Published in final edited form as:

Cancer J. 2010 ; 16(4): 304–310. doi:10.1097/PPO.0b013e3181eb33d7.

Cellular Vaccine Approaches

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Abstract

Therapeutic cancer vaccines aim to generate immunologic targeting of cancer cells through the induction of effective cellular and antibody-mediated responses specific for antigens selectively expressed by the tumor. Exploiting the adaptive immune system as a targeted tool against cancer is appealing in its capacity for exact specificity and avoidance of unintended tissue damage seen by other conventional agents such as chemotherapy. There are a multitude of challenges to designing effective vaccine strategies. The components of a vaccine strategy start with the challenges of selecting immunogenic, tumor-specific antigen targets, choosing a platform with which to deliver the antigens, and enhancing the immunostimulatory context in which the vaccines are delivered. Although understanding the components of effective T-cell activation is essential, successful effector T cells can only be produced if there is also an understanding of the natural processes that tumors exploit to down-modulate active immune responses. These processes are normally used to down-regulate excessive tissue-destructive immune responses against infectious agents once the infecting agent is cleared or to prevent autoimmunity. Advances in molecular and cellular technologies continue to provide insights into the regulation of immune responses both to infectious agents and to cancer that may be manipulated to tip the balance in favor of tumor regression over immune tolerance. This review focuses primarily on cellular vaccines. For the purpose of this review, cellular vaccines are defined as vaccines that use whole cells or cell lysates either as the source of antigens or the platform in which to deliver the antigens. Dendritic cell (DC)-based vaccines focus on ex vivo antigen delivery to DCs. Other platforms such as GVAX (tumor cells genetically engineered to produce granulocyte-macrophage colony-stimulating factor) aim to deliver tumor antigens in vivo in an immune stimulatory context to endogenous DCs. Because data continue to emerge regarding the importance of the maturation status of DCs and the importance of the particular subset of DCs being targeted, these insights will be integrated into vaccine strategies that are likely to produce more effective vaccines.

Keywords

cancer vaccine; cellular; dendritic cell; T cell

Therapeutic cancer vaccines aim to generate immunologic responses against cancer cells through the induction of effective cellular and antibody-mediated responses. If successful, these responses can potentially result in immunologic memory, providing the capacity to protect against recurrence even after vaccination course has been completed. Exploiting the adaptive immune system as a targeted tool against cancer is appealing in its capacity for exact specificity and potential avoidance of collateral tissue damage seen by other conventional agents such as chemotherapy. The specificity may be in the antibody

recognition of cell surface proteins or in the CD8⁺ cytolytic T cells or CD4⁺ helper T cells recognition of peptides in association with class I and II major histocompatibility complexes (MHC), respectively. Cells may present peptide/MHC complexes to T cells after processing of intracellular antigens or after antigen-presenting cells engulf extracellular antigens, process them, and present peptides on the host's endogenous MHC molecules via cross-presentation.

There are a multitude of challenges to designing effective vaccine strategies. The components of a vaccine strategy start with the nuances of selecting immunogenic, tumor-specific antigen targets, choosing a platform with which to deliver the antigens, and enhancing the immunostimulatory context in which the vaccines are delivered. Preclinical and clinical examples of tumor antigen-specific T-cell-mediated responses associated with tumor regressions have been documented that support the existence of these T cells in individuals with cancer, as well as their ability to become activated and kill tumors. Although understanding the components of effective T cell activation is essential, successful effector T cells can only be produced if there is also an understanding of the processes that have evolved to counter an active immune response. These processes normally down-regulate an active immune response against infectious agents once the infecting agent is cleared to prevent collateral tissue damage or induce tolerance to prevent autoimmunity. Advances in molecular and cellular technologies continue to provide insights into the regulation of immune responses both to infectious agents and to cancer that may be manipulated to tip the balance in favor of tumor regression over immune tolerance.

The first US Food and Drug Administration approval in history for a therapeutic cancer vaccine has recently been granted. This approval was the result of randomized clinical trial of Sipuleucel-T (Provenge; Dendreon, Inc.), an autologous dendritic cell (DC)-based vaccine loaded with a prostatic acid phosphatase (PAP)-granulocyte-macrophage colony-stimulating factor (GM-CSF) fusion protein used to treat men with advanced castrate-resistant prostate cancer (CRPC). The trial demonstrated a 4-month benefit relative to a control arm that received no treatment. This trial contrasts with the "failure" of prostate GVAX (Cell Genesys/Biosante), an allogeneic tumor cell-based vaccine. Both successes and failures have shed some light on important considerations in both vaccine design and trial design before moving forward with definitive studies. Another recent success for immunotherapy was the demonstration of improved survival in metastatic melanoma patients treated with an antibody that blocks a fundamental inhibitory pathway mediated by the CTLA-4 receptor. This is the first agent to ever demonstrate a survival benefit in advanced melanoma in a randomized clinical trial. The CTLA-4 and programmed death (PD)-1 pathways are among the growing lists of immune inhibitory pathways (sometimes termed immune checkpoints) that are being blocked by monoclonal antibodies in clinical development to enhance antitumor immunity. In addition to single agents, they are beginning to be used in combination with vaccines. CTLA-4 competes with CD28 for binding with costimulatory molecules on DCs.¹ PD-1 ligation of PD-L1/B7-H1 and PD-L2/B7-DC has been shown to down-regulate T-cell activation.²⁻⁴ Both molecules are implicated in the dampening of intended T-cell responses targeting infections and cancer, and unintended T-cell responses that may result in autoimmunity. The outcomes of years of research focused on understanding the role of these pathways in regulating T-cell responses are starting to be reflected in signals of clinical activity including objective tumor regressions.

