Novel Pancreatic Cancer Vaccines Could Unleash the Army Within
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**Background:** Despite recent progress with novel chemotherapy regimens, pancreatic ductal adenocarcinoma remains the fourth leading cause of cancer death in the United States. Innovative approaches to treatment of this disease are needed to accelerate progress.

**Methods:** A review was conducted of the results of 2 pancreatic cancer vaccine programs with results that have shown promise in early-phase clinical trials.

**Results:** In a phase 2 trial, a cell-based allogeneic pancreatic cancer vaccine exploiting the hyperacute rejection response targeted against alpha-1,3 galactosyl epitopes (algenpantucel-L) has shown improvement in disease-free and overall survival rates in the adjuvant setting compared with a historical control. This vaccine has advanced to ongoing phase 3 trials. Compared with GVAX alone, a second whole-cell vaccine employing GM-CSF–expressing pancreatic cancer cells (GVAX) to enhance the antigen presentation in a priming phase followed by a Listeria-based vaccine targeting mesothelin in a boost phase improved survival rates. This vaccine platform is undergoing additional phase 2 testing.

**Conclusions:** Allogenic whole-cell pancreatic adenocarcinoma vaccines show promise in early-phase trials and have the potential to improve survival rates by unleashing antitumor immunity.

**Introduction**
Pancreatic ductal adenocarcinoma (PDA) remains near the top of the list of leading causes of death from cancer. With 46,420 new cases and 39,590 deaths, the rate of fatality remains above 85%. The apparent intrinsic resistance of PDA is highlighted by the long list of conventional and targeted chemotherapy agents that have failed to produce clinically significant improvements in survival rates among patients with this malignancy. In the 15 years between the initial approval of gemcitabine for PDA and the successful phase 3 trials of the multiagent regimens 5-fluorouracil/folinic acid/oxaliplatin/irinotecan and gemcitabine/nab-paclitaxel, overall survival (OS) rates for patients with advanced disease have improved from 6 months to 11 months. Five-year survival rates for patients with early-stage PDA who undergo resection followed by adjuvant therapy have improved from approximately 10% to 20%. Given this slow rate of progress, it is understandable that a desire exists for investigating a different approach to this disease. Encouraging results with 2 new pancreatic cancer vaccine therapies give hope that immunotherapy can achieve a leap in progress.

**Amenable to Immunotherapy?**
Several clinical observations suggest that the enhancement of antitumor immunity in PDA may provide a clinical benefit. The accumulation of CD8+ T cells in human PDA correlates with improved survival rates. Numerous tumor-associated antigens (TAAs) have been identified in PDA. Among the most promising are mesothelin, mucin 1 (MUC1), and Kirsten rat sarcoma (Kras). Antibodies to TAAs are found in the serum of patients with PDA, and the presence of these antibodies correlates with survival. In addition, the presence of PDA is associated with immunosuppression characterized by elevated levels of CD4+ CD25+ Foxp3+ regulatory T cells and CD11b+ CD14+ CD33+ myeloid-derived suppressor cells, which downregulate antitumor immune responses. In animal models of pancreatic cancer, these cells may represent 50% of the leukocytes infiltrating the tumor. The elevation of myeloid-derived suppressor cells is an independent prognostic factor in PDA. Finally, several vaccine strategies have shown efficacy in preclinical animal models.
What Is Required for an Effective Antitumor Immune Response?

The sequence of events leading to tumor rejection has been organized into a conceptual framework called the cancer-immunity cycle. The cytotoxic T-lymphocyte (CTL)–mediated elimination of tumor cells is an antigen-dependent process. Ideal TAAs are present on tumor cells alone and absent — or at least expressed at reduced levels — in normal cells. Whole exome sequencing of patient-derived tumor cells can identify a complete set of mutated genes and their respectively abnormal protein products. As this technology is applied to additional tumor types, the research has become clear that no 2 patients with similar cancer diagnoses have an identical set of mutations. The antigen specificity of tumor-infiltrating lymphocytes capable of mediating tumor rejection has been compared with the unique library of mutations in a given patient’s tumor for some cancers. Researchers are finding that the TAAs to which CTLs respond are peptides derived from these mutated proteins. In particular, a subset of mutant peptides, which are capable of binding to the major histocompatibility complex (MHC) with high affinity for antigen presentation, are relevant mutations for antitumor immunity. Genome projects that sequence pancreatic cancer have identified an average of 25 to 45 mutations per patient, ranging from 1 to 116. In addition, the expression of nonmutated antigens, such as mesothelin, telomerase, survivin, MUC1, human epidermal growth factor receptor 2, and carcinoembryonic antigen, are upregulated.

