50}

Use of GM-CSF transduced irradiated bystander K562 cells admixed with autologous tumor has been developed to ease preparation and improve scalability. In 10 advanced glioma patients receiving autologous GVAX, vigorous humoral responses to tumor-associated angiogenic cytokines were observed along with increased expression of CTLA-4, PD-1, 4-1BB, and OX40 by CD4\(^+\) T cells and PD-1 and 4-1BB by CD8\(^+\) T cells. However, no objective responses were noted.\textsuperscript{52} This approach has also been explored in NSCLC. In one study, tumors were harvested from 86 patients, tumor cell processing was successful in 76, and 49 proceeded to vaccination.\textsuperscript{53} Compared with autologous GVAX vaccines prepared by transduction of individual tumors with an adenoviral GM-CSF vector, GM-CSF secretion was approximately 25-fold higher with the bystander GVAX vaccine technology. Serum GM-CSF pharmacokinetics were consistent with secretion of GM-CSF from vaccine cells for up to 4 days with associated transient leukocytosis confirming the bioactivity of vaccine-secreted GM-CSF.
TABLE 1  GVAX in solid tumors

| Clinical trial       | Malignancy                  | Vaccine formulation                              | Clinical results                       |
|----------------------|-----------------------------|-------------------------------------------------|----------------------------------------|
| Simons et al.47       | Renal cell carcinomaN = 16  | AutologousRetroviral vector                      | 1 PR                                   |
| Soiffer et al.36      | Metastatic melanomaN = 21  | AutologousRetroviral vector                      | 1 PR                                   |
| Simons et al.48       | Prostate cancerN = 8        | AutologousRetroviral vector                      | No CR or PR                            |
| Salgia et al.49       | NSCLCN = 25                 | AutologousRetroviral vector                      | No CR or PR                            |
| Soiffer et al.46      | Metastatic melanomaN = 34  | AutologousAdenoviral vector                      | 1 CR, 1 PR                             |
| Goldberg et al.50     | SarcomaN = 11               | AutologousAdenoviral vector                      | No CR or PR                            |
| Curry et al.52        | Recurrent gliomaN = 10      | GM-K562 cells admixed with autologous glioma cells | No CR or PR                            |
| Nemunaitis et al.53   | NSCLCN = 49                 | GM-K562 cells admixed with autologous NSCLC cells | No CR or PR                            |
| Small et al.57        | Prostate cancerN = 55       | Allogeneic prostate cancer lines transfected with GM-CSF | No CR or PR                            |
| Higano et al.48       | Metastatic prostate cancerN = 80 | Allogeneic prostate cancer lines transfected with GM-CSF (3 doses) | Median survival in high-dose group was 35 months, 20 months in mid-dose group, and 23 months in low-dose group |
| Le et al.66           | Metastatic refractory PDACN = 90 | Allogeneic PDAC lines, transfected with GM-CSF + cyclophosphamide | OS in GVAX/Cy/CRS-207 arm was 6.1 months vs. 3.9 months in GVAX/Cy alone |
| Obradovic et al.68    | High-risk prostate cancer(T1c-3b)N = 48 | Allogeneic prostate cancer lines transfected with GM-CSF + cyclophosphamide + degarelix vs. degarelix alone | Time-to-PSA-relapse and time-to-next-therapy superior in GVAX/Cy + degarelix subjects vs. degarelix-only subjects (p = 0.15 and 0.16, respectively) |
| Le et al.67           | Metastatic, refractory PDACN = 213 | Allogeneic PDAC lines, transfected with GM-CSF + cyclophosphamide +/- CRS-207 vs. chemotherapy | Median OS was 3.7 months in subjects with GVAX/Cy/CRS-207, 5.4 months in subjects with CRS-207 only, and 4.6 months in patients with single-agent chemotherapy (p = NS) |
| Wu et al.74           | Metastatic refractory PDACN = 82 | Allogeneic prostate cancer lines transfected with GM-CSF + ipilimumab vs. FOLFIRINOX | OS 9.4 months ipilimumab + GVAX 9.4 months vs. 14.7 months FOLFIRINOX |
| Yarchoan et al.77     | Metastatic mismatch repair proficient (MMRp) colorectal CA | Allogeneic colorectal cancer lines plus GM-K562 + cyclophosphamide + pembrolizumab | No CR or PR |
| Tsujikawa et al.76    | Metastatic refractory PDACN = 93 | Two allogeneic PDAC lines expressing GM-CSF + cyclophosphamide + CRS-207 +/- nivolumab | Median OS with and without nivolumab was 5.9 months and 6.1 months, respectively. Two of 51 subjects with nivolumab and one of 42 subjects without had objective responses |

