Advances in inducing adaptive immunity using cell-based cancer vaccines: Clinical applications in pancreatic cancer

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Abstract

The incidence of pancreatic ductal adenocarcinoma (PDA) is on the rise, and the prognosis is extremely poor because PDA is highly aggressive and notoriously difficult to treat. Although gemcitabine- or 5-fluorouracil-based chemotherapy is typically offered as a standard of care, most patients do not survive longer than 1 year. Therefore, the development of alternative therapeutic approaches for patients with PDA is imperative. As PDA cells express numerous tumor-associated antigens that are suitable vaccine targets, one promising treatment approach is cancer vaccines. During the last few decades, cell-based cancer vaccines have offered encouraging results in preclinical studies. Cell-based cancer vaccines are mainly generated by presenting whole tumor cells or dendritic cells to cells of the immune system. In particular, several clinical trials have explored cell-based cancer vaccines as a promising therapeutic approach for patients with PDA. Moreover, chemotherapy and cancer vaccines can synergize to result in increased efficacies in patients with PDA. In this review, we will discuss both the effect of cell-based cancer vaccines and advances in terms of future strategies of cancer vaccines for the treatment of PDA patients.

Key words: Pancreatic cancer; Dendritic cell; Whole tumor cell; Cancer vaccine; Cytotoxic T lymphocyte
Core tip: Chemotherapy and cell-based cancer vaccines such as dendritic cell- and whole tumor cell-based cancer vaccines can synergize to result in increased efficacies in patients with pancreatic ductal adenocarcinoma (PDA). Moreover, cell-based cancer vaccines and immune checkpoint inhibitors can be used to block inhibitory ligand/receptor interactions by acting on certain cancer cells or T cells, allowing an enhancement of the antitumor immune response in specific tumors, including PDA. Therefore, the blockade of immune regulatory checkpoints combined with cell-based cancer vaccines and/or chemotherapy may be effective in inducing adaptive antitumor immunity in patients with PDA.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA), which is derived from glandular tissue of the pancreas, accounts for approximately 95% of pancreatic cancer and is one of the most lethal cancers because of a propensity for metastatic spread[1,2]. Although the definitive treatment for early-stage PDA is surgical resection, this is only possible in approximately 15% of cases[3], as most patients with PDA present in an advanced stage at the time of diagnosis. Additionally, despite surgical resection, radiation and/or chemotherapy, patients with PDA have an overall 5-year survival of only 5% due to local recurrence and metastasis[1,2,4]. PDA cells grow rapidly and spread outside of the pancreas, including into the liver, lung, bone, and brain, through lymphatic and/or blood vessels. The current standard chemotherapy for patients with advanced PDA is gemcitabine. Gemcitabine can also be combined with nab-paclitaxel[5] or erlotinib[6], resulting in improved survival. Moreover, a multi-chemotherapy regimen (FOLFIROX) consisting of 5-fluorouracil, folinic acid, oxaliplatin and irinotecan has been associated with significant improvement in survival for patients with advanced PDA[7]. However, the currently used chemotherapeutic agents have still failed to demonstrate satisfactory clinical advantages in patients with advanced PDA. It has been well demonstrated that PDA is relatively resistant to chemotherapy, so new therapeutic strategies are urgently needed to improve pancreatic cancer treatment. Regarding potential targets for cancer vaccines, PDA cells express numerous tumor-associated antigens (TAAs), such as Wilms’ tumor gene 1 (WT1)[8], mucin 1 (MUC1)[9], human telomerase reverse transcriptase (hTERT)[10], mutated K-Ras[11], survivin[12], carinoembryonic antigen (CEA)[13], epidermal growth factor receptor 2 (HER-2)[14], and p53[15]. Therefore, cancer vaccines targeting these TAAs may be an alternative approach for treating patients with PDA.

