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TOPIC HIGHLIGHT

2016 Pancreatic Cancer: Global view

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.

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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 (FOLFIRINOX) 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], carcinoembryonic 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

Table 1 Cell-based cancer vaccines

Cell	Antigen source	Ref.
Dendritic cells	Whole tumor cell lysates	[33]
	MHC class Ⅰ restricted antigenic peptides	[33,56,60,61]
	MHC class Ⅰ and Ⅱ restricted antigenic peptides	[31,57,62]
	Dying or dead tumor cells	[34]
	mRNA encoding tumor associated antigens	[35,36]
	cDNA	[37]
	Exosomes	[38]
	Fusions generated with whole tumor cells	[39-43]
Immunogenic whole tumor cells	A GM-CSF-secreting, irradiated, allogeneic PDA cell line	[76-82]
Cancer stem cells	Cancer stem-like cell-associated antigens	[107-109]

GM-CSF: Granulocyte macrophage colony-stimulating factor; PDA: Pancreatic ductal adenocarcinoma; MHC: Major histocompatibility complex.

cell-based cancer vaccines, such as either DCs loaded with TAAs or modified whole tumor cells, is essential to 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 [*e.g.*, transforming growth factor (TGF)-β, 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-β 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[28]; and (4) DCs may have impaired function in patients with advanced PDA[29]. Therefore, *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 monocyte-derived 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-killed and lyophilized preparations of a low-virulence strain (Su) of *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 *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]
			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]
			Phase Ⅰ	7 patients with pancreatic cancer	These patients showed MUC1-specific immune responses; however, there was no significant clinical benefit.	[48]
		DCs transfected with MUC1 cDNA	Phase Ⅰ/Ⅱ	10 patients with pancreatic cancer	MUC1 specific immune responses were observed in 4 of 10 patients.	[49]
	WT1	DCs loaded with MHC class Ⅰ restricted WT1 peptides	Retrospective analysis	49 patients with pancreatic cancer refractory to standard treatment	The median survival time from vaccines was 360 d. Erythema reaction at the vaccination site was a prognostic factor for a significant survival benefit.	[56]
		DCs loaded 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 sever 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	All 3 PDA patients showed injection site reactivity and remained alive without recurrence at more than 2.5 yr from the original diagnosis	[68]
	DCs transfected with an adenovirus encoding IL-12		Phase Ⅰ	3 patients with pancreatic cancer	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.	[73]
	penicillin-killed and lyophilized preparations of a low virulence strain (Su) of Streptococcus pyogenes (OK-432)-activated DCs and CD3-stimulated LAK cells		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.	[75]
Peripheral blood mononuclear cells (PBMCs)	K-ras	irradiated PBMCs were used as antigen-presenting cells and loaded with K-ras peptide	Phase Ⅰ	9 patients with pancreatic cancer	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]

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[9,44]. It has been reported MUC1-

targeted cancer vaccines were effective in inducing antitumor immunity in murine pancreatic cancer models[45]. 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[47]. Moreover, 7 PDA patients were vaccinated with DCs/MUC1 peptide in a phase Ⅰ trial[48]; 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[50,51]. 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 oncogenicity; (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)[31]. 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[56]. 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 chemoimmunotherapy 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-

ssion[64]. Therapeutic strategies targeting such antigens involved in tumor growth resulted in antitumor immune responses in a mouse study[65]. 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)[66]. In this clinical study, DCs/hTERT mRNA vaccination was specifically administered to a PDA patient with relapsed disease[67]. 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[67]. In one clinical trial, 3 patients with resected PDA received neoadjuvant therapy, including DCs loaded with CEA mRNA (DCs/CEA mRNA), for 6 mo[68]. In this trial, all 3 PDA patients showed injection site reactivity and remained alive and without recurrence at more than 2.5 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[67]. The results may be at least partly associated with the immunosuppressive effects of the tumor micro-environment. 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[69], targeting mutant K-ras-specific immune responses may influence the clinical benefits of treatment for PDA patients[70]. To induce K-ras-specific antitumor immunity, irradiated peripheral blood mononuclear cells (PBMCs) were used as APCs and loaded with a K-ras epitope[71]. 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[72]. An alternative strategy for clinical trials of DC-based cancer vaccines is use of IL-12-secreting DCs[73]. The main source of IL-12 in humans is DCs, and IL-12 acts as a major orchestrator of the T helper 1 (Th1)-type immune response against cancer when present directly in the tumor[74]. Therefore, 3 PDA patients were vaccinated with DCs transfected with an adenovirus encoding the IL-12 gene (DCs/IL-12)[73]. 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[73]. 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[75]. 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[75]. 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[76-82] (Table 3). GVAX recruits and activates DCs and promotes presentation of TAAs by DCs for activation of CD4+ and CD8+ T cells[83,84]. Early clinical trials demonstrated that vaccination with GVAX enhances CD8+ CTL responses against multiple mesothelin-specific epitopes that have been correlated with survival benefits[76-78]. 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[80]. Recently, 39 PDA patients received GVAX alone or in combination with low-dose cyclophosphamide (Cy) to deplete Tregs[80]. 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

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]

candidates for immune checkpoint therapies than vaccine-naive patients[79]. 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[85]. In a clinical trial, although GVAX alone also failed to show clinical benefits in PDA patients, infiltration of activated T cells expressing CTL-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[79]. 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-year OS was 27%. Among patients with OS > 4.3 mo, there was an increase in the peak mesothelin-specific T cell count and enhancement of the T cell repertoire[79]. Moreover, immunosuppressive pathways in the tumor microenvironment were overcome by the addition of the GVAX vaccine and low-dose Cy for PD-1 blockade. Therefore, combining anti-PD-1 or 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].