This review focuses primarily on cellular vaccines. For the purpose of this review, cellular vaccines are defined as vaccines that use whole cells or cell lysates either as the source of antigens or the platform in which to deliver the antigens. DC-based vaccines focus on ex vivo antigen delivery to DCs. Other platforms such as GVAX (tumor cells genetically

engineered to produce GM-CSF) aim to deliver tumor antigens in vivo in an immune stimulatory context to endogenous DCs. Because data continue to emerge regarding the importance of the maturation status of DCs and the importance of the particular subset of DCs being targeted, these insights will be integrated into vaccine development and are likely to produce more effective vaccines.

DC-BASED VACCINES

Our understanding of DC biology is rapidly evolving and is influencing the development of DC-based vaccines. Much of our understanding comes from its role in inducing immune responses against infectious agents, particularly viruses. Banchereau et al.,⁵ in his review of cancer vaccines, succinctly describes the function of DCs and their various subsets. DCs collect antigen from various tissues and carry them to secondary lymphoid organs to ultimately activate antigen-specific T cells. Myeloid DCs and plasmacytoid DCs are the 2 main subsets of DCs. Through toll-like receptor (TLR) 7 and 9, plasmacytoid DCs recognize viral nucleic acids and secrete type I interferon (IFN). Three myeloid DC subsets localize to the skin. Langerhans cells (LCs) are found in the epidermis and CD1a⁺DCs and CD14⁺DCs are found in the dermis. CD14⁺ DCs produce interleukin (IL)-1 β , IL-6, IL-8, IL-10, IL-12, GM-CSF, membrane cofactor protein-1, and tumor growth factor- β . LCs produce IL-15, which is a growth and maintenance factor for CD8⁺ T cells and natural killer cells. LCs loaded with MART-1, gp-100, and tyrosinase are more efficient at CD8⁺ T-cell activation in vitro than CD14⁺ DCs loaded with these peptides. LCs are more efficient in cross-presentation and prime higher avidity T cells with reported greater capacity for cell kill. Although DC biology is complicated, it is clear that these cells are the critical regulators of adaptive T-cell and B-cell responses. These findings have provided the rationale for ex vivo antigen loading of DC as vaccines.

DCs have been loaded with tumor antigens in the form of peptides, proteins, tumor lysates, and mRNAs. Alternatively, they have been fused with tumor cells or infected with viral vectors encoding tumor-associated antigens. However, clinical development has at least 3 feasibility considerations that will require additional study before a DC approach will be generalizable as a therapeutic modality. The first consideration is the lack of standardized methods for the reliable production of functioning DC. Currently, it is difficult to demonstrate that each preparation has the same levels of processed and presented antigen, and activates an equivalent immune response after administration. This is true for multiple preparations given to one patient and for different preparations given to many patients. Quality control in the processing of cellular products is critical to the integrity of the product. Large amounts of autologous peripheral blood mononuclear cells must be cultured in the presence of several cytokines making their off-the-shelf marketability challenging. There are critical issues not only in ensuring the proper maturation status of the DCs but also in the precise selection of appropriate subsets of DCs required to elicit the desired response. The other 2 considerations include the significant cost of manufacturing the product and the significant labor required to produce a viable product within a short time frame. However, despite these technical hurdles, there is a DC-based vaccine, Sipuleucel-T, which recently received Food and Drug Administration approval based on a successful phase III trial showing improvement in overall survival (OS) in men with asymptomatic or minimally symptomatic metastatic CRPC. The key to manufacturing feasibility of Sipuleucel-T is the absence of DC purification in the preparation. The preparation of a Sipuleucel-T product involves a leukapheresis to obtain the peripheral blood of the patient. The leukapheresed specimen is then transferred to the company manufacturing facility. The cell pellet containing DCs (CD54⁺), T lymphocytes (CD3⁺), B lymphocytes (CD19⁺), monocytes (CD14⁺), and natural killer cells (CD56⁺) is exposed to PA2024, an engineered antigen-cytokine fusion protein consisting of PAP and GM-CSF. The GM-CSF facilitates uptake of

the fusion protein by DCs and promotes DC stimulation. PAP is the tumor antigen used in this vaccine approach. The final product is transported to the patient at 4°C and infused intravenously within 8 hours of formulation. Because the product is a mixture of cell types, the precise mechanism of action has not been established. As suggested by the name, it is not clear that induction of antiprstate cancer responses involve in vivo activation of T cells by the loaded DCs in the preparation. It is also possible that T cells in the preparation are activated ex vivo and that this therapy actually represents adoptive T-cell therapy. The paucity of available immunologic data to date precludes mechanistic dissection of this immunotherapy.