INDUCTION OF ANTITUMOR IMMUNE RESPONSES

Cancer cells degrade endogenous antigens into short peptides (usually 8-10 amino acids) and present them via major histocompatibility complex (MHC) class I molecules. These cells express numerous TAA-derived peptides on their cell surface as a result of malignant transformation. Meanwhile, T cells with the αβ T cell receptor (TCR) express CD4+ T cell or CD8+ T cell lineage markers[16]. Interaction of the TCR on CD8+ cytotoxic T lymphocytes (CTLs) with the complexes of antigenic peptides and MHC class I molecules on tumor cells is a critical event in the T cell-mediated antitumor immune response. However, induction of CD8+ CTLs also requires antigenic peptides to be presented on the surface of antigen-presenting cells (APCs) in the context of MHC class I molecules. It has become clear that dendritic cells (DCs) are the most potent APCs in the human body and play a pivotal role in the initiation, programming, and regulation of antitumor immune responses[17]. DCs can process endogenously synthesized antigens into peptides, which are presented on the cell surface as peptide/MHC class I complexes, but require activation signals to differentiate and eventually migrate to the regional lymph nodes, where they are recognized by the αβ TCR on CD8+ T cells[17]. Moreover, DCs capture and process exogenous antigens and present peptide/MHC class I complexes through an endogenous pathway via a process known as antigen cross-presentation[18]. This cross-presentation is essential for the initiation of CD8+ CTL responses[19]. In contrast, exogenous antigens from the extracellular environment are captured and delivered to the compartments of the endosome/lysosome, where they are degraded into antigenic peptides, which are then complexed with MHC class II and recognized by the αβ TCR of CD4+ T cells[17]. Finally, mature DCs can present TAAs to naive CD4+ and CD8+ T cells in the regional lymph nodes; these T cells then differentiate into activated T cells. It is well known that in the induction of efficient CD8+ CTL responses against cancer cells, CD4+ T cells are essential for the priming of CD8+ CTLs through activation of APCs and production of interleukin (IL)-2 and interferon (IFN)-γ[20]. CD4+ T cells also play an important role in the maintenance and infiltration of CD8+ CTLs at a tumor site[21]. Therefore, activation of antigen-specific CD4+ and CD8+ T cell responses by
Pancreatic ductal adenocarcinoma (PDA) and other pancreatic cancer cells induce efficient antitumor immunity against pancreatic cancer cells\[22\].

PDA cells can evade immune control through several mechanisms. One major mechanism is the immunosuppressive tumor microenvironment. The microenvironment in pancreatic cancer in particular consists of PDA cells and stroma cells, such as cancer-associated fibroblasts (CAFs), tolerogenic DCs, myeloid-derived suppressor cells (MDSCs), immunosuppressive tumor-associated macrophages (TAMs), and regulatory T cells (Tregs). Importantly, PDA cells themselves induce immune suppression through production of immunosuppressive substances such as cytokines \([\text{e.g.}]\), transforming growth factor (TGF)-\(\beta\), IL-10, and IL-6, vascular endothelial growth factor (VEGF), Fas ligand (Fas-L), programmed cell death-1 (PD-1) ligand (PD-L1) and indoleamine-2, and 3-dioxygenase (IDO))\[22,23\]. These immunosuppressive cells inhibit antitumor immunity by various mechanisms, including depletion of arginine and elaboration of reactive oxygen species (ROS) and nitrogen oxide (NO)\[22,23\].

The pancreatic cancer microenvironment not only contributes to pancreatic cancer-induced immune suppression but also might be closely related to the extent of disease. For example, T cells producing IL-22 were significantly increased in PDA tissue, and this increase was significantly associated with tumor staging and poor prognosis\[24\]. Moreover, Tregs, MDSCs, and T helper 17 (Th17) cells in intratumoral tissue elicited strong immune suppression in patients\[25,26\]. As a result, CD8+ CTL function in patients with advanced PDA is impaired by IL-10 and TGF-\(\beta\) from Tregs. Therefore, DC-based cancer vaccines against PDA cells that cause induction of TAA-specific CD4+ and CD8+ T cells combined with depletion of immunosuppressive cells may tip the balance in favor of immunostimulation.

### DC-BSAED CANCER VACCINES

The aim of cancer vaccines is to induce efficient antitumor immunity. Peptide vaccines are frequently used because they are simple, safe, and economical. However, certain obstacles prevent the use of peptide vaccines from becoming widespread. The drawbacks of peptide vaccines are related to numerous factors: (1) the limited number of known synthesized short peptides cannot be presented via many MHC molecules\[27\]; (2) monoclonal CD8+ CTLs may be ineffective in reacting to PDA cells\[28\]; (3) certain TAAs and MHC class I molecules are occasionally down-regulated, which may occur during tumor progression\[29\]; and (4) DCs may have impaired function in patients with advanced PDA\[29\]. Therefore, \textit{in vitro}-generated mature DCs have been developed as cancer vaccines because of their powerful ability to induce antigen-specific CD4+ T cells and CD8+ CTL responses in preclinical and clinical studies\[30\]. To date, the majority of DC-based cancer vaccines have been generated using monocytederived DCs. Immature DCs can be generated by a single leukapheresis after culture in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4. In our laboratory, immature DCs are activated for vaccines by incubation with penicillin- and lyophilized preparations of a low-virulence strain (Su) of \textit{Streptococcus pyogenes} (OK-432) and with prostaglandin E2 (PGE2), after which a large number of DCs can be cryopreserved in ready-for-use aliquots\[31\]. Several strategies have been used to develop DC-based cancer vaccines to elicit efficient antitumor immune responses (Table 1). To induce DC presentation of TAAs, DCs have been loaded with TAAs in the form of tumor lysates\[32\], antigenic peptides\[33\], dying or dead tumor cells\[34\], mRNA\[35,36\], cDNA\[37\], or exosomes\[38\] or have been fused with whole tumor cells to form hybrid cells\[39\]. The strategy of fusing DCs and whole tumor cells is based on the facts that DCs are potent APCs and that whole tumor cells express abundant TAAs, including both known and unidentified TAAs\[40-42\].