Whole tumor cells can be genetically modified to produce cytokines to inhibit tumor cell production of immunosuppressive cytokines, such as TGF-β, IL-10, IL-6, and VEGF. In particular, TGF-β has a critical role in 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-β 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-β, IL-10 and VEGF by human PDA cells is significantly limited upon exposure to

pharmaceutical-grade ethanol, without decreased expression of MHC class I and MUC1[91]. 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[92]. 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[93,94]. In addition, high-mobility group box 1 (HMGB1)[95,96] and pentraxin-3 (PTX3)[97] 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[98]. 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[98-101]. We also reported that up-regulated presentation of WT1 peptide *via* MHC class I molecules on PDA cells is induced by exposure of the cells to gemcitabine and/or S-1[102]. Importantly, WT1-specific CTLs can more efficiently lyse gemcitabine-treated PDA cells than untreated cells[102]. 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[103]. Therefore, cancer vaccines can synergize with chemotherapy in targeting PDA cells[104]. 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[105]. Importantly, cancer patients who have previously received cancer vaccines could also benefit more from subsequent chemotherapy than those patients who are not vaccinated[106].

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[107,108]. 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[108,109]. 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[108]. 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[110]. Therefore, immune checkpoint inhibitors, such as CTLA-4, PD-1 and anti-PD-L1 antibody, may be an efficient means for treating cancer patients[110]. 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[111]. However, single-agent immune checkpoint inhibitors, such as CTLA-4, PD-1, and anti-PD-L1 antibody, elicit limited adaptive immune responses in PDA patients due to the non-immunogenic tumor microenvironment, which provides a formidable barrier to CTL infiltration at baseline[85]. Therefore, cell-based cancer vaccines may prime PDA patients for treatment with better candidate checkpoint inhibitors[112]. Combining a blockade of multiple inhibitory pathways with cell-based cancer vaccines may synergistically decrease T cell anergy and improve clinical benefits.

REFERENCES

1 **Michl P**, Gress TM. Current concepts and novel targets in advanced pancreatic cancer. *Gut* 2013; **62**: 317-326 [PMID: 23112132 DOI: 10.1136/gutjnl-2012-303588]
2 **Arslan C**, Yalcin S. Current and future systemic treatment options in metastatic pancreatic cancer. *J Gastrointest Oncol* 2014; **5**: 280-295 [PMID: 25083302 DOI: 10.3978/j.issn.2078-6891.2014.030]
3 **Saif MW**. Advancements in the management of pancreatic