The registration study was based on the results of 2 phase II trials of Sipuleucel-T in patients with CRPC who showed a greater than 25% decline in prostate-specific antigen (PSA) in 30% and 15% of patients. Immune responses correlated with improved time to progression (TTP). Phase I and II trials have demonstrated T-cell responses to PA2024, PAP, and GM-CSF. Antibodies have also been detected.^{6–8} Two sequential phase III placebo-controlled studies were subsequently conducted in patients with metastatic CRPC, with a primary end point of TTP. In the first trial, D9901 (n = 127), there was a trend toward improved TTP in patients receiving Sipuleucel-T (11.7 vs. 10.0 weeks; $P = 0.052$). Although there was a survival benefit at 36 months, this was not a prespecified primary efficacy end point (35% vs. 11% were alive at 36 months, $P = 0.005$). The second trial, D9902A, was actually a truncated study group in which the TTP end point showed a trend toward improvement in the entire group but was significant in the subjects with a Gleason score ≤ 7 .

Integrated data from the D9901 and D9902A were presented that again suggested a survival benefit but failed to show significance for the predetermined clinical end point.⁹ In this combined data set, a total of 225 patients were randomized to Sipuleucel-T (n = 147) or placebo (n = 78). There was a 33% reduction in the risk of death (HR 1.50; 95% CI 1.10–2.05; $P = 0.011$). There was only a 4.8% PSA response in the combined analysis. Median survival was 23.2 versus 18.9 months and the percentage alive at 36 months was 33% versus 15% in favor of the treatment groups. Cumulative CD54 up-regulation, a measure of product potency, correlated with OS.

As a result of these studies, Dendreon pursued a new study, D9902B, also known as the IMmunotherapy for Prostate Adeno Carcinoma Treatment (IMPACT) trial, which favored enrollment of subjects with a Gleason score ≤ 7 . OS was the primary end point. Five hundred twelve patients were enrolled in this study. On April 28, 2009, data presented at the American Urological Association annual meeting showed that, despite absence of clinical response to Sipuleucel-T or affect on TTP, the study met its primary end point of survival benefit.¹⁰ Subjects in the treatment group experienced a longer median survival (25.8 vs. 21.7 months) and greater than 36 month OS (31.7% vs. 23%). The final analysis after 349 events demonstrated a median OS benefit of 4.1 months (HR 0.759; 95% CI 0.606–0.951; $P = 0.017$) (www.dendreon.com). Interestingly, there was no difference in median time to objective disease progression.

In contrast to the Sipuleucel-T study, a phase III study of an autologous peptide-loaded DC vaccine reported in stage IV melanoma failed to meet its primary end point.¹¹ In this study, the primary end point was objective response and the comparison arm was not placebo but dacarbazine (DTIC). This trial was performed by the DC study group of the Dermatologic Cooperative Oncology Group (DeCOG). DC vaccines were loaded with MHC class I and II-restricted peptides. DCs were generated from peripheral blood mononuclear cells obtained via leukapheresis and cultured with GM-CSF and IL-4 and then matured with tumor necrosis factor (TNF)- α , IL-1 β , IL-6, and prostaglandin E2. At the time of the first interim analysis, objective response was low (DTIC: 5.5%, DC: 3.8%). The Data Safety and

Monitoring Board recommended closure of the study. No significant differences between the 2 arms, either in OS or in progression-free survival (PFS), were found. Unscheduled subset analyses revealed that only in the DC-arm did those patients with an initial unimpaired general health status (Karnofsky = 100) or an human leukocyte antigen (HLA)-A2⁺/HLA-B44⁻ haplotype survive significantly longer than patients with a Karnofsky index <100 or other HLA haplotypes. Interestingly, improved outcomes in HLA-A2⁺ vaccinated patients using an allogeneic melanoma vaccine (Melacine; Corixa Corporation) in the adjuvant setting had previously been reported.¹² The investigators pointed out the following weaknesses in the DeCOG study: vaccine quality was variable in terms of number and maturation status; the subcutaneous route of administration was less effective than intradermal or intranodal routes; and nonspecific “helper proteins” used in previous studies had been omitted from this study. While they acknowledge the deficiencies in unscheduled subset analyses of small numbers, they made interesting observations about potential selection or stratification criteria for future studies.

While the optimal preparation of DCs and appropriate selection of tumor antigens has yet to be defined, both the prostate cancer and melanoma studies suggest that clinical parameters that may define less aggressive cancer biology may be useful in selecting a patient population that is more likely to benefit from immunotherapy. Combining proper patient selection and focusing on longer term follow-up endpoints such as OS may result in a separation of the curve at later time points at which the patients with more indolent disease are represented.