Therefore, DC-tumor fusion cells can process a broad array of TAAs and present them \textit{via} MHC class I and class II in the context of co-stimulatory molecules\[40-42\]. Moreover, many adjuvants, including Toll-like receptor (TLR)3, TLR9, synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG, polyinosinic:polycytidylic acid (polyI: C), IL-2, IL-12, and IL-18, have been used in DC-based cancer vaccines to maximize antitumor immune responses in preclinical studies\[43\].

The field of cancer vaccines for PDA is currently in an active state of clinical investigation. In particular, the development of DC-based cancer treatments is of great importance. Clinical trials of DC-based cancer vaccines for PDA patients have been conducted (Table 2), including clinical trials for an MUC1-targeted DC-
Table 2  Clinical trials of dendritic cell-based cancer vaccines in pancreatic cancer patients

| Cell-based cancer vaccines | Targets | Vaccines | Phase | Patients | Results | Ref. |
|---------------------------|---------|----------|-------|----------|---------|------|
| Dendritic cells (DCs)     | MUC1    | DCs loaded with MUC1 peptide | Phase I / II | 12 pancreatic or biliary cancer patients following surgical resection | These patients have been followed for more than 4 yr after vaccination, and 4 of them were alive without recurrence. | [46] |
| DCs transfected with MUC1 cDNA | Phase I | 16 patients with pancreatic cancer | | 2 of 15 patients with resected PDA were alive and disease free at 32 and 61 mo. | | [47] |
| | | 7 patients with pancreatic cancer | | These patients showed MUC1-specific immune responses; however, there was no significant clinical benefit. | | [48] |
| | DCs loaded with MHC class I restricted WT1 peptides | Phase I / II | 10 patients with pancreatic cancer | MUC1 specific immune responses were observed in 4 of 10 patients. | | [49] |
| | DCs transfected with MHC class I restricted WT1 peptides | Retrospective analysis | 255 patients with pancreatic cancer refractory to standard treatment | The median survival time from diagnosis was 16.5 mo. Erythema reaction at the vaccination site was a prognostic factor for a significant survival benefit. | | [60] |
| | DCs loaded with MHC class I restricted WT1 peptides | Phase I | 10 patients with pancreatic cancer | The therapy was feasible, tolerable and effective in PDA patients without liver metastases. | | [61] |
| | DCs loaded with MHC class I and class II restricted WT1 peptides | Phase I | 7 patients with pancreatic cancer | WT1 peptide-specific delayed-type hypersensitivity (DTH) was detected in 4 of 7 patients with PDA vaccinated with DC/WT1-I/II and in 0 of 3 patients with PDA vaccinated with DC/WT1-I or DC/WT1-II. All 3 PDA patients with strong WT1-specific DTH reactions had a median OS of 717 d. A patient with multiple liver metastases has remained alive for over 1000 d and received more than 71 vaccinations. | | [31,62,63] |
| hTERT | DCs transfected with hTERT mRNA | Phase I | A patient who could not receive chemotherapy due to severe neutropenia | Vaccination was associated with induction of strong immune responses to multiple hTERT epitopes. The patient had been vaccinated with DC/hTERT mRNA alone for 3 yr and resulted in no evidence of active disease. | | [66] |
| CEA | DCs loaded with CEA mRNA | Phase I | 3 patients with resected pancreatic cancer following neoadjuvant vaccine therapy | Intratumoral DC injections were guided by ultrasound. Vaccines induced a significantly increased infiltration of CD8+ T cells in some patients. A partial response was observed in 1 of 3 patients. | | [68] |
| | DCs transfected with an adenovirus encoding IL-12 | Phase I | 3 patients with pancreatic cancer | Intratumoral injection of OK432-activated DCs, followed by intravenous infusion of CD3-stimulated LAK cells. One patient had a partial response and 2 had stable disease for over 6 mo. The median OS was 478 d. Only one patient showed a positive cellular immune response. The worse prognosis of PDA patients on this immunization protocol using PBMCs as APCs may be associated with impaired induction of an antitumor immune responses. | | [70] |
| penicillin-killed and lyophilized preparations of a low virulence strain (Su) of Streptococcus pyogenes (OK-432)-activated DCs and CD3-stimulated LAK cells | K-ras irradiated PBMCs were used as antigen-presenting cells and loaded with K-ras peptide | Phase I | 5 patients with pancreatic cancer | Intratumoral injection of OK432-activated DCs, followed by intravenous infusion of CD3-stimulated LAK cells. One patient had a partial response and 2 had stable disease for over 6 mo. The median OS was 478 d. Only one patient showed a positive cellular immune response. The worse prognosis of PDA patients on this immunization protocol using PBMCs as APCs may be associated with impaired induction of an antitumor immune responses. | | [71] |
| Peripheral blood mononuclear cells (PBMCs) | 9 patients with pancreatic cancer | | | | |