cancer: 2013. *JOP* 2013; **14**: 112-118 [PMID: 23474549 DOI: 10.6092/1590-8577/1481]
4 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010; **60**: 277-300 [PMID: 20610543 DOI: 10.3322/caac.20073]
5 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]
6 **Kordes S**, Pollak MN, Zwinderman AH, Mathôt RA, Weterman MJ, Beeker A, Punt CJ, Richel DJ, Wilmink JW. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Oncol* 2015; **16**: 839-847 [PMID: 26067687 DOI: 10.1016/s1470-2045(15)00027-3]
7 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]
8 **Oji Y**, Nakamori S, Fujikawa M, Nakatsuka S, Yokota A, Tatsumi N, Abeno S, Ikeba A, Takashima S, Tsujie M, Yamamoto H, Sakon M, Nezu R, Kawano K, Nishida S, Ikegame K, Kawakami M, Tsuboi A, Oka Y, Yoshikawa K, Aozasa K, Monden M, Sugiyama H. Overexpression of the Wilms' tumor gene WT1 in pancreatic ductal adenocarcinoma. *Cancer Sci* 2004; **95**: 583-587 [PMID: 15245594 DOI: 10.1111/j.1349-7006.2004.tb02490.x]
9 **Torres MP**, Chakraborty S, Souchek J, Batra SK. Mucin-based targeted pancreatic cancer therapy. *Curr Pharm Des* 2012; **18**: 2472-2481 [PMID: 22372499 DOI: 10.2174/13816128112092472]
10 **Seki K**, Suda T, Aoyagi Y, Sugawara S, Natsui M, Motoyama H, Shirai Y, Sekine T, Kawai H, Mita Y, Waguri N, Kuroiwa T, Igarashi M, Asakura H. Diagnosis of pancreatic adenocarcinoma by detection of human telomerase reverse transcriptase messenger RNA in pancreatic juice with sample qualification. *Clin Cancer Res* 2001; **7**: 1976-1981 [PMID: 11448913]
11 **Gjertsen MK**, Bakka A, Breivik J, Saeterdal I, Solheim BG, Søreide O, Thorsby E, Gaudernack G. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. *Lancet* 1995; **346**: 1399-1400 [PMID: 7475823 DOI: 10.1016/S0140-6736(95)92408-6]
12 **Wobser M**, Keikavoussi P, Kunzmann V, Weininger M, Andersen MH, Becker JC. Complete remission of liver metastasis of pancreatic cancer under vaccination with a HLA-A2 restricted peptide derived from the universal tumor antigen survivin. *Cancer Immunol Immunother* 2006; **55**: 1294-1298 [PMID: 16315030 DOI: 10.1007/s00262-005-0102-x]
13 **Yamamoto K**, Mine T, Katagiri K, Suzuki N, Kawaoka T, Ueno T, Matsueda S, Yamada A, Itoh K, Yamana H, Oka M. Immunological evaluation of personalized peptide vaccination for patients with pancreatic cancer. *Oncol Rep* 2005; **13**: 874-883 [PMID: 15809753 DOI: 10.3892/or.13.5.874]
14 **Komoto M**, Nakata B, Amano R, Yamada N, Yashiro M, Ohira M, Wakasa K, Hirakawa K. HER2 overexpression correlates with survival after curative resection of pancreatic cancer. *Cancer Sci* 2009; **100**: 1243-1247 [PMID: 19432892 DOI: 10.1111/j.1349-7006.2009.01176.x]
15 **Maacke H**, Kessler A, Schmiegel W, Roeder C, Vogel I, Deppert W, Kalthoff H. Overexpression of p53 protein during pancreatitis. *Br J Cancer* 1997; **75**: 1501-1504 [PMID: 9166944 DOI: 10.1038/bjc.1997.256]
16 **Boon T**, Coulie PG, Van den Eynde B. Tumor antigens recognized by T cells. *Immunol Today* 1997; **18**: 267-268 [PMID: 9190110]
17 **Steinman RM**. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; **9**: 271-296 [PMID: 1910679 DOI: 10.1146/annurev.iy.09.040191.001415]
18 **Berard F**, Blanco P, Davoust J, Neidhart-Berard EM, Nouri-Shirazi M, Taquet N, Rimoldi D, Cerottini JC, Banchereau J, Palucka AK. Cross-priming of naive CD8 T cells against melanoma antigens using dendritic cells loaded with killed allogeneic melanoma cells. *J Exp Med* 2000; **192**: 1535-1544 [PMID: 11104796 DOI: 10.1084/jem.192.11.1535]
19 **Melief CJ**. Cancer immunotherapy by dendritic cells. *Immunity* 2008; **29**: 372-383 [PMID: 18799145 DOI: 10.1016/j.immuni.2008.08.004]
20 **Hermans IF**, Ritchie DS, Yang J, Roberts JM, Ronchese F. CD8+ T cell-dependent elimination of dendritic cells in vivo limits the induction of antitumor immunity. *J Immunol* 2000; **164**: 3095-3101 [PMID: 10706699 DOI: 10.4049/jimmunol.164.6.3095]
21 **Marzo AL**, Kinnear BF, Lake RA, Frelinger JJ, Collins EJ, Robinson BW, Scott B. Tumor-specific CD4+ T cells have a major "post-licensing" role in CTL mediated anti-tumor immunity. *J Immunol* 2000; **165**: 6047-6055 [PMID: 11086036 DOI: 10.4049/jimmunol.165.11.6047]
22 **Koido S**, Homma S, Takahara A, Namiki Y, Tsukinaga S, Mitobe J, Odahara S, Yukawa T, Matsudaira H, Nagatsuma K, Uchiyama K, Satoh K, Ito M, Komita H, Arakawa H, Ohkusa T, Gong J, Tajiri H. Current immunotherapeutic approaches in pancreatic cancer. *Clin Dev Immunol* 2011; **2011**: 267539 [PMID: 21922022 DOI: 10.1155/2011/267539]
23 **Koido S**, Homma S, Hara E, Namiki Y, Takahara A, Komita H, Nagasaki E, Ito M, Ohkusa T, Gong J, Tajiri H. Regulation of tumor immunity by tumor/dendritic cell fusions. *Clin Dev Immunol* 2010; **2010**: 516768 [PMID: 21048993 DOI: 10.1155/2010/516768]
24 **Niccolai E**, Taddei A, Ricci F, Rolla S, D'Elios MM, Benagiano M, Bechi P, Bencini L, Ringressi MN, Pini A, Castiglione F, Giordano D, Satolli MA, Coratti A, Cianchi F, Bani D, Prisco D, Novelli F, Amedei A. Intra-tumoral IFN-γ-producing Th22 cells correlate with TNM staging and the worst outcomes in pancreatic cancer. *Clin Sci* (Lond) 2016; **130**: 247-258 [PMID: 26590104 DOI: 10.1042/cs20150437]
25 **Bazhin AV**, Shevchenko I, Umansky V, Werner J, Karakhanova S. Two immune faces of pancreatic adenocarcinoma: possible implication for immunotherapy. *Cancer Immunol Immunother* 2014; **63**: 59-65 [PMID: 24129765 DOI: 10.1007/s00262-013-1485-8]
26 **Amedei A**, Niccolai E, Benagiano M, Della Bella C, Cianchi F, Bechi P, Taddei A, Bencini L, Farsi M, Cappello P, Prisco D, Novelli F, D'Elios MM. Ex vivo analysis of pancreatic cancer-infiltrating T lymphocytes reveals that ENO-specific Tregs accumulate in tumor tissue and inhibit Th1/Th17 effector cell functions. *Cancer Immunol Immunother* 2013; **62**: 1249-1260 [PMID: 23640603 DOI: 10.1007/s00262-013-1429-3]
27 **Kanodia S**, Kast WM. Peptide-based vaccines for cancer: realizing their potential. *Expert Rev Vaccines* 2008; **7**: 1533-1545 [PMID: 19053209 DOI: 10.1586/14760584.7.10.1533]
28 **Waldmann TA**. Immunotherapy: past, present and future. *Nat Med* 2003; **9**: 269-277 [PMID: 12612576 DOI: 10.1038/nm0303-269]
29 **Koido S**, Hara E, Homma S, Namiki Y, Komita H, Takahara A, Nagasaki E, Ito M, Sagawa Y, Mitsunaga M, Uchiyama K, Satoh K, Arihiro S, Ohkusa T, Gong J, Tajiri H. Dendritic/pancreatic carcinoma fusions for clinical use: Comparative functional analysis of healthy- versus patient-derived fusions. *Clin Immunol* 2010; **135**: 384-400 [PMID: 20226739 DOI: 10.1016/j.clim.2010.02.003]
30 **Steinman RM**, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; **449**: 419-426 [PMID: 17898760 DOI: 10.1038/nature06175]
31 **Koido S**, Homma S, Okamoto M, Takakura K, Mori M, Yoshizaki S, Tsukinaga S, Odahara S, Koyama S, Imazu H, Uchiyama K, Kajihara M, Arakawa H, Misawa T, Toyama Y, Yanagisawa S, Ikegami M, Kan S, Hayashi K, Komita H, Kamata Y, Ito M, Ishidao T, Yusa S, Shimodaira S, Gong J, Sugiyama H, Ohkusa T, Tajiri H. Treatment with chemotherapy and dendritic cells pulsed with multiple Wilms' tumor 1 (WT1)-specific MHC class I/II-restricted epitopes for pancreatic cancer. *Clin Cancer Res* 2014; **20**: 4228-4239 [PMID: 25056373 DOI: 10.1158/1078-0432.