Most of these mutant and overexpressed proteins are intracellular and released upon tumor cell necrosis. These released proteins undergo capture, phagocytosis, or receptor-mediated endocytosis by antigen-presenting cells (APCs) that can present on MHC classes I and II. Peptides that are 9 to 10 amino acids in length are generated from these proteins and then bind to the MHC and are presented to T cells. The initial priming step of the cancer-immunity cycle requires tumor cell lysis to release a mixture of TAAs to APCs in an environment of immunostimulatory cytokines. Even when this occurs, APCs may fail to phagocytose and process released TAAs.

Algenpantucel-L is designed to harness the activity of hyperacute graft rejection to enhance these initial steps, thus leading to better immune priming (Fig 1). Hyperacute graft rejection after xenotransplantation results in lysis of foreign cells within minutes. The antigen that triggers this response is galactosyl-alpha-1,3-galactose (alpha gal) on the cell surface glycoproteins of mammalian cells, with the exception of humans and Old World primates. The absence of this antigen on human cells is due to the inactivation of the GGT1A gene for alpha-1,3-galactosyltransferase about 20 million years ago during the evolution of primates. The human pseudogene contains a 2 base-pair frameshift mutation. Pre-existing high titer antibodies to alpha gal represent 1% to 2% of all circulating antibodies. Binding of these antibodies to alpha gal on nonhuman transplanted cells activates complement-mediated lysis and antibody-dependent cell-mediated cytotoxicity.

Anti–alpha gal antibody bound to autologous tumor cells modified by transfection with the GGT1A gene to express alpha gal targets those tumor cells for opsonization by APCs via the antibody Fc-gamma receptor. This allows the APCs to phagocytose the entire tumor cell with their complete library of TAAs. These APCs then migrate to regional lymph nodes and process and present TAA peptides to CD8+ cytotoxic T cells in association with MHC class I as well as CD4+ T cells in association with MHC class II.

The algenpantucel-L vaccine consists of 2 human pancreatic cancer cell lines (HAPA-1 and HAPA-2) modified to express alpha gal by retroviral transduction of the murine GGT1A gene. These 2 cell lines were selected for their representation of known TAAs for pancreatic cancer (eg, mesothelin, carcinoembryonic antigen). The engineered cells are irradiated and administered as intradermal injections. In a phase 1 study, algenpantucel-L was administered to 7 participants. No dose-limiting toxicities were seen. A phase 2 study was conducted in 70 patients in the adjuvant setting in which the primary end point was 12-month disease-free survival (DFS). Beginning 6 weeks after R0 or R1 resection of PDA, vaccination with either 100 million or 300 million cells commenced. Chemotherapy with gemcitabine and chemoradiation with fluorouracil as a radiosensitizer was given according to positive feedback loop. In principle, the process can continue until every tumor cell is eliminated, resulting in complete regression of the tumor.
the RTOG-9704 standard. Vaccine injections were given every 2 weeks during chemotherapy and chemoradiation for up to 14 vaccinations. Restaging computed tomography scans were obtained at the end of treatment and then every 3 months for 1 year, then every 6 months for 2 years, and then yearly thereafter. The 12-month DFS rate was 62% for the entire cohort. There appeared to be a dose effect because the 12-month DFS rate for the cohort receiving 300 million cells was 81% compared with 51% for those receiving 100 million cells. The 12-month OS rate for the entire cohort was 86% (96% at a dose of 300 million cells and 79% at a dose of 100 million cells). This compared favorably with the 12-month OS rate of the RTOG-9704 historical control (69%), despite a larger proportion of patients with node-positive disease in the algenpantucel-L study (81%) compared with RTOG-9704 (68%). At 3 years, the DFS and OS rates were 26% and 39%, respectively. The most frequent adverse event related to the vaccine was injection site reaction (grades 1/2) and was seen in 51% of participants. Grade 3 events related to the vaccine were lymphopenia (6%), injection site reaction (3%), and leukopenia (3%). No grade 4 events were seen. With regard to immune parameters, 90% of patients showed increases in anti-alpha gal antibodies with elevated titers for more than 200 days. Antimesothelin and anti-carcinoembryonic antigen antibodies were also detected. Elevation in antimesothelin antibodies correlated with OS.

Based on these results, a randomized phase 3 trial at more than 70 centers was initiated in 2010 and enrolled 722 patients in 2013. Survival analysis is in progress (NCT01072981). A second phase 3 study called the Pancreatic Immunotherapy with Algenpantucel-L for Locally Advanced Non-Resectable trial has also been initiated and will evaluate the activity of algenpantucel-L combined with standard chemotherapy and chemoradiation in 280 patients with borderline resectable and locally advanced PDA (NCT01836432).