MHC: Major histocompatibility complex; PDA: Pancreatic ductal adenocarcinoma; APC: Antigen-presenting cells; IL-12: Interleukin-12; WT1: Wilms’ tumor gene 1; MUC1: Mucin 1; hTERT: Human telomerase reverse transcriptase; CEA: Carcinoembryonic antigen.

Based cancer vaccination regimen. MUC1 is a TAA consisting of a polymorphic, glycosylated type I transmembrane protein present in glandular epithelium and overexpressed in 90% of PDAs. Importantly, MUC1 is associated with poor prognosis, enhanced metastasis and chemoresistance[44,46]. It has been reported MUC1-
targeted cancer vaccines were effective in inducing antitumor immunity in murine pancreatic cancer models\(^{30}\). Therefore, several groups have conducted clinical trials with DCs loaded with MUC1 peptide (DCs/MUC1 peptide) or transfected with MUC1 cDNA (DCs/MUC1 cDNA). In a phase I/II clinical trial, following surgical resection, 12 patients with pancreatic or biliary cancer were vaccinated with MUC1 peptide-loaded DCs. These patients were followed for more than 4 years after vaccination, at which point 4 were alive and without recurrence\(^{46}\). In another phase I study of 16 patients with PDA who were vaccinated with DCs/MUC1 peptide, 2 of 15 patients with resected PDA were alive and disease free at 32 or 61 mo\(^{67}\). Moreover, 7 PDA patients were vaccinated with DCs/MUC1 peptide in a phase I trial\(^{46}\); these patients showed MUC1-specific immune responses, although there was no significant clinical benefit. MUC1-specific immune responses were also observed in 4 of 10 PDA patients following vaccination with DCs/MUC1 cDNA in a phase I/II trial\(^{49}\). Although the MUC1-targeted DC-based cancer vaccination regimen was safe and a significant MUC1-specific immune response was observed in several enrolled PDA patients, further investigation is warranted.