In addition to focusing on improving clinical trial designs, there is a considerable effort to enhance the potency of DC-based vaccines. Improving this effort is based on our evolving understanding of DC biology. Developing DC vaccine strategies must take into account both the maturation status and the subsets of DCs being targeted. In vitro studies suggest that the cocktail of maturation signals delivered to DCs can influence their differentiation and their capacity to elicit T-cell responses. In vitro GM-CSF and IL-4 DCs activated with a cocktail of IFN- α , polyinosinic-polycytidylic acid, IL-1 β , TNF- α , and IFN- γ induce up to 40-fold higher number of melanoma-specific CTLs than “gold standard” DCs matured by IL-1 β /TNF- α /IL-6/prostaglandin E2.¹³ Continued optimization of DCs development may translate into more potent vaccines. However, a major limitation of ex vivo-loaded DC vaccines is that, although ex vivo DC culture and maturation enhances in vitro T-cell activation, it may impair in vivo trafficking to lymph nodes at which the key antigen presentation steps occur. Thus, optimization must also involve analysis of trafficking of reinjected DCs and ultimate antigen presentation in lymph nodes, a study that has never been performed clinically.

WHOLE CELL VACCINE APPROACHES

Both autologous and allogeneic whole tumor cells are under clinical development as another form of cellular vaccine. In this approach, the whole tumor cell is the source of immunogens to induce an antitumor immune response. The advantage of using a whole tumor cell approach is that the tumor antigens do not have to be prospectively identified and multiple antigens can be simultaneously targeted. Early studies using irradiated whole tumor cells alone had not been shown to be effective. However, the cloning of multiple cytokines and chemokines and the development of gene delivery systems, together with an expansion in our knowledge how cytokines and chemokines work together to induce systemic immunity, led to the development of genetically modified whole tumor cells approaches. This second generation of whole tumor cell vaccines has undergone extensive preclinical and clinical testing. Tumor cells expressing a number of cytokines, chemokines, and costimulatory molecules have been shown to demonstrate varying degrees of antitumor immunity in mouse models and in early clinical trials.

One cytokine-expressing whole tumor cell approach that has undergone significant preclinical and clinical testing is GVAX (Cell Genesys). This approach consists of either autologous or allogeneic tumor cells genetically modified to express GM-CSF. GM-CSF works locally in a paracrine manner to recruit and activate antigen-presenting cells and to promote uptake of irradiated tumor cells for cross-presentation. There is a significant body of preclinical data supporting its antitumor efficacy especially in combination strategies with agents such as T regulatory modifying doses of cyclophosphamide, anti-CTLA-4, and anti-PD-1.¹⁴⁻¹⁶ Autologous tumor cells are thought to provide a more accurate panel of immunogens representative of an individual patient's tumor. However, allogeneic approaches have become the preferred approach in some disease types as it is much less cumbersome to use cell lines when compared with individually obtained and manipulated tumor specimens. A major concern of using an allogeneic approach is that the induced immune responses may be predominantly directed against the allogeneic HLA molecules. Several preclinical models have shown that an allogeneic allele does not diminish and may enhance the antitumor immune response. Li et al¹⁷ also addressed this question in a mouse model by comparing a GM-CSF-secreting B16F1 cell line as autologous immunotherapy and the same cell line modified to express the MHC molecule K^d as the allogeneic counterpart. The antitumor effects of both therapies were similar. When combined with anti-PD-1, tumor-specific and allogeneic immune responses were equally enhanced.

GM-CSF-secreting whole tumor cell approaches have been in clinical development in many cancers including breast, pancreatic, colorectal, and prostate cancer patients. Cell Genesys launched and recently terminated 2 phase III trials of prostate GVAX. Prostate GVAX consists of 2 prostate cancer cell lines, LNCaP and PC-3, transfected with a GM-CSF gene. These registration studies were based on earlier studies that showed antibody responses to filamin B increased during treatment and correlated with survival if the antibody was induced by week 12 and sustained at week 24. PSA responses were also seen. OS was improved compared with predicted survival times using Halabi prediction models.^{18,19} The first phase III study, Vaccine Immunotherapy with Allogeneic Prostate Cancer Cell Lines (VITAL)-1, was a phase III trial designed to compare GVAX to docetaxel plus prednisone in asymptomatic, CRPC.²⁰ In contrast to the Sipuleucel-T studies, the comparator was not a placebo group. Docetaxel plus prednisone has been shown to provide an OS benefit in patients with symptomatic CRPC. The primary end point of VITAL-1 was OS. Although patients in the vaccine arm demonstrated improved OS when compared with standard therapy, the difference did not reach significance.