ccr-14-0314]
32 **Mackensen A**, Herbst B, Chen JL, Köhler G, Noppen C, Herr W, Spagnoli GC, Cerundolo V, Lindemann A. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. *Int J Cancer* 2000; **86**: 385-392 [PMID: 10760827 DOI: 10.1002/(SICI)1097-0215(20000501)86:3<385::AID-IJC13>3.3.CO;2-K]
33 **Nestle FO**, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998; **4**: 328-332 [PMID: 9500607]
34 **Palucka AK**, Ueno H, Connolly J, Kerneis-Norvell F, Blanck JP, Johnston DA, Fay J, Banchereau J. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. *J Immunother* 2006; **29**: 545-557 [PMID: 16971810 DOI: 10.1097/01.cji.0000211309.90621.8b]
35 **Nair SK**, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. *Nat Biotechnol* 1998; **16**: 364-369 [PMID: 9555728 DOI: 10.1038/nbt0498-364]
36 **Koido S**, Kashiwaba M, Chen D, Gendler S, Kufe D, Gong J. Induction of antitumor immunity by vaccination of dendritic cells transfected with MUC1 RNA. *J Immunol* 2000; **165**: 5713-5719 [PMID: 11067929 DOI: 10.4049/jimmunol.165.10.5713]
37 **Gong J**, Chen L, Chen D, Kashiwaba M, Manome Y, Tanaka T, Kufe D. Induction of antigen-specific antitumor immunity with adenovirus-transduced dendritic cells. *Gene Ther* 1997; **4**: 1023-1028 [PMID: 9415307 DOI: 10.1038/sj.gt.3300496]
38 **Pitt JM**, Charrier M, Viaud S, André F, Besse B, Chaput N, Zitvogel L. Dendritic cell-derived exosomes as immunotherapies in the fight against cancer. *J Immunol* 2014; **193**: 1006-1011 [PMID: 25049431 DOI: 10.4049/jimmunol.1400703]
39 **Gong J**, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nat Med* 1997; **3**: 558-561 [PMID: 9142127 DOI: 10.1038/nm0597-558]
40 **Koido S**, Ohana M, Liu C, Nikrui N, Durfee J, Lerner A, Gong J. Dendritic cells fused with human cancer cells: morphology, antigen expression, and T cell stimulation. *Clin Immunol* 2004; **113**: 261-269 [PMID: 15507391 DOI: 10.1016/j.clim.2004.08.004]
41 **Koido S**, Gong J. Characterization of structure and direct antigen presentation by dendritic/tumor-fused cells as cancer vaccines. *Anticancer Res* 2013; **33**: 347-354 [PMID: 23393323]
42 **Koido S**, Homma S, Okamoto M, Namiki Y, Takakura K, Uchiyama K, Kajihara M, Arihiro S, Imazu H, Arakawa H, Kan S, Komita H, Ito M, Ohkusa T, Gong J, Tajiri H. Fusions between dendritic cells and whole tumor cells as anticancer vaccines. *Oncoimmunology* 2013; **2**: e24437 [PMID: 23762810 DOI: 10.4161/onci.24437]
43 **Koido S**, Homma S, Okamoto M, Namiki Y, Takakura K, Uchiyama K, Kajihara M, Arihiro S, Imazu H, Arakawa H, Kan S, Komita H, Kamata Y, Ito M, Ohkusa T, Gong J, Tajiri H. Strategies to improve the immunogenicity of anticancer vaccines based on dendritic cell/malignant cell fusions. *Oncoimmunology* 2013; **2**: e25994 [PMID: 24228229 DOI: 10.4161/onci.25994]
44 **Xu H**, Inagaki Y, Seyama Y, Du G, Wang F, Kokudo N, Tang W. Expression of KL-6/MUC1 in pancreatic cancer tissues and its potential involvement in tumor metastasis. *Oncol Rep* 2011; **26**: 371-376 [PMID: 21617869 DOI: 10.3892/or.2011.1315]
45 **Mukherjee P**, Ginardi AR, Madsen CS, Sterner CJ, Adriance MC, Tevethia MJ, Gendler SJ. Mice with spontaneous pancreatic cancer naturally develop MUC-1-specific CTLs that eradicate tumors when adoptively transferred. *J Immunol* 2000; **165**: 3451-3460 [PMID: 10975866 DOI: 10.4049/jimmunol.165.6.3451]
46 **Lepisto AJ**, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, Geller BA, Schmotzer A, Potter DP, Whiteside T, Finn OJ, Ramanathan RK. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. *Cancer Ther* 2008; **6**: 955-964 [PMID: 19129927]
47 **Ramanathan RK**, Lee KM, McKolanis J, Hitbold E, Schraut W, Moser AJ, Warnick E, Whiteside T, Osborne J, Kim H, Day R, Troetschel M, Finn OJ. Phase I study of a MUC1 vaccine composed of different doses of MUC1 peptide with SB-AS2 adjuvant in resected and locally advanced pancreatic cancer. *Cancer Immunol Immunother* 2005; **54**: 254-264 [PMID: 15372205 DOI: 10.1007/s00262-004-0581-1]
48 **Rong Y**, Qin X, Jin D, Lou W, Wu L, Wang D, Wu W, Ni X, Mao Z, Kuang T, Zang YQ, Qin X. A phase I pilot trial of MUC1-peptide-pulsed dendritic cells in the treatment of advanced pancreatic cancer. *Clin Exp Med* 2012; **12**: 173-180 [PMID: 21932124 DOI: 10.1007/s10238-011-0159-0]
49 **Pecher G**, Häring A, Kaiser L, Thiel E. Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial. *Cancer Immunol Immunother* 2002; **51**: 669-673 [PMID: 12439613 DOI: 10.1007/s00262-002-0317-z]
50 **Oji Y**, Ogawa H, Tamaki H, Oka Y, Tsuboi A, Kim EH, Soma T, Tatekawa T, Kawakami M, Asada M, Kishimoto T, Sugiyama H. Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Jpn J Cancer Res* 1999; **90**: 194-204 [PMID: 10189890]
51 **Nakatsuka S**, Oji Y, Horiuchi T, Kanda T, Kitagawa M, Takeuchi T, Kawano K, Kuwae Y, Yamauchi A, Okumura M, Kitamura Y, Oka Y, Kawase I, Sugiyama H, Aozasa K. Immunohistochemical detection of WT1 protein in a variety of cancer cells. *Mod Pathol* 2006; **19**: 804-814 [PMID: 16547468 DOI: 10.1038/modpathol.3800588]
52 **Cheever MA**, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res* 2009; **15**: 5323-5337 [PMID: 19723653 DOI: 10.1158/1078-0432.ccr-09-0737]
53 **Sugiyama H**. Cancer immunotherapy targeting Wilms' tumor gene WT1 product. *Expert Rev Vaccines* 2005; **4**: 503-512 [PMID: 16117707 DOI: 10.1586/14760584.4.4.503]
54 **Oka Y**, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, Elisseeva OA, Oji Y, Kawakami M, Ikegame K, Hosen N, Yoshihara S, Wu F, Fujiki F, Murakami M, Masuda T, Nishida S, Shirakata T, Nakatsuka S, Sasaki A, Udaka K, Dohy H, Aozasa K, Noguchi S, Kawase I, Sugiyama H. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci USA* 2004; **101**: 13885-13890 [PMID: 15365188 DOI: 10.1073/pnas.0405884101]
55 **Oji Y**, Kitamura Y, Kamino E, Kitano A, Sawabata N, Inoue M, Mori M, Nakatsuka S, Sakaguchi N, Miyazaki K, Nakamura M, Fukuda I, Nakamura J, Tatsumi N, Takakuwa T, Nishida S, Shirakata T, Hosen N, Tsuboi A, Nezu R, Maeda H, Oka Y, Kawase I, Aozasa K, Okumura M, Miyoshi S, Sugiyama H. WT1 IgG antibody for early detection of nonsmall cell lung cancer and as its prognostic factor. *Int J Cancer* 2009; **125**: 381-387 [PMID: 19384943 DOI: 10.1002/ijc.24367]
56 **Kimura Y**, Tsukada J, Tomoda T, Takahashi H, Imai K, Shimamura K, Sunamura M, Yonemitsu Y, Shimodaira S, Koido S, Homma S, Okamoto M. Clinical and immunologic evaluation of dendritic cell-based immunotherapy in combination with gemcitabine and/or S-1 in patients with advanced pancreatic carcinoma. *Pancreas* 2012; **41**: 195-205 [PMID: 21792083 DOI: 10.1097/MPA.0b013e31822398c6]
57 **Koido S**, Homma S, Okamoto M, Takakura K, Gong J, Sugiyama H, Ohkusa T, Tajiri H. Chemoimmunotherapy targeting Wilms' tumor 1 (WT1)-specific cytotoxic T lymphocyte and helper T cell responses for patients with pancreatic cancer. *Oncoimmunology* 2014; **3**: e958950 [PMID: 25941581 DOI: 10.4161/21624011.2014.958950]
58 **Nishida S**, Koido S, Takeda Y, Homma S, Komita H, Takahara A, Morita S, Ito T, Morimoto S, Hara K, Tsuboi A, Oka Y, Yanagisawa S, Toyama Y, Ikegami M, Kitagawa T, Eguchi H, Wada H, Nagano