**WT1**

The WT1 antigen is also one of the most widely expressed TAAs in various tumor types, including PDA\(^{31,51}\). Importantly, WT1 has been ranked by the National Cancer Institute (NCI) as the number 1 target for cancer vaccines based on several factors: (1) therapeutic function; (2) immunogenicity; (3) the role of the antigen in oncogenesis; (4) specificity; (5) the expression level and percentage of antigen-positive cells; (6) stem cell expression; (7) the number of patients with antigen-positive cancers; (8) the number of antigenic epitopes; and (9) the cellular location of antigen expression\(^{52}\). WT1 has been found to be oncogenic, rather than tumor suppressive, in tumorigenesis\(^{53}\). Moreover, both cellular and humoral immune responses against the WT1 protein are naturally elicited in cancer patients, indicating that the WT1 gene product is highly immunogenic\(^{54,55}\). Therefore, we and other groups have been performing clinical trials of the efficacy of WT1-targeted cancer vaccines for patients with PDA\(^{31,56-63}\). Four clinical reports about the use of DCs loaded with WT1 peptides combined with standard chemotherapy, such as gemcitabine, to treat advanced PDA patients have been published\(^{31,56,60,61}\). The vaccines can be mainly classified into 2 groups: (1) DCs loaded with MHC class I-restricted WT1 peptides (DC/WT1-I\(^{56,60,61}\)) and (2) DCs loaded with multiple MHC class I- and class II-restricted WT1 peptides (DC/WT1-I/II\(^{51}\)). Both DC/WT1-I and DC/WT1-I/II vaccinations are associated with significant induction of WT1-specific CD8+ T cells in circulating blood. In one study, Kobayashi et al\(^{60}\) analyzed 255 PDA patients who received standard chemotherapy combined with DC-based cancer vaccines, including DC/WT1-I. The median survival time (MST) from diagnosis was 16.5 mo. Interestingly, an erythema reaction at the vaccination site was a prognostic factor for a significant survival benefit. DC/WT1-I-based cancer vaccines alone or combined with lymphokine-activated killer (LAK) cells were also retrospectively analyzed in 49 PDA patients\(^{66}\). Among all 49 patients, 2 had complete remission, 5 had a partial response, and 10 had stable disease. The survival of patients receiving DC-based cancer vaccines and standard chemotherapy (gemcitabine and/or S-1, an oral fluoropyridine) plus LAK cells was significantly longer than the survival of those receiving the vaccine in combination with chemotherapy but no LAK cells. Moreover, a prospective clinical trial using DC/WT1-I combined with gemcitabine demonstrated that the therapy was feasible, tolerable and effective in PDA patients without liver metastases\(^{61}\). We also conducted a phase I study of chemoinmunotherapy using DC/WT1-I/II vaccines and standard chemotherapy (gemcitabine and/or S-1) in 7 advanced PDA patients\(^{31,57,62}\). The combination therapy was well tolerated, and WT1-specific IFN-γ-producing CD4+ and CD8+ T cells were significantly increased following treatment with DC/WT1-I/II. WT1 peptide-specific delayed-type hypersensitivity (DTH) was detected in 4 of the 7 patients with PDA who were vaccinated with DC/WT1-I/II and in 0 of the 3 patients with PDA who were vaccinated with DC/WT1-I or DCs loaded with MHC class II-restricted WT1 peptides (DC/WT1-II). Moreover, the MST and the median progression-free survival (PFS) of the patients with PDA who were vaccinated with DC/WT1-I/II were significantly longer than the MST and PFS of those receiving the DC/WT1-I or DC/WT1-II vaccine. In addition, the WT1-specific DTH-positive patients who received DC/WT1-I/II showed significantly improved overall survival (OS) and PFS compared with the negative-control patients. In particular, all 3 PDA patients with strong WT1-specific DTH reactions had a median OS of 717 d. Surprisingly, a patient with multiple liver metastases remained alive for more than 1000 d and received more than 71 vaccinations; this patient had strong WT1-specific DTH reactions throughout the vaccination period\(^{63}\). The combination of DC/WT1-I/II and chemotherapy induced long-term WT1-specific CD4+ and CD8+ T cell responses. DC/WT1-I/II may elicit not only effector but also long-lived effector memory and central memory T cells, all of which are capable of recognizing WT1-positive PDA cells and which are therefore associated with long-term stable disease\(^{57}\).

**hTERT**

hTERT, the catalytic subunit of a functional telomerase complex, is also widely expressed in most human tumors and plays an essential role in tumor progre-
sston. Therapeutic strategies targeting such antigens involved in tumor growth resulted in antitumor immune responses in a mouse study. As loss of telomerase activity may inhibit the progression of PDA cells, hTERT is a widely applicable target for triggering CTL responses. It was demonstrated that hTERT-specific immune responses were safely induced in a PDA patient vaccinated with DCs transfected with hTERT mRNA (DCs/hTERT mRNA). In this clinical study, DCs/hTERT mRNA vaccination was specifically administered to a PDA patient with relapsed disease. The patient could not receive chemotherapy due to severe neutropenia and thus was vaccinated with DCs/hTERT mRNA alone for 3 years, which resulted in no evidence of active disease. The vaccinated patient also showed induction of strong immune responses to multiple hTERT epitopes. Therefore, hTERT-targeted DC-based cancer vaccines may be an effective approach for treating patients with PDA.

CEA
PDA cells widely express CEA, a glycosylated protein, so induction of CEA-specific immune responses may be associated with survival benefits. In one clinical trial, 3 patients with resected PDA received neoadjuvant therapy, including DCs loaded with CEA mRNA (DCs/CEA mRNA), for 6 mo. In this trial, all 3 PDA patients showed injection site reactivity and remained alive and without recurrence at more than 5 years from the original diagnosis. Although CEA-targeted cancer vaccinations induce strong CEA-specific immune responses, they usually fail to eradicate the tumor in most patients with advanced disease. The results may be at least partly associated with the immunosuppressive effects of the tumor microenvironment. Therefore, to improve the clinical efficacy of CEA-targeted cancer vaccines, we need to design improved strategies that can overcome the immunosuppressive tumor microenvironment.