VITAL-2 was the second phase III study and was conducted in symptomatic CRPC.²¹ One arm received GVAX with docetaxel and the other received docetaxel and prednisone. VITAL-2 was closed early because of increased deaths in the GVAX combination arm compared with the control arm (67 vs. 47, respectively). The initial reported difference of 20 deaths actually decreased to 9 deaths at the final analysis (December 2008). The discrepancy was due to the slower reporting of deaths in patients receiving the standard therapy only. There were no increases in severity or new toxicities in the GVAX plus docetaxel arm that could explain an imbalance in deaths. Eighty-five percent of deaths were reported as due to prostate cancer in both arms, and there was no trend in the causes of death in the remaining patients. The decision to omit the potentially immunosuppressive drug, prednisone, in the GVAX arm may have contributed to an insignificant decrease in OS when compared with the combination of chemotherapy and prednisone. Interestingly, the experimental combination of GVAX and docetaxel had not previously been studied in patients before entering phase III testing. Notably, chemotherapy agents can have synergistic or antagonistic effects on immunotherapy depending on the agent, relative timing of their administration, and selected dose. It is likely that the dose of docetaxel used in this study inhibited a

vaccine-induced immune response because docetaxel does affect white blood cell counts and was given at the time of vaccination.

VITAL-1 was terminated soon after the VITAL-2 results were reported based on the results of a previously unplanned futility analysis, which indicated that the trial had <30% chance of meeting its predefined primary end point of improved OS. However, the final Kaplan-Meier survival curves for the 2 treatment arms suggest a late favorable effect of GVAX immunotherapy on patient survival with the curve for GVAX patients crossing above the chemotherapy curve at approximately the same time median survival was reached in both treatment arms (21 months). Additionally, the data suggest that patients with Halabi predicted OS ≥ 18 months may have a more favorable response to the immunotherapy. Chemotherapy may be particularly important in patients with more rapidly progressing disease. Although noninferiority was not the predefined end point, it is worth noting that a potential benefit of an immunotherapy approach lies in the toxicity profile despite similar OS outcomes. Although it is only speculation, perhaps using a placebo group as the comparator arm in asymptomatic patients or selecting better prognosis patients (as was done for the Dendreon vaccine) may have resulted in a “positive” study. With the technically “negative” studies and the reality that single-agent prostate GVAX is unlikely to significantly improve on survival outcomes with docetaxel and prednisone, it is unlikely that single-agent prostate GVAX will be tested again in a large study. However, academic centers will likely continue to test the agent strategically in various combinations driven by preclinical data and in selected indications.

Although much attention has been directed toward prostate GVAX, intriguing results with a variety of other GVAX-based vaccines continue to stimulate interests in this platform. Recent studies have been reported using irradiated, autologous, GM-CSF-secreting leukemia cell vaccines early after stem cell transplantation. Drug-induced lymphopenia results in a change in cytokine milieu, characterized by high levels of IL-7, IL-12, and IL-15, which favors reconstitution of effector T cells relative to regulatory T cells. In addition, mucosal damage may stimulate TLR signaling in DCs promoting maturation. In a study using GVAX after allogeneic transplantation in high-risk myelodysplastic syndrome and acute myelogenous leukemia, 10 of 15 subjects achieved extended DFS.²² Long-term responders generated DTH responses and bone marrow eosinophil and T-cell infiltration. In a second study, investigators used autologous leukemia cells admixed with GM-CSF-secreting K562 cells accompanied by immunotherapy-primed lymphocytes after transplant.²³ Posttreatment induction of DTH reactions to autologous leukemia cells was associated with longer 3-year RFS (100% vs. 48%). A decrease in WT1 transcripts in blood was noted in 69% of patients after the first immunotherapy dose and was also associated with longer 3-year RFS (61% vs. 0%). Another example of the use of this platform in hematologic malignancies is the report that K562/GM-CSF immunotherapy reduced tumor burden in chronic myelogenous leukemia patients with residual disease on imatinib mesylate.²⁴ Seven of 19 patients became BCR-ABL PCR-undetectable despite long durations of previous imatinib mesylate therapy. A GM-CSF-secreting allogeneic pancreatic cancer vaccine has finished phase I and II testing. A phase II adjuvant study of the vaccine integrated into 5FU-based radiation resulted in a median OS of 24.8 months.²⁵ Median OS of a historical control cohort was 20.3 months. Postimmunotherapy induction of mesothelin-specific CD8⁺ T cells correlated with DFS. Although direct comparisons cannot be made, it is interesting that the avidity or quality of the T cells is more potent in the adjuvant compared with the metastatic patients. However, even in the metastatic setting, cyclophosphamide can enhance the generation of higher avidity T cells and in one study correlated with improved PFS.²⁶

OTHER WHOLE CELL VACCINE APPROACHES

Other whole tumor cell vaccine approaches have made it to phase III testing. The vaccines consist of tumor cells coadministered with adjuvants. One of the first to be completed was that of Melacine. Melacine is a lysate of 2 melanoma cell lines given with Detox adjuvant (altered mycobacterium cell wall skeleton and monophosphoryl lipid A). It was tested in comparison with a chemotherapy regimen referred to as the Dartmouth regimen in stage IV melanoma. The response rate was <10%. Survival was comparable but with less toxicity and this led to Canadian approval for stage IV melanoma. Given that there is not a proven OS benefit with the Dartmouth regimen, there is also an unclear OS benefit to Melacine, and Melacine is not approved in the United States. In an adjuvant phase III trial, Southwest Oncology Group 9035, conducted in stage IIA disease there was no statistical difference in DFS when compared with an observation control arm. Five class I HLA types appeared in responders analyzed in the above 2 trials.^{12,27} Preplanned analysis suggests that certain classes of HLA class I types may benefit such as HLA A2 and C3 patients. A study restricted to A2/C3 haplotypes could potentially enrich for responders. However, this study has not been performed. Corixa, the biotechnology company that had been developing the agent, was acquired by GlaxoSmithKline in July 2005.