H, Nakata J, Nakae Y, Hosen N, Oji Y, Tanaka T, Kawase I, Kumanogoh A, Sakamoto J, Doki Y, Mori M, Ohkusa T, Tajiri H, Sugiyama H. Wilms tumor gene (WT1) peptide-based cancer vaccine combined with gemcitabine for patients with advanced pancreatic cancer. *J Immunother* 2014; **37**: 105-114 [PMID: 24509173 DOI: 10.1097/CJI.0000000000000020]

59 **Kaida M**, Morita-Hoshi Y, Soeda A, Wakeda T, Yamaki Y, Kojima Y, Ueno H, Kondo S, Morizane C, Ikeda M, Okusaka T, Takaue Y, Heike Y. Phase 1 trial of Wilms tumor 1 (WT1) peptide vaccine and gemcitabine combination therapy in patients with advanced pancreatic or biliary tract cancer. *J Immunother* 2011; **34**: 92-99 [PMID: 21150717 DOI: 10.1097/CJI.0b013e3181fb65b9]

60 **Kobayashi M**, Shimodaira S, Nagai K, Ogasawara M, Takahashi H, Abe H, Tanii M, Okamoto M, Tsujitani S, Yusa S, Ishidao T, Kishimoto J, Shibamoto Y, Nagaya M, Yonemitsu Y. Prognostic factors related to add-on dendritic cell vaccines on patients with inoperable pancreatic cancer receiving chemotherapy: a multicenter analysis. *Cancer Immunol Immunother* 2014; **63**: 797-806 [PMID: 24777613 DOI: 10.1007/s00262-014-1554-7]

61 **Mayanagi S**, Kitago M, Sakurai T, Matsuda T, Fujita T, Higuchi H, Taguchi J, Takeuchi H, Itano O, Aiura K, Hamamoto Y, Takaishi H, Okamoto M, Sunamura M, Kawakami Y, Kitagawa Y. Phase I pilot study of Wilms tumor gene 1 peptide-pulsed dendritic cell vaccination combined with gemcitabine in pancreatic cancer. *Cancer Sci* 2015; **106**: 397-406 [PMID: 25614082 DOI: 10.1111/cas.12621]

62 **Takakura K**, Koido S, Kan S, Yoshida K, Mori M, Hirano Y, Ito Z, Kobayashi H, Takami S, Matsumoto Y, Kajihara M, Misawa T, Okamoto M, Sugiyama H, Homma S, Ohkusa T, Tajiri H. Prognostic markers for patient outcome following vaccination with multiple MHC Class I/II-restricted WT1 peptide-pulsed dendritic cells plus chemotherapy for pancreatic cancer. *Anticancer Res* 2015; **35**: 555-562 [PMID: 25550602]

63 **Tsukinaga S**, Kajihara M, Takakura K, Ito Z, Kanai T, Saito K, Takami S, Kobayashi H, Matsumoto Y, Odahara S, Uchiyama K, Arakawa H, Okamoto M, Sugiyama H, Sumiyama K, Ohkusa T, Koido S. Prognostic significance of plasma interleukin-6/-8 in pancreatic cancer patients receiving chemoimmunotherapy. *World J Gastroenterol* 2015; **21**: 11168-11178 [PMID: 26494971 DOI: 10.3748/wjg.v21.i39.11168]