Again, although evidence of vaccine-induced immune activation was observed, no objective tumor responses were noted.

Given the practical difficulties of obtaining autologous tumor tissue, particularly from surgically remote sites, there has been interest in generating vaccination from off-the-shelf cell sources. This source of tumor antigens is obtained from irradiated GM-CSF transduced allogeneic cell lines and, as such, run the risk of being rejected by the host. In the initial study of patients with advanced pancreatic cancer, there were no objective responses among 14 vaccinated patients although several did experience prolonged survival associated with evidence of immune activation in vivo. Allogeneic GVAX has been administered following pancreaticoduodenectomy to be followed by systemic 5-FU chemotherapy and booster vaccination. In this single-arm trial, median disease-free survival was 17 months and OS 24 months. Post-GVAX induction of mesothelin-specific CD8+ T cells correlated with disease-free survival in HLA-0101 and HLA-0201 subjects. Subsequent reports suggest that presurgical GVAX administration induced intratumoral tertiary lymphoid aggregates in 33 of 39 patients 2 weeks after vaccine treatment. Microarray analysis of microdissected aggregates identified gene-expression signatures in five signaling pathways involved in regulating immune-cell activation and trafficking that were associated with improved postvaccination responses. A suppressed Treg pathway and an enhanced Th17 pathway within these aggregates were associated with improved survival.

Subsequent studies explored the same concept with advanced prostate cancer using transfected allogeneic prostate cancer lines.
In one report, vaccine was administered in three different dosages to three groups of subjects. Median survival in maximum-dose group was 35 months, 20 months in the mid-dose group, and 23 months in low-dose group. PSA levels stabilized in 15/80 patients. A dosage-dependent increase in antibody response to ≥1 cancer cell line was observed in 16/18 of maximum-dose subjects, 13/18 of mid-dose subjects, and 10/23 of low-dose subjects. GVAX has been combined with chemotherapy (docetaxel) as neoadjuvant therapy prior to radical prostatectomy with no clear toxicity though of uncertain added benefit. A phase 3 clinical trial of this approach in advanced prostate carcinoma patients failed to demonstrate improved clinical outcome with an allogeneic prostate carcinoma cells secreting GM-CSF combined with docetaxel versus docetaxel alone.60

Allogeneic GM-CSF-secreting tumor vaccines have also been tested in breast cancer patients. In a phase I dose-ranging study, 28 patients with metastatic breast cancer were treated with a HER2-positive, GM-CSF-secreting tumor vaccine, along with various doses of cyclophosphamide and doxorubicin. The vaccine produced optimal HER2-specific immunity in combination with low-dose cyclophosphamide and doxorubicin. In a further feasibility study, a combination of a HER2-positive GM-CSF-secreting allogeneic vaccine produced a 6-month clinical benefit rate of 55%, median PFS of 7 months, and median OS of 42 months in 20 patients receiving weekly trastuzumab therapy.62