Canvaxin (CancerVax) is another whole cell vaccination approach that entered phase III testing. It is an allogeneic melanoma vaccine (irradiated cells from 3 melanoma cell lines) given intradermally with BCG. Early studies suggested that DTH responses to vaccine cells could be used as a surrogate marker for benefit. A small proportion of patients with stage IV measurable disease have also had responses to Canvaxin. In a nonrandomized phase II study in 136 patients with stage IIIA/IV melanoma, median OS was 23 months compared with 7 months for historical controls. In 40 patients with disease who could be followed up, there were 3 CRs and 6 “regressions.” Survival has correlated with vaccine DTH responses and antibody induction in several studies.^{28–30}

A phase III study in stage III and resectable stage IV melanoma patients receiving Canvaxin versus BCG alone was discontinued once the Data and Safety Monitoring Board ruled that it was determined that the Canvaxin-treated patients displayed a trend toward lower OS than patients treated with BCG alone.

There is one agent that is an autologous cellular vaccine administered with BCG known as OncoVax which has been authorized for commercial use in Switzerland. The major effect of the agent was seen in patients with stage II colon cancer, with a significantly longer recurrence-free period ($P = 0.011$) and 61% risk reduction for recurrences. Recurrence-free survival was significantly longer (42% risk reduction for recurrence or death, $P = 0.032$). However, there was only a trend toward improved OS.³¹ An ECOG study, E5283, concluded that there was no benefit for stage II or III patients.³² However, in this study, vaccine manufacturing was not centralized and felt to be of poor quality as evidenced by quality-control assessments and fewer patients with DTH responses as compared with subjects in other studies. In addition, a booster vaccine was not added until the subsequent study.³³

FUTURE PERSPECTIVES

With a long list of unsuccessful phase III trials with cellular vaccines, various aspects of the proposed experimental therapy must be considered. One question that is being asked is whether the preclinical data are robust enough to justify moving forward into a human trial? Also, is the early phase clinical trial data robust enough to justify moving forward into larger, definitive studies? Another important question is whether a vaccine in the context of an immune adjuvant is likely to be sufficient to provide a clinical benefit in the face of

tolerance induction and checkpoint ligand expression by tumors? Thus, will only combinatorial strategies using T regulatory cell modifiers, immune checkpoint inhibitors, immune stimulation agonists, immune stimulating chemotherapy, or radiation regimens be potent enough to translate into clinical benefit for less immunogenic tumors? Certainly, the preclinical models suggest that this is likely to be the case. Preclinical studies also suggest that immunotherapy will be more likely successful in the adjuvant setting when the disease burden is low and the immune tolerance mechanisms are less pronounced. Interestingly, current reported phase III adjuvant trials in melanoma have been negative. Yet tumor responses have been reported using DC vaccines and immune checkpoint blockade in advanced melanoma patients.

Although the negative outcomes from completed studies using cellular vaccines have been sobering, each individual vaccination platform and strategy has to be evaluated on its own scientific merit. However, outside observers are becoming skeptical of the potential of therapeutic cancer vaccines. Yet, it is only through these studies that new insights are gained that might influence future vaccine and trial design. Dendreon reported an OS improvement, whereas most of the other agents that have completed phase III testing have not. Is the scientific rationale supporting the use of Sipuleucel-T more robust than that of other agents that have failed testing? Is it the platform, the antigen, the patient population, or the trial design that contributed to the improved OS in that study? Each of these factors likely contributed.

An illustrative example of the elements of late-stage clinical trial development that affect the success or failure of cancer vaccines comes from the comparison of the Dendreon's successful Sipuleucel-T IMPACT trial versus the Cell Genesys' failed GVAX VITAL-1 trial, both were performed in men with CRPC. The GVAX vaccine phase III trial compared GVAX alone with the standard of care in the United States for men with advanced CRPC – taxotere + prednisone (T + P). T + P is known to provide a small but real (3–4 months) enhancement in overall patient survival relative to no treatment. For the VITAL-1 trial, Cell Genesys set statistically significant survival benefit relative to T + P. This relatively high bar was set based on comparisons of survival to historical data with T + P in an extremely small phase II GVAX trial. An interim analysis of VITAL-1 was performed, showing no statistical difference in median survival between the GVAX and the T + P arms. An interesting trend was observed in which there was a slightly lower initial death rate in the T + P arm; however, the curves crossed at about 90 weeks such that the GVAX arm demonstrated superiority to T + P for patients surviving beyond 90 weeks. Because the trial was terminated prematurely, there were not enough patients followed up long enough to conclude that there was any statistical significance to these trends.