64 **Beatty GL**, Vonderheide RH. Telomerase as a universal tumor antigen for cancer vaccines. *Expert Rev Vaccines* 2008; **7**: 881-887 [PMID: 18767939 DOI: 10.1586/14760584.7.7.881]

65 **Adotévi O**, Mollier K, Neuveut C, Dosset M, Ravel P, Fridman WH, Tartour E, Charneau P, Wain-Hobson S, Langlade-Demoyen P. Targeting human telomerase reverse transcriptase with recombinant lentivector is highly effective to stimulate antitumor CD8 T-cell immunity in vivo. *Blood* 2010; **115**: 3025-3032 [PMID: 20130242 DOI: 10.1182/blood-2009-11-253641]

66 **Suso EM**, Dueland S, Rasmussen AM, Vetrhus T, Aamdal S, Kvalheim G, Gaudernack G. hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes. *Cancer Immunol Immunother* 2011; **60**: 809-818 [PMID: 21365467 DOI: 10.1007/s00262-011-0991-9]

67 **Turriziani M**, Fantini M, Benvenuto M, Izzi V, Masuelli L, Sacchetti P, Modesti A, Bei R. Carcinoembryonic antigen (CEA)-based cancer vaccines: recent patents and antitumor effects from experimental models to clinical trials. *Recent Pat Anticancer Drug Discov* 2012; **7**: 265-296 [PMID: 22630596 DOI: 10.2174/157489212801820020]

68 **Morse MA**, Nair SK, Boczkowski D, Tyler D, Hurwitz HI, Proia A, Clay TM, Schlom J, Gilboa E, Lyerly HK. The feasibility and safety of immunotherapy with dendritic cells loaded with CEA mRNA following neoadjuvant chemoradiotherapy and resection of pancreatic cancer. *Int J Gastrointest Cancer* 2002; **32**: 1-6 [PMID: 12630764 DOI: 10.1385/ijgc:32:1:1]

69 **Gnoni A**, Licchetta A, Scarpa A, Azzariti A, Brunetti AE, Simone G, Nardulli P, Santini D, Aieta M, Delcuratolo S, Silvestris N. Carcinogenesis of pancreatic adenocarcinoma: precursor lesions. *Int J Mol Sci* 2013; **14**: 19731-19762 [PMID: 24084722 DOI: 10.3390/ijms141019731]

70 **Wedén S**, Klemp M, Gladhaug IP, Møller M, Eriksen JA, Gaudernack G, Buanes T. Long-term follow-up of patients with resected pancreatic cancer following vaccination against mutant K-ras. *Int J Cancer* 2011; **128**: 1120-1128 [PMID: 20473937 DOI: 10.1002/ijc.25449]

71 **Carbone DP**, Ciernik IF, Kelley MJ, Smith MC, Nadaf S, Kavanaugh D, Maher VE, Stipanov M, Contois D, Johnson BE, Pendleton CD, Seifert B, Carter C, Read EJ, Greenblatt J, Top LE, Kelsey MI, Minna JD, Berzofsky JA. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. *J Clin Oncol* 2005; **23**: 5099-5107 [PMID: 15983396 DOI: 10.1200/jco.2005.03.158]

72 **Koido S**, Ohkusa T, Homma S, Namiki Y, Takakura K, Saito K, Ito Z, Kobayashi H, Kajihara M, Uchiyama K, Arihiro S, Arakawa H, Okamoto M, Gong J, Tajiri H. Immunotherapy for colorectal cancer. *World J Gastroenterol* 2013; **19**: 8531-8542 [PMID: 24379570 DOI: 10.3748/wjg.v19.i46.8531]

73 **Mazzolini G**, Alfaro C, Sangro B, Feijoó E, Ruiz J, Benito A, Tirapu I, Arina A, Sola J, Herraiz M, Lucena F, Olagüe C, Subtil J, Quiroga J, Herrero I, Sádaba B, Bendandi M, Qian C, Prieto J, Melero I. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. *J Clin Oncol* 2005; **23**: 999-1010 [PMID: 15598979 DOI: 10.1200/jco.2005.00.463]

74 **Lasek W**, Zagożdżon R, Jakobisiak M. Interleukin 12: still a promising candidate for tumor immunotherapy? *Cancer Immunol Immunother* 2014; **63**: 419-435 [PMID: 24514955 DOI: 10.1007/s00262-014-1523-1]

75 **Hirooka Y**, Itoh A, Kawashima H, Hara K, Nonogaki K, Kasugai T, Ohno E, Ishikawa T, Matsubara H, Ishigami M, Katano Y, Ohmiya N, Niwa Y, Yamamoto K, Kaneko T, Nieda M, Yokokawa K, Goto H. A combination therapy of gemcitabine with immunotherapy for patients with inoperable locally advanced pancreatic cancer. *Pancreas* 2009; **38**: e69-e74 [PMID: 19276867 DOI: 10.1097/MPA.0b013e318197a9e3]

76 **Jaffee EM**, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, Goemann M, Coleman J, Grochow L, Donehower RC, Lillemoe KD, O'Reilly S, Abrams RA, Pardoll DM, Cameron JL, Yeo CJ. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 2001; **19**: 145-156 [PMID: 11134207]

77 **Laheru D**, Lutz E, Burke J, Biedrzycki B, Solt S, Onners B, Tartakovsky I, Nemunaitis J, Le D, Sugar E, Hege K, Jaffee E. Allogeneic granulocyte macrophage colony-stimulating factor-secreting tumor immunotherapy alone or in sequence with cyclophosphamide for metastatic pancreatic cancer: a pilot study of safety, feasibility, and immune activation. *Clin Cancer Res* 2008; **14**: 1455-1463 [PMID: 18316569 DOI: 10.1158/1078-0432.ccr-07-0371]

78 **Lutz E**, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, Sugar E, Piantadosi S, Cameron JL, Solt S, Onners B, Tartakovsky I, Choi M, Sharma R, Illei PB, Hruban RH, Abrams RA, Le D, Jaffee E, Laheru D. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. *Ann Surg* 2011; **253**: 328-335 [PMID: 21217520 DOI: 10.1097/SLA.0b013e3181fd271c]