KRAS
As the KRAS gene is mutated in up to 95% of PDA cells, targeting mutant K-ras-specific immune responses may influence the clinical benefits of treatment for PDA patients. To induce K-ras-specific antitumor immunity, irradiated peripheral blood mononuclear cells (PBMCs) were used as APCs and loaded with a K-ras epitope. In this clinical trial, 9 patients with PDA, all with KRAS mutations, were vaccinated. Only one patient showed a positive cellular immune response, resulting in a median OS of 60 d. The worse prognosis of PDA patients subjected to an immunization protocol using PBMCs as APCs may be associated with impaired induction of antitumor immune responses per se. The vaccination protocol could be improved using mature DCs instead of PBMCs.

DCs combination therapy
The major cytokines currently in use or under evaluation for use in cancer vaccines are IFN-α, IL-2, GM-CSF, and IL-12. An alternative strategy for clinical trials of DC-based cancer vaccines is use of IL-12-secreting DCs. The main source of IL-12 in humans is DCs, and IL-12 acts as a major orchestrator of the Th1-type immune response against cancer when present directly in the tumor. Therefore, 3 PDA patients were vaccinated with DCs transfected with an adenovirus encoding the IL-12 gene (DCs/IL-12). The intratumoral DC injections were mainly guided by ultrasound. DCs/IL-12 induced significantly increased infiltration of CD8+ T cells in certain patients, and a partial response was observed in 1 of the 3 patients with PDA. As the DCs were not loaded with TAAs, cross-presentation of TAAs by the DCs in the patients must have been induced by IL-12. Another group reported administering gemcitabine and an endoscopic ultrasound-guided fine-needle injection of OK432-activated DCs into tumors in 5 PDA patients, followed by intravenous infusion of CD3-stimulated LAK cells. Three of the 5 patients demonstrated effective responses: 1 had a partial response, and 2 had long-term stable disease for more than 6 mo. The median OS was 478 d in this phase I trial. In the patient with partial remission, induction of tumor antigen-specific CTLs was observed.

WHOLE TUMOR CELL-BASED CANCER VACCINES
Whole tumor cells can be genetically modified to produce cytokines to enhance antitumor responses. A GM-CSF-secreting, irradiated, allogeneic PDA cell line (GVAX) has been investigated in multiple phase I and II studies (Table 3). GVAX recruits and activates DCs and promotes presentation of TAAs by DCs for activation of CD4+ and CD8+ T cells. Early clinical trials demonstrated that vaccination with GVAX enhances CD8+ CTL responses against multiple mesothelin-specific epitopes that have been correlated with survival benefits. As cancer vaccines alone have usually failed to demonstrate significant clinical activity in advanced PDA patients, PDAs are considered as non-immunogenic tumors, which is due to the immunosuppressive tumor microenvironment. Recently, 39 PDA patients received GVAX alone or in combination with low-dose cyclophosphamide (Cy) to deplete Tregs. Importantly, 33 of the 39 patients treated with GVAX showed the formation of vaccine-induced lymphoid aggregates. Moreover, the post-GVAX CTL infiltration and aggregate formation resulted in up-regulation of immunosuppressive regulatory mechanisms, including the PD-1/PD-L1 pathway. Therefore, GVAX-vaccinated PDA patients are better...
candidates for immune checkpoint therapies than vaccine-naïve patients\(^{[29]}\). In a mouse study, a GVAX vaccine combined with anti-PD-1 antibody blockade improved murine survival compared with anti-PD-1 antibody or GVAX alone\(^{[86]}\). In a clinical trial, although GVAX alone also failed to show clinical benefits in PDA patients, infiltration of activated T cells expressing CTLA-4 associated antigen 4 (CTLA-4) and PD-1 was induced by GVAX\(^{[80]}\). The efficiency of immune checkpoint-targeting agents is dependent on induction of adaptive immune responses\(^{[86]}\). Thus, they conducted combination therapy with inhibition of the CTLA-4 pathway using ipilimumab (anti-CTLA-4) and GVAX in metastatic PDA patients\(^{[29]}\). Three of 15 patients had evidence of prolonged disease stabilization (31, 71, or 81 wk), and 7 patients experienced a decline in carbohydrate antigen 19-9 (CA19-9). In 2 of these patients, disease stabilization occurred after an initial period of progression. The median OS was 5.7 mo and 1 yr OS was 27%. Among patients with OS > 4.3 mo, there was an increase in the peak mesothelin-specific T cells and enhancement of the T-cell repertoire. In a mouse study, several strategies to down-regulate immunosuppressive mechanisms, so down-regulation of TGF-β activates DCs and increases TAA-specific CTL induction. In mouse studies, several strategies to inhibit the production of TGF-β by cancer cells were developed. For example, TGF-β1 production by cancer cells was inhibited by the administration of neutralizing antibodies\(^{[87,88]}\) and small interfering RNAs (siRNAs)\(^{[89]}\) or constructs coding for a soluble variant of the TGF-β receptor\(^{[90]}\). We have previously demonstrated that the production of TGF-β1, IL-10 and VEGF by human PDA cells is significantly limited upon exposure to anti-PD-L1 antibody therapy with cancer vaccines such as GVAX may be effective therapy for PDA patients. In addition, they demonstrated that GVAX coupled with low-dose Cy followed by treatment with CRS-207 (live-attenuated Listeria monocytogenes expressing mesothelin) induced innate and adaptive immunity in 61 PDA patients. Mesothelin-specific CD8+ CTL responses enhanced by GVAX/Cy/CRS-207 were associated with longer OS (n = 61, 9.7 mo) compared with the responses enhanced by GVAX/Cy (n = 29, 4.6 mo)\(^{[81]}\).