6 | COMBINATORIAL THERAPY WITH GVAX

The disappointing clinical results of GVAX trials despite generation of potent humoral and cellular immune reactivity both in vivo and ex vivo led investigators to consider combining GVAX with therapies that might augment vaccine-induced responses. One such strategy would be to administer lympho-depleting chemotherapy in conjunction with vaccination to leverage lymphopenia-induced proliferation and homeostatic expansion and to suppress regulatory T cells. A study of nine patients with metastatic colorectal carcinoma received cyclophosphamide, allogeneic colorectal cancer cell lines and K562-secreting GM-CSF. Six patients who had undergone hepatic metastasectomy with curative intent survived more than 3 years, four of whom were disease free more than 3 years. Four patients demonstrated enhanced production of anti-MUC1 IgG antibodies in their postvaccination sera. A study of fully resected Stages III–IV melanoma patients treated with GVAX alone (n = 12) or with (n = 8) cyclophosphamide (Cy/GVAX) to deplete regulatory T cells demonstrated an increase in serum GM-CSF concentrations and levels of activated circulating monocytes but no clear impact of lymphodepletion. A randomized trial in 87 patients with pancreatic cancer prior to and after resection comparing GVAX alone to GVAX with different schedules of cyclophosphamide demonstrated no clear survival advantage to the addition of cyclophosphamide. The study did suggest, however, that neoadjuvant/adjuvant GVAX was superior to historical results with adjuvant GVAX alone.65

Combining Cy/GVAX with CRS-207, a live-attenuated Listeria monocytogenes-expressing mesothelin that induces innate and adaptive immunity has been prospectively examined in a randomized trial in which 61 pancreatic cancer patients received two doses of Cy/GVAX, followed by four doses of CRS-207 or six doses of Cy/GVAX every 3 weeks. OS was 6.1 months in the combination versus 3.9 months for Cy/GVAX alone (p = 0.02). For patients receiving at least three doses (two doses of Cy/GVAX plus one of CRS-207 or three of Cy/GVAX), OS was 9.7 versus 4.6 months (p = 0.02). Enhanced mesothelin-specific CD8 T-cell responses were associated with improved outcome, independent of treatment arm. A subsequent three-arm randomized study of Cy/GVAX + CRS-207, CRS-207 alone, and standard chemotherapy in 213 patients reported median OS of 3.7 months in subjects with GVAX/Cy/CRS-207, 5.4 months in subjects with CRS-207 only, and 4.6 months in patients with single-agent chemotherapy, indicating no beneficial clinical contribution from this GVAX formulation.67

In a small randomized trial of men with high-risk prostate cancer undergoing radical prostatectomy, neoadjuvant treatment of androgen-deprivation therapy with degarelix alone (n = 16) was compared with degarelix plus Cy/GVAX (n = 32). Both therapies were associated with a CD8+ T-cell infiltration, although accompanied by an increase in regulatory T cells. There was a therapeutic signal in the Cy/GVAX cohort as evidenced by a modest improvement in time-to-PSA progression and time-to-next treatment. The authors concluded that combination therapy should be studied further in conjunction with an agent that depletes regulatory T cells.

One of the first combinatorial approaches in the context of GVAX that showed therapeutic synergy was blockade of the inhibitory receptor CTLA-4. In mice, a combination of GVAX and CTLA-4 blockade eradicated established B16 tumors that were unaffected by either treatment alone. Further interest in evaluating vaccination with checkpoint inhibitors was prompted by a report in 2003, in which CTLA-4 blockade appeared to enhance vaccine-induced tumor infiltration with checkpoint T cells. A high ratio of CD8+ T cells/FoxP3+ Tregs in tumor biopsies after therapy was associated with immune-mediated tumor destruction. GVAX has since been coupled with checkpoint blockade in multiple settings. Allogeneic GVAX plus ipilimumab combination has been explored in patients with metastatic prostate cancer with some reported clinical responses. Improved survival was associated with the induction of antibody responses to prostate carcinoma-associated antigens. A small randomized study in pancreatic cancer compared GVAX plus ipilimumab (CTLA-4 blockade) at 10 mg/kg to ipilimumab alone. Seven of 15 patients receiving GVAX plus ipilimumab experienced declines in CA19-9 levels compared to no patients in the ipilimumab-alone arm. Median OS 5.7 versus 3.6 months (p = 0.072) and 1-year OS (27% vs 7%, p = 0.014) seemed to favor the combined arm. However, results from a prospective randomized trial that compared GVAX plus ipilimumab to FOLFIRINOX-based chemotherapy in advanced pancreatic cancer suggested no benefit of GVAX/ipilimumab. Forty subjects who received ipilimumab + GVAX had an OS of 9.38 months compared to 42 subjects that received FOLFIRINOX who had an OS of 14.7 months. Only one complete or partial response was noted in the GVAX arm and three in the chemotherapy arm. PFS was reported as 2.4 months in the GVAX/ipilimumab arm versus 5.55 months in the FOLFIRINOX arm.74
Experimental models had suggested a synergy between vaccine-induced immune responses and PD-1/PD-L1 pathway blockade. However, when nivolumab was added to patients receiving Cy/GVAX with CRS-207, there was no significant improvement in response or median survival. As well, the addition of pembrolizumab to Cy/GVAX in patients with mismatch repair proficient advanced colorectal cancer induced no responses, with a median PFS of only 82 days.