79 **Le DT**, Lutz E, Uram JN, Sugar EA, Onners B, Solt S, Zheng L, Diaz LA, Donehower RC, Jaffee EM, Laheru DA. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. *J Immunother* 2013; **36**: 382-389 [PMID: 23924790 DOI: 10.1097/CJI.0b013e31829fb7a2]

80 **Lutz ER**, Wu AA, Bigelow E, Sharma R, Mo G, Soares K, Solt S, Dorman A, Wamwea A, Yager A, Laheru D, Wolfgang CL, Wang J, Hruban RH, Anders RA, Jaffee EM, Zheng L. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic

foci of immune regulation. *Cancer Immunol Res* 2014; **2**: 616-631 [PMID: 24942756 DOI: 10.1158/2326-6066.cir-14-0027]
81 **Le DT**, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, Morse M, Zeh H, Cohen D, Fine RL, Onners B, Uram JN, Laheru DA, Lutz ER, Solt S, Murphy AL, Skoble J, Lemmens E, Grous J, Dubensky T, Brockstedt DG, Jaffee EM. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. *J Clin Oncol* 2015; **33**: 1325-1333 [PMID: 25584002 DOI: 10.1200/jco.2014.57.4244]
82 **Hardacre JM**, Mulcahy M, Small W, Talamonti M, Obel J, Krishnamurthi S, Rocha-Lima CS, Safran H, Lenz HJ, Chiorean EG. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. *J Gastrointest Surg* 2013; **17**: 94-100; discussion p. 100-1 [PMID: 23229886 DOI: 10.1007/s11605-012-2064-6]
83 **Dranoff G**. GM-CSF-based cancer vaccines. *Immunol Rev* 2002; **188**: 147-154 [PMID: 12445288 DOI: 10.1034/j.1600-065X.2002.18813.x]
84 **Jinushi M**, Hodi FS, Dranoff G. Enhancing the clinical activity of granulocyte-macrophage colony-stimulating factor-secreting tumor cell vaccines. *Immunol Rev* 2008; **222**: 287-298 [PMID: 18364009 DOI: 10.1111/j.1600-065X.2008.00618.x]
85 **Soares KC**, Rucki AA, Wu AA, Olino K, Xiao Q, Chai Y, Wamwea A, Bigelow E, Lutz E, Liu L, Yao S, Anders RA, Laheru D, Wolfgang CL, Edil BH, Schulick RD, Jaffee EM, Zheng L. PD-1/PD-L1 blockade together with vaccine therapy facilitates effector T-cell infiltration into pancreatic tumors. *J Immunother* 2015; **38**: 1-11 [PMID: 25415283 DOI: 10.1097/cji.0000000000000062]
86 **Topalian SL**, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* 2012; **24**: 207-212 [PMID: 22236695 DOI: 10.1016/j.coi.2011.12.009]
87 **Terabe M**, Ambrosino E, Takaku S, O'Konek JJ, Venzon D, Lonning S, McPherson JM, Berzofsky JA. Synergistic enhancement of CD8+ T cell-mediated tumor vaccine efficacy by an anti-transforming growth factor-beta monoclonal antibody. *Clin Cancer Res* 2009; **15**: 6560-6569 [PMID: 19861451 DOI: 10.1158/1078-0432.CCR-09-1066]
88 **Ueda R**, Fujita M, Zhu X, Sasaki K, Kastenhuber ER, Kohanbash G, McDonald HA, Harper J, Lonning S, Okada H. Systemic inhibition of transforming growth factor-beta in glioma-bearing mice improves the therapeutic efficacy of glioma-associated antigen peptide vaccines. *Clin Cancer Res* 2009; **15**: 6551-6559 [PMID: 19861464 DOI: 10.1158/1078-0432.CCR-09-1067]
89 **Conroy H**, Galvin KC, Higgins SC, Mills KH. Gene silencing of TGF-β1 enhances antitumor immunity induced with a dendritic cell vaccine by reducing tumor-associated regulatory T cells. *Cancer Immunol Immunother* 2012; **61**: 425-431 [PMID: 22193988 DOI: 10.1007/s00262-011-1188-y]
90 **Zhang M**, Berndt BE, Chen JJ, Kao JY. Expression of a soluble TGF-beta receptor by tumor cells enhances dendritic cell/tumor fusion vaccine efficacy. *J Immunol* 2008; **181**: 3690-3697 [PMID: 18714045]
91 **Koido S**, Homma S, Okamoto M, Namiki Y, Takakura K, Takahara A, Odahara S, Tsukinaga S, Yukawa T, Mitobe J, Matsudaira H, Nagatsuma K, Kajihara M, Uchiyama K, Arihiro S, Imazu H, Arakawa H, Kan S, Hayashi K, Komita H, Kamata Y, Ito M, Hara E, Ohkusa T, Gong J, Tajiri H. Augmentation of antitumor immunity by fusions of ethanol-treated tumor cells and dendritic cells stimulated via dual TLRs through TGF-β1 blockade and IL-12p70 production. *PLoS One* 2013; **8**: e63498 [PMID: 23717436 DOI: 10.1371/journal.pone.0063498]
92 **Gabrilovich DI**. Combination of chemotherapy and immunotherapy for cancer: a paradigm revisited. *Lancet Oncol* 2007; **8**: 2-3 [PMID: 17196504 DOI: 10.1016/S1470-2045(06)70985-8]
93 **Obeid M**, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini JL, Castedo M, Mignot G, Panaretakis T, Casares N, Métivier D, Larochette N, van Endert P, Ciccosanti F, Piacentini M, Zitvogel L, Kroemer G. Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nat Med* 2007; **13**: 54-61 [PMID: 17187072 DOI: 10.1038/nm1523]
94 **Vacchelli E**, Aranda F, Eggermont A, Galon J, Sautès-Fridman C, Cremer I, Zitvogel L, Kroemer G, Galluzzi L. Trial Watch: Chemotherapy with immunogenic cell death inducers. *Oncoimmunology* 2014; **3**: e27878 [PMID: 24800173 DOI: 10.4161/onci.27878]