**Table 3 Clinical trials of whole tumor cell-based cancer vaccines in pancreatic cancer patients**

| Cell-based cancer vaccines | Vaccine  | Phase | Patients | Results | Ref.  |
|---------------------------|---------|-------|----------|---------|-------|
| Whole tumor cell          | GM-CSF-secreting allogeneic pancreatic cancer cell lines (GVAX) and chemoradiotherapy | Phase II | 14 patients with resected pancreatic cancer | 3 patients were disease free at least 25 mo after diagnosis | [76] |
|                           | GVAX (arm A)/GM-CSF vaccine and cyclophosphamide (arm B) | Phase II | 50 patients with pancreatic cancer (2 arm) | Median OS: 2.3 mo in arm A, 4.3 mo in arm B | [77] |
|                           | GVAX and chemoradiotherapy | Phase II | 60 patients with resected pancreatic cancer | Induction of mesothelin-specific CD8+ T cells correlated with disease-free survival. Median OS: 24.8 mo | [78] |
| Ipilimumab (anti-CTLA-4 monoclonal antibody) alone (arm 1), Ipilimumab and GVAX (arm 2) | Phase II | 30 patients with pancreatic cancer (2 arm: 1:1) | Three of 15 patients had evidence of prolonged disease stabilization (31, 71, and 81 wk) and 7 patients experienced CA19-9 declines (arm 1). In 2 of these patients, disease stabilization occurred after an initial period of progression. The median OS was 5.7 mo and 1 yr OS was 27%. Among patients with OS > 4.3 mo, there was an increase in the peak mesothelin-specific T cells and enhancement of the T-cell repertoire. | [79] |
| GVAX                      | Phase II | 39 patients with pancreatic cancer | GVAX treatment was associated with the formation of vaccine-induced intratumoral tertiary lymphoid aggregates in 33 of 39 patients. Enhanced CD8+ CTL responses against multiple mesothelin-specific epitopes that have been correlated with survival benefits were also found. | [80] |
| GVAX with low-dose cyclophosphamide (Cy) followed by CRS-207 (live-attenuated Listeria monocytogenes-expressing mesothelin) (arm A), GVAX + Cy (arm B) | Phase II | 90 patients with pancreatic cancer | Enhanced mesothelin-specific CD8+ CTL responses were associated with longer OS. Median OS was 9.7 mo (arm A, n = 61). | [81] |
| Algenpantucel-L (2 pancreatic cancer cell lines that have been modified to express alpha-gal) | Phase II | 62 patients with resected pancreatic cancer | The 12-mo disease-free survival was 62%, and the 12-mo overall survival was 86%; the phase III study is ongoing. | [82] |
pharmaceutical-grade ethanol, without decreased expression of MHC class I and MUC1. Therefore, whole tumor cells genetically modified to express immunosuppressive cytokines, such as GM-CSF, and to inhibit immunosuppressive cytokines, such as TGF-β, are better candidates for the generation of DC-based cancer vaccines for PDA patients.