7 | GVAX IN HEMATOLOGIC MALIGNANCIES AND HEMATOPOIETIC CELL TRANSPLANTATION

GVAX strategies have also been evaluated in blood cancers. In patients with chronic myelogenous leukemia with persistent disease despite imatinib therapy, 19 patients received the K562-based GM-CSF secreting GVAX vaccine. Thirteen of 19 experienced progressive declines in disease burden, seven of whom became undetectable by PCR. A randomized study comparing K562 GVAX versus interferon plus GM-CSF for patients failing to achieve molecular remission after tyrosine kinase therapy in CML has been initiated (NCT0036349). A smaller trial of K562 GVAX in MDS demonstrated safety and feasibility but no clear clinical responses. In myeloma patients receiving lenalidomide, vaccination with allogeneic myeloma lines, K562/GM, and Prevnar-13 converted eight of 15 myeloma patients from a near CR to a full CR with tumor-specific immunity demonstrated.

Several GVAX strategies have been examined after hematopoietic stem cell transplantation. Treatment with GM-CSF secreting myeloid leukemia cell vaccine prior to autologous-BMT improves the survival of leukemia-challenged mice. The lymphopenic milieu after transplantation fosters a surge of homeostatic cytokines, including IL-7, IL-12, and IL-15 that promote T-cell activation and expansion. Regulatory T cells are also relatively deficient early after HCT, setting the stage for what be optimal conditions for GVAX. GVAX has been tested in the autologous transplant setting in humans. Patients with newly diagnosed acute myelogenous leukemia (AML), tumor cells were harvested and cryopreserved. Patients in remission then went on to receive induction chemotherapy and one round of consolidation followed by stem cell collection. Approximately 2 weeks later, patients received vaccination with thawed irradiated autologous AML cells admixed with K562/GM cells, followed 2 weeks later by a second leukapheresis to collect “primed” lymphocytes (target $10^8$ CD3+ T cells/kg). Peripheral blood stem cells and “primed” lymphocytes were reinfused after conditioning on transplantation day 0. Beginning at 6 weeks after transplantation, eight additional vaccinations were planned at 3-week intervals over a 6-month period. For the 28 vaccinated patients, the relapse-free survival and OS rates were 61.8% and 73.4%, respectively. Posttreatment induction of delayed type hypersensitivity reactions to autologous leukemia cells was associated with longer 3-year RFS rate (100% vs. 48%). These results, though hard to interpret in the absence of a sense of molecular risk of these leukemia, were very encouraging.