**GVAX + CRS-207**

Another whole-cell vaccine platform that has recently shown promise is the sequential 2-vaccine program with GVAX and CRS-207 (Fig 2). GVAX, like algenpan-
tucel-L, is an irradiated allogeneic vaccine composed of 2 human PDA cell lines (Panc 10.05 and Panc 6.03) that have been modified by transfection of a plasmid containing the human GM-CSF gene.\(^9\) When they are intradermally injected, these cells secrete high levels of granulocyte–macrophage colony-stimulating factor (GM-CSF) at the vaccination site. In animal models, GM-CSF is the most potent cytokine at attracting APCs and promoting their differentiation. APCs from tumor-bearing hosts show reduced antigen-presenting activity. When treated with GM-CSF, antigen-presenting activity is rescued and these cells migrate to regional lymph nodes and activate CD4\(^+\) and CD8\(^+\) T cells.

In a phase 1 study, 14 patients were treated with escalating doses of GVAX.\(^{19}\) No dose-limiting toxicities were seen. Delayed-type hypersensitivity responses to injection of irradiated autologous tumor cells was seen in 3 patients treated with at least 100 million cells. Although DFS was not a primary end point in the study, DFS longer than 25 months was noted in these 3 patients. The results from that trial led to a phase 2 study in 60 patients with R0 and R1 resected PDA.\(^{20}\) Patients received 500 million vaccine cells starting 8 to 10 weeks following surgery. Fluorouracil-based chemoradiation was then given. In patients who remained free of disease, 3 additional vaccine treatments were given 1 month apart. The primary end point was DFS, with OS and induction of antitumor immune response as secondary end points. The rate of DFS at 12 months was 67.4% and the median DFS rate was 17.3 months. OS at 12 months was 85% with a median OS rate of 24.8 months. Postvaccination induction of antimesothelin CD8\(^+\) T cells in HLA-A1+ and HLA-A2+ patients correlated with DFS.

CRS-207 is a genetically engineered strain of Listeria monocytogenes. L. monocytogenes is a gram-positive bacterium that is an intracellular pathogen capable of cell-to-cell spread by virtue of the actA virulence gene and invasion of nonphagocytic cells via the inlB gene. CRS-207 is a live-attenuated strain with deletions of both of these virulence genes. It has also been engineered to express mesothelin. Therefore, CRS-207 is able to directly deliver the TAA mesothelin to the intracellular compartment of APCs for processing and presentation on MHC classes I and II. These APCs can then activate effector T cells. L. monocytogenes also induces an inflammatory cytokine response that further recruits APCs.

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Fig 2.— GVAX + CRS-207–mediated tumor immunity. CTL = cytotoxic T-lymphocyte, GM-CSF = granulocyte–macrophage colony-stimulating factor, MHC = major histocompatibility complex, PDA = pancreatic ductal adenocarcinoma, TAA = tumor-associated antigen.
CRS-207 was evaluated in a phase 1 study of escalating doses in 17 patients with progressed mesothelioma, PDA, non–small-cell lung cancer, and ovarian cancer. The maximum tolerated dose was 1 × 10^9 CFU given for up to 4 doses. Evidence was suggestive of antimesothelin T-cell responses. In the study, 6 of 17 patients survived for at least 15 months, and 3 of these participants had PDA. However, it is worth noting that some of these patients were participants in prior GVAX trials, a fact suggestive of the synergy between the 2 vaccines, with GVAX priming an immune response and CRS-207 later boosting that response.

Therefore, a phase 2 study of 90 patients with PDA and progressive disease or intolerant of chemotherapy was conducted. Participants were randomized 1:2 to GVAX alone for 6 doses every 3 weeks or 2 doses of GVAX followed by 4 doses of CRS-207. In prior studies of GVAX, large numbers of immunosuppressive regulatory T cells were seen at vaccine sites. Because cyclophosphamide treatment reduces the number and activity of regulatory T cells, low-dose cyclophosphamide was given during this study as an immune modulator prior to GVAX. The primary end point was OS. With a median follow-up of 7.8 months, the OS rate in those assigned to the GVAX arm was 3.9 months; for those assigned to the GVAX + CRS-207 arm, the OS rate was 6.1 months (hazard ratio [HR] 0.54; P = .011). In patients who received at least 3 doses of the vaccine, the median OS rates were 4.6 months for the GVAX arm and 9.7 months for the GVAX + CRS-207 arm (HR 0.44; P = .0074). Based on these promising results, a phase 2b trial was initiated. Study researchers intend to enroll 240 patients with metastatic PDA in the second line or greater to GVAX in combination with CRS-207, CRS-207 alone, or chemotherapy alone (NCT02004262).

These 2 vaccine platforms have accomplished proof of principle that cell-based vaccines for pancreatic cancer can induce immune responses to relevant TAs. These immune responses appear to correlate with rates of DFS and OS. Definitive proof of clinically significant effectiveness will depend on the results of ongoing randomized trials. If such efficacy is demonstrated, then efforts to augment the benefit by combining the vaccines with immune checkpoint inhibition can be expected to unleash the antitumor army within.

References