95 **Apetoh L**, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, Yang H, Amigorena S, Ryffel B, Barrat FJ, Saftig P, Levi F, Lidereau R, Nogues C, Mira JP, Chompret A, Joulin V, Clavel-Chapelon F, Bourhis J, André F, Delaloge S, Tursz T, Kroemer G, Zitvogel L. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007; **13**: 1050-1059 [PMID: 17704786 DOI: 10.1038/nm1622]
96 **Bianchi ME**, Beltrame M, Paonessa G. Specific recognition of cruciform DNA by nuclear protein HMG1. *Science* 1989; **243**: 1056-1059 [PMID: 2922595 DOI: 10.1126/science.2922595]
97 **Rovere P**, Peri G, Fazzini F, Bottazzi B, Doni A, Bondanza A, Zimmermann VS, Garlanda C, Fascio U, Sabbadini MG, Rugarli C, Mantovani A, Manfredi AA. The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. *Blood* 2000; **96**: 4300-4306 [PMID: 11110705]
98 **Nowak AK**, Robinson BW, Lake RA. Gemcitabine exerts a selective effect on the humoral immune response: implications for combination chemo-immunotherapy. *Cancer Res* 2002; **62**: 2353-2358 [PMID: 11956096]
99 **Suzuki E**, Kapoor V, Jassar AS, Kaiser LR, Albelda SM. Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res* 2005; **11**: 6713-6721 [PMID: 16166452 DOI: 10.1158/1078-0432.CCR-05-0883]
100 **Kan S**, Hazama S, Maeda K, Inoue Y, Homma S, Koido S, Okamoto M, Oka M. Suppressive effects of cyclophosphamide and gemcitabine on regulatory T-cell induction in vitro. *Anticancer Res* 2012; **32**: 5363-5369 [PMID: 23225438]
101 **Soeda A**, Morita-Hoshi Y, Makiyama H, Morizane C, Ueno H, Ikeda M, Okusaka T, Yamagata S, Takahashi N, Hyodo I, Takaue Y, Heike Y. Regular dose of gemcitabine induces an increase in CD14+ monocytes and CD11c+ dendritic cells in patients with advanced pancreatic cancer. *Jpn J Clin Oncol* 2009; **39**: 797-806 [PMID: 19797418 DOI: 10.1093/jjco/hyp112]
102 **Takahara A**, Koido S, Ito M, Nagasaki E, Sagawa Y, Iwamoto T, Komita H, Ochi T, Fujiwara H, Yasukawa M, Mineno J, Shiku H, Nishida S, Sugiyama H, Tajiri H, Homma S. Gemcitabine enhances Wilms' tumor gene WT1 expression and sensitizes human pancreatic cancer cells with WT1-specific T-cell-mediated antitumor immune response. *Cancer Immunol Immunother* 2011; **60**: 1289-1297 [PMID: 21607557 DOI: 10.1007/s00262-011-1033-3]
103 **Everson RG**, Antonios JP, Lisiero DN, Soto H, Scharnweber R, Garrett MC, Yong WH, Li N, Li G, Kruse CA, Liau LM, Prins RM. Efficacy of systemic adoptive transfer immunotherapy targeting NY-ESO-1 for glioblastoma. *Neuro Oncol* 2016; **18**: 368-378 [PMID: 26330563 DOI: 10.1093/neuonc/nov153]
104 **Koido S**, Homma S, Takahara A, Namiki Y, Komita H, Uchiyama K, Ito M, Gong J, Ohkusa T, Tajiri H. Immunotherapy synergizes with chemotherapy targeting pancreatic cancer. *Immunotherapy* 2012; **4**: 5-7 [PMID: 22149993 DOI: 10.2217/imt.11.150]
105 **Kan S**, Koido S, Okamoto M, Hayashi K, Ito M, Kamata Y, Komita H, Nagasaki E, Homma S. Up-regulation of HER2 by gemcitabine enhances the antitumor effect of combined gemcitabine and trastuzumab emtansine treatment on pancreatic ductal adenocarcinoma cells. *BMC Cancer* 2015; **15**: 726 [PMID: 26475267 DOI: 10.1186/s12885-015-1772-1]
106 **Chiang CL**, Benencia F, Coukos G. Whole tumor antigen vaccines. *Semin Immunol* 2010; **22**: 132-143 [PMID: 20356763 DOI: 10.1016/j.smim.2010.02.004]
107 **Xu Q**, Liu G, Yuan X, Xu M, Wang H, Ji J, Konda B, Black KL, Yu JS. Antigen-specific T-cell response from dendritic cell vaccination using cancer stem-like cell-associated antigens. *Stem Cells* 2009; **27**: 1734-1740 [PMID: 19536809 DOI: 10.1002/

stem.102]
108 **Weng D**, Song B, Durfee J, Sugiyama V, Wu Z, Koido S, Calderwood SK, Gong J. Induction of cytotoxic T lymphocytes against ovarian cancer-initiating cells. *Int J Cancer* 2011; **129**: 1990-2001 [PMID: 21154809 DOI: 10.1002/ijc.25851]
109 **Engelmann K**, Shen H, Finn OJ. MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1. *Cancer Res* 2008; **68**: 2419-2426 [PMID: 18381450 DOI: 10.1158/0008-5472.CAN-07-2249]
110 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]
111 **Mahoney KM**, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov* 2015; **14**: 561-584 [PMID: 26228759 DOI: 10.1038/nrd4591]
112 **De Remigis A**, de Gruijl TD, Uram JN, Tzou SC, Iwama S, Talor MV, Armstrong TD, Santegoets SJ, Slovin SF, Zheng L, Laheru DA, Jaffee EM, Gerritsen WR, van den Eertwegh AJ, Le DT, Caturegli P. Development of thyroglobulin antibodies after GVAX immunotherapy is associated with prolonged survival. *Int J Cancer* 2015; **136**: 127-137 [PMID: 24832153 DOI: 10.1002/ijc.28973]

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