GVAX elicits potent antitumor immunity after experimental allogeneic transplantation in murine models. Studies in humans demonstrated induction of immune reactivity despite concurrent immune-suppressive medications without induction of graft-versus-host disease. Studies have included patients with advanced leukemia entering transplant with active leukemia. In the first study, autologous leukemia cells were harvested and transduced with an adenoviral vector. Patients underwent a reduced intensity transplantation with busulfan and fludarabine conditioning and tacrolimus plus methotrexate for GVHD prophylaxis. Between Day 28 and 42 after transplantation, patients with adequate hematopoietic count recovery and no active GVHD received thawed transduced vaccine administered six times over 9 weeks. Tacrolimus was continued during this period and then tapered. Vaccination elicited local and systemic reactions that were qualitatively similar to those previously observed in nontransplanted, immunized solid tumor patients. Infiltration of CD3+ T cells and other inflammatory cells targeting leukemia cells was observed after vaccination. The frequencies of acute and chronic GVHD were not increased, nine of 10 subjects who completed six vaccinations achieved durable complete remissions, with a median follow-up of over 2 years. Six long-term responders showed marked decreases in the levels of soluble NKG2D ligands, and three demonstrated normalization of cytotoxic lymphocyte NKG2D expression as a function of treatment. Vaccinated patients entering HCT despite having $>5\%$ marrow blasts had a lower incidence of disease relapse (38% vs. 59%, $p = 0.001$) at 2 years and improved PFS (44% vs. 25%, $p = 0.019$) compared with a large contemporaneous control cohort of MDS/AML patients transplanted without vaccination. This trial has set the stage for a prospective double-blind randomized phase 2 trial in which patients with high-risk AML/MDS will receive either patient-derived GVAX versus sham vaccine following allogeneic transplantation (NCT 01773395). This trial should provide definitive evidence as to the safety and efficacy (clinical outcome and immunogenicity) of GVAX in this setting.

These observations established the safety and immunogenicity of irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after allogeneic HCT. Two subsequent studies evaluated the K562/GM bystander formulation in conjunction with nontransduced tumor cells, one in chronic lymphocytic leukemia (CLL) and one in AML/MDS. In a study in patients with CLL, 18 patients received vaccinations with GM-K562 admixed with autologous CLL cells early after RIC HCT, with a 2-year PFS and OS of 82% and 88%, respectively. Vaccination expanded CD8+ T cells that reacted against autologous tumor, but not against nonmalignant recipient cells, in contrast to T cells from nonvaccinated CLL patients undergoing HCT. Further analysis showed that 17% of the CD8+ T-cell clones isolated from four vaccinated patients solely reacted against CLL-associated antigens. In a study of 33 patients with advanced AML/MDS receiving GM-K562 and autologous tumor vaccination, cumulative incidence of grades 2–4 acute and chronic GVHD were 24% and 33%, respectively. PFS and OS at 5 years were 39% and 39%. Postvaccination antibody responses to angiopoietin-2 was associated with superior OS (hazard ratio [HR], 0.43, $p = 0.031$) and PFS (HR, 0.5, $p = 0.036$). Patients transplanted with active disease had more frequent angiopoietin-2 antibody responses (62.5% vs. 20%, $p = 0.029$) than those transplanted...
These studies confirm that GM-K562/leukemia cell vaccination induces biologic activity, even in patients transplanted with active MDS/AML.

8 | FUTURE OF GVAX

Despite the excitement engendered by experimental animal models and early-phase human trials, GVAX has not lived up to its promise in inducing clinically meaningful outcomes in cancer patients. Perhaps this is related to the dual and sometimes opposing effects of GM-CSF in inducing recruitment of Treg and MDSCs. Efforts to improve GVAX efficacy might focus on DC activation. The adjuvant activity of CpG ODN in this context is under study. A formulation of GVAX coupled with LPS also induced superior protective immunity against tumor challenge that was associated with enhanced DC activation and increased T-cell tumor infiltration.91,92 The formulation of a TLR-7 agonist with GVAX further activated pDCs, which in turn potentiated antitumor responses. The stimulator of interferon (IFN) genes (STING) pathway, a TLR-independent pathway can also be exploited to enhance immune activation. GVAX formulated with a STING agonist (STINGVAX) generated enhanced antitumor immunity compared to GVAX alone, and PD-1 blockade further potentiated tumor control.93 Linking GVAX with tetanus toxoid is being tested in a cervical cancer model.94 A neoantigen-based GVAX combination is being explored in colorectal cancer.95 New checkpoint combinatorial strategies incorporating agonist OX40 antibodies appear effective in murine glioma.96,97 The pro- and anti-inflammatory properties of GM-CSF are being exploited by a codon-modified GM-CSF tumor cell vaccine to maximize inflammatory and suppress inhibitory effects.98

Although GVAX clinical studies to date have been disappointing, these studies have taught the field much about the subtleties of tumor immunity. A deeper understanding of the checks and balances regulating GVAX-mediated immune responses will be necessary if it is to play a pivotal role in cancer therapy.

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