CELL-BASED CANCER VACCINES COMBINED WITH CHEMOTHERAPY

Cytotoxic chemotherapy has been known to blunt immune responses because of the toxic effects of these treatments on dividing bone marrow progenitor cells, including lymphocytes. However, increasing evidence has suggested that cancer vaccines have the possibility of achieving better effects if combined with chemotherapy. Cancer cells undergoing immunogenic apoptosis due to chemotherapy express calreticulin (CRT), which is a Ca2+-binding chaperone on the cell surface that mediates efficient phagocytosis by DCs. In addition, high-mobility group box 1 (HMGB1) and pentraxin-3 (PTX3) are released from late-stage dying cancer cells to activate DCs and modulate immune responses via a TLR4-dependent signaling pathway. Therefore, necrotic or apoptotic tumor cells induced by chemotherapeutic agents enhance immunogenicity and can be effectively taken up by DCs, resulting in efficient processing of TAAs for presentation to T cells. For example, a standard cytotoxic agent for PDA, gemcitabine, can enhance the cross-presentation of TAAs by DCs as well as CTL induction. Moreover, Cy and gemcitabine can each augment the antitumor effects by depleting immunosuppressive cells such as Tregs, B cells and MDSCs as well as by inducing the proliferation of DCs, all of which potentially enhances the antitumor immune response. We also reported that up-regulated WT1 peptide via MHC class I molecules on PDA cells is induced by exposure of the cells to gemcitabine and/or S-1. Importantly, WT1-specific CTLs can more efficiently lyse gemcitabine-treated PDA cells than untreated cells. Certain TAAs that are not usually expressed on cancer cells may be uncovered by treating cancer cells with chemotherapeutic agents; these antigens are good targets for cancer vaccines because they can be effectively recognized by antigen-specific CTLs. Therefore, cancer vaccines can synergize with chemotherapy in targeting PDA cells. In addition, our recent reports indicate that the combination of gemcitabine and trastuzumab conjugated to a cytotoxic agent (T-DM1) may be a promising modality for the treatment of PDA cells with low human epidermal growth factor 2 (HER2) expression as a result of the unique HER2-up-regulating effect of gemcitabine. Importantly, cancer patients who have previously received cancer vaccines could also benefit more from subsequent chemotherapy than those patients who are not vaccinated.

Although conventional treatments such as chemotherapy can eradicate certain cancer cells, the remaining cancer stem cells (CSCs) can lead to tumor relapse. Although CSCs have been implicated in chemoresistance, these remaining CSCs are still attractive targets for cancer vaccines. Therefore, it is desirable to develop a novel therapy that selectively targets CSCs via cancer vaccines, which can be combined with conventional chemotherapy. Indeed, expression of TAAs such as MUC1 is up-regulated in CSCs by chemotherapy, and CSCs are efficiently lysed by MUC1-specific CTLs. CSC-loaded DC-based cancer vaccines may be an alternative approach. We have reported that DCs fused with CSC cells induced CSC-specific CD4+ and CD8+ T cells with high production of IFN-γ, which is predominantly produced by Th1 cells. Therefore, developing surgery/chemotherapy targeting the bulk of cancer cells combined with cell-based cancer vaccines targeting CSCs is highly desirable.

CONCLUSION

CTLA-4 and PD-1 are well-described co-inhibitory molecules that are highly expressed by TAAs-specific CTLs and associated with impaired antitumor immune responses. In contrast, PD-L1, which binds to PD-1, is not constitutively expressed in tumor cells but is induced in response to IFN-γ produced by activated T cells. Therefore, immune checkpoint inhibitors, such as CTLA-4, PD-1 and anti-PD-L1 antibody, may be an efficient means for treating cancer patients. Indeed, antibodies can be used to block inhibitory ligand/receptor interactions by acting on certain cancer cells (e.g., anti-PD-L1) or T cells (e.g., anti-CTLA-4 or anti-PD-1), allowing enhancement of the antitumor immune response in specific tumors. However, single-agent immune checkpoint inhibitors, such as CTLA-4, PD-1, and anti-PD-L1 antibody, elicit limited adoptive immune responses in PDA patients due to the non-immunogenic tumor microenvironment, which provides a formidable barrier to CTL infiltration at baseline. Therefore, cell-based cancer vaccines may prime PDA patients for treatment with better candidate checkpoint inhibitors. Combining a blockade of multiple inhibitory pathways with cell-based cancer vaccines may synergistically decrease T cell anergy and improve clinical benefits.

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