Dendreon followed a very different strategy of development for Sipuleucel-T. Ultimately, the IMPACT trial also set OS as the primary end point. The control arm was not T + P but rather reinfused PMBC not incubated in antigen or GM-CSF. The overall cohort was skewed toward earlier stage disease (~75% Gleason score ≤7) as compared with the Cell Genesys VITAL-1 patient cohort (~50% Gleason score ≤7). Further analysis of the characteristics of the enrolled patients and survival curves supports the notion that, within the broad scope of “advanced castrate resistant prostate cancer,” the IMPACT cohort had relatively earlier stage disease at the time of enrollment than the VITAL-1 cohort. Ultimately, the vaccine arms in both trials demonstrated similar survival, whereas the control arm (T + P) of the VITAL-1 trial exhibited roughly 4 months better survival than the IMPACT control arm.

There are multiple areas of consideration as future generations of vaccine approaches are designed and tested. These include (1) developing new standards for assessing response

criteria and defining study endpoints; (2) considering patient selection and identifying the patient population most likely to benefit from the therapy; and (3) considering ways to optimize the potency of vaccine approaches.

Response Criteria/Study Endpoints

Recent clinical studies have provided evidence that traditional response criteria may be inadequate to test for efficacy of immunotherapeutic approaches. Conventional drug development practices have taken the perspective that response rate as determined by tumor regression defines clinical utility. However, there are a few chemotherapeutic and targeted agents that have been approved on the basis of demonstrating clinical benefit using criteria such as quality of life improvement even when they did not meet accepted tumor response rates. In addition, a number of cancer therapies that were approved based on conventional response rate did not affect OS (eg, DTIC for metastatic melanoma). OS and clinical benefit are likely better endpoints for immunotherapy trials. Supporting this concept is the example of the incongruity between progression and survival endpoints for the phase III Sipuleucel-T study. Additionally, a phase II ProstVAC study showed no difference in PFS but did show an improvement in OS. However, the primary end point of this study was PFS and not OS. This study was a double-blind phase II trial of ProstVac-VF, a vaccinia and fowlpox-based vaccine, versus an empty vector in 122 men with metastatic CRPC failed to show a significant difference in the primary end point of PFS. However, updated 3 year results revealed an OS benefit (24.5 vs. 16.0 months, $P = 0.016$). Patients with a Halabi predicted survival of >18 months (median predicted survival of 20.9 months) met or exceeded a survival of 37.3 months, which generated the hypothesis that patients with more indolent metastatic CRPC may best benefit from vaccine therapy.³⁴ Agents such as ipilimumab have shown that immunotherapy can take months to demonstrate tumor shrinkage radiographically. In fact, some studies have shown that tumors can progress before shrinking or may stabilize or grow more slowly. Based on the ipilimumab experience, Wolchok et al³⁵ proposed the use of immune-related response criteria (irRC) that allow for transient increases in sizes of lesions and the emergence of new lesions as long as the best response is a decline in total tumor burden. Waiting for delayed responses can be clinically challenging in terms of decision making for individual patients.

OS is a difficult end point to use in early phase trials and it is important to continue to explore surrogate immune endpoints. Of course, this is with the caveat that no immune end point has yet been statistically validated to correlate with clinical benefit or OS in the published literature. Although immune responses have not strictly correlated with clinical responses, it would be difficult to imagine that a vaccine that could not induce an immune response could be of value. Induction of tumor antigen-targeted antibodies, DTH, and CD8-specific T-cell responses should therefore be analyzed as a standard part of early stage vaccine trials. There is also an increasing attempt to correlate T-cell quality measures such as avidity, poly-functional cytokine release, T-cell persistence, cytolytic capacity, and antigen repertoire, with vaccine outcomes. The hope is that better surrogates could provide tools to define more effective regimens to move forward in clinical development.

Patient Selection

Immunotherapies are likely to take longer to produce an effect and therefore it is essential to select the appropriate patient population. A difference between an experimental arm and a control arm may not be evident unless the curves are followed for a prolonged time as 2 curves may be superimposed during the initial time points reflecting the rapid progression of a subset of patients. Patient selection may include patients with smaller disease burden, clinical features of less aggressive disease, or chemotherapy naive only patients. As an

example, Gleason scores and Halabi-predicted survivals have been used in prostate cancer with this rationale in mind.

The pursuit of selection and predictive markers will likely intensify potential efficacy signals. A study using a DC-based vaccine pulsed with allogeneic tumor lysate against renal cell carcinoma showed that prevaccination samples from patients with stable disease showed an enhanced T_H1 response (IFN γ) compared with samples from patients with progressive disease who showed a mixed T_H1/T_H2 response (IFN γ IL-4, IL-5) to tumor lysate-pulsed DCs.³⁶ A gene signature derived from pretreatment tumor biopsies predicted clinical benefit in a study of a recombinant MAGE-A3 fusion protein (EORTC 16032-18031) in the treatment of melanoma.³⁷ A DC vaccine study in colon cancer patients identified a prevaccination plasma protein signature (sgp130, CXCL11, CD62L, membrane cofactor protein-1, and IL-20) that predicted responses to DC vaccination.³⁸ Much work has to be done before correlative studies validating prediction markers can be realized. In the meantime, clinical parameters such as earlier disease settings, prediction models, pathology predictors, and HLA status could potentially be used to enrich for potential responders.

Optimization of Vaccination Strategies

While patient selection can influence the outcome of a study, it is most important to focus on improving the efficacy of a vaccine. Identification of tumor antigens that are necessary for survival, bind to MHC molecules, and can induce T-cell responses is critical. Large efforts using genomic and proteomic approaches are currently underway to more rapidly identify the most clinically relevant targets that may also be associated with the cancer biology. Other areas of optimization include improving DC maturation, vaccine consistency, route of administration, booster vaccinations, and incorporation of adjuvants.

Adjuvant selection, dose, and route of administration can affect outcomes. In a study adding GM-CSF to Canvaxin, the GM-CSF arm showed enhanced humoral (anti-TA90 response) and diminished cellular responses (DTH).³⁹ There was a trend toward reduced survival in the GM-CSF arm. Postulated mechanisms include the presence of GM-CSF receptors on vascular endothelial cells facilitating tumor growth or the induction of myeloid-derived suppressor cells. The dose of GM-CSF used in this study was considered high at 400 μ g/d. Suppressive effects have been reported with high doses of GM-CSF. Slinghuff reported on the use of a multi-peptide vaccine with or without GM-CSF.⁴⁰ Although the study was not adequately powered to be conclusive, worse T-cell responses and survival were seen in the GM-CSF arm. GM-CSF was given locally and once a week. The effective daily dose was thought to be <20 μ g/d, because it was given as a slow-release formulation. However, 110 μ g is still likely to provide a higher peak GM-CSF level than a GVAX preparation that before irradiation releases 100 ng/10⁶ cells/24 h. Target serum GM-CSF levels in GVAX studies are in the picogram per milliliter range. It should be pointed out that all these studies used soluble GM-CSF protein, which diffuses away from the vaccination site within a few hours. This contrasts with the local pharmacokinetics of GM-CSF gene-transduced vaccines (GVAX), which continuously secrete GM-CSF at the vaccine site for days.

New adjuvants have been explored in phase I testing and are thought to enhance potency of vaccines including TLR agonists such as CpG ODNs (oligodeoxynucleotides) and imiquimod. Furthermore, combination strategies are underway that use conventional therapies such as chemotherapy and radiation that seem to synergize with vaccinations. When using these strategies, great care has to be taken to test optimal dose and sequence. Timing and dose of cyclophosphamide and doxorubicin relative to a GM-CSF-secreting breast cancer vaccine were shown to influence the induction of HER2-specific immunity.⁴¹ Although some agents such as gemcitabine may have effects on myeloid-derived suppressor populations and radiation may alter tumor-cell phenotype and up-regulate the expression of

some tumor-associated antigens, costimulatory molecules, cytokine receptors, MHC class I and Fas molecules, dose and timing, are a few of the critical parameters that may be altered when using potentially immunosuppressive agents.

Another area of intense study is the role of modulating immune signals that may either enhance or inhibit vaccine-induced antitumor immunity. Cancer patients often exhibit multiple mechanisms of immune tolerance that can affect the efficacy of a vaccine. As one example, multiple vaccinations with a GM-CSF secreting tumor in a murine model resulted in increased frequency and absolute number of the baseline CD4⁺Foxp3⁺ regulatory T cells in a cancer bearing host that was associated with a decreased therapeutic efficacy. This vaccine inhibition effect was reversed by anti-CD4 administration before each booster.⁴² In support of this concept, the DeCOG and CancerVax groups reported trends toward an inferior OS in their multidose vaccine studies. Targeting regulatory T cells is an active area of investigation. As a second example, low-dose cyclophosphamide and ONTAK, an engineered protein combining interleukin-2 and diphtheria toxin, are being used in clinical studies to decrease regulatory T cells in the context of vaccination.

Another important area of combinatorial strategies is the combination of vaccines with agents that enhance T-cell costimulation or inhibit immune checkpoints. Preclinical studies have combined vaccines with agonist antibodies to OX-40 and 41BB that enhance T-cell stimulation and have combined vaccines with checkpoint blockade using anti-CTLA-4 and anti-PD-1.^{15,16,43,44} In murine models, the least immunogenic tumor models are best treated with combination strategies and it would stand to reason that in the human cancer setting in which tolerance to tumor antigens has years to develop, a combination strategy would more likely be effective than a single-agent vaccine. In ongoing clinical studies, anti-CTLA-4 is being combined with a variety of vaccine platforms in a number of disease indications. These clinical grade antibodies are all in various phases of testing in human subjects. Thus, vaccine platforms have been generated on the basis of sound scientific rationale but the technology to clone genes and dissect regulatory pathways lagged behind. As more agents targeting these pathways become available for clinical testing, there is hope that combinations will provide new therapeutic approaches for patients with cancer.

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