Priming the pancreatic cancer tumor microenvironment for checkpoint-inhibitor immunotherapy

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Keywords: pancreatic cancer, tumor immunotherapy, tumor vaccines

Pancreatic ductal adenocarcinoma (PDAC) is highly resistant to chemotherapy and radiation therapy. While surgical resection provides the best opportunity for a cure, only around 20% of patients are candidates for surgery, and as many as 80% of patients recur following surgical resection and adjuvant therapy. Median survival for patients with unresectable metastatic PDAC remains <1 y, and the overall 5 y survival rate for PDAC is only 6%.1

Cancer immunotherapy is considered to be one of the biggest breakthroughs for cancer treatment in the last decade. Ipilimumab, a monoclonal antibody that blocks the immune checkpoint cytotoxic T lymphocyte antigen-4 (CTLA-4) was approved by the United States FDA for the treatment of advanced melanoma.2 More recently, other checkpoint inhibitors including Programmed-Death-1 (PD-1) and Programmed-Death-1 Ligand-1 (PD-L1) blocking antibodies were shown to induce objective responses in approximately 20–30% of patients with several cancers, including melanoma, renal cell carcinoma, and non-small cell lung cancer (NSCLC).3,4 Despite the success of blocking CTLA-4 and PD-1 as single therapy in several cancers, treatment of patients with PDAC with these single agents has been ineffective.6

One difference between tumors that have responded to checkpoint-inhibitors and PDAC is the immune status of the TME. Cancers that have responded to checkpoint-inhibitors tend to be naturally infiltrated with effector lymphocytes7 and are generally considered to be ‘immunogenic’ neoplasms. PDAC, on the other hand, is similar to many other non-immunogenic’ neoplasms, which has slowed the development and application of immune-based therapies for these diseases.

Our group has developed a vaccine (GVAX) for the treatment of PDAC consisting of two allogeneic PDAC cell lines engineered to secrete GM-CSF.9,10 GVAX is designed to induce immune responses against a broad range of PDAC-associated antigens, including the commonly expressed PDAC antigen mesothelin. Studies evaluating GVAX in patients with both resected and metastatic PDAC have shown that GVAX induces enhanced mesothelin-specific T cell responses in a subset of patients that are associated with longer survival.11,12 Prior work has also shown that combining GVAX with low-dose cyclophosphamide (Cy) to deplete CD4+ T regulatory cells (Tregs) results in more robust mesothelin-specific T cell responses and longer survival in patients with metastatic PDAC compared to GVAX alone.11 Although our prior work demonstrates that GVAX treatment induces peripheral T cell responses that can be enhanced with low-dose Cy, the studies were not designed to directly evaluate the effects of GVAX treatment on the PDAC TME. Therefore, we designed a neo-adjuvant and adjuvant clinical trial comparing GVAX given as single agent, or in combination with low dose Cy.13 The first treatment was given 2 weeks prior to surgery providing the first opportunity to study how the PDAC TME is altered by GVAX-based immunotherapy.

Immunohistochemical analysis (IHC) of resected tumor tissue revealed the formation of intratumoral tertiary lymphoid aggregates in 33 (84.6%) of 39 vaccinated patients that are not observed in tumors from GVAX-naive patients. The aggregates were composed of naïve and activated T cells, B cells and innate antigen-presenting cells (APCs); and resembled ectopic lymph node-like structures observed in
immunotherapy naïve patients with melanoma, colon cancer and NSCLC. Lymphoid aggregates formed regardless of whether GVAX was given with or without Cy. However, lower numbers of FoxP3+ Tregs were observed in tumors from patients treated with the combination of GVAX+Cy indicating that low-dose Cy reduces Treg levels within the TME. In contrast to primary and secondary lymphoid structures, tertiary lymphoid structures develop in response to antigen exposure. Thus, their formation demonstrates that GVAX induces an adaptive immune response within the PDAC TME.

Treatment with GVAX induced interferon gamma (IFNγ)-production in T effector cells infiltrating PDACs, but also induced the upregulation of immunosuppressive regulatory mechanisms, including upregulation of the PD-1/PD-L1 pathway (Fig. 1). In unvaccinated patients, only a small percentage of PDAC tumor cells expressed low levels of membranous PD-L1. By contrast, moderate membranous expression of PD-L1 by tumor cells was observed in patients treated with GVAX. Lymphoid aggregates were also infiltrated with innate immune cells expressing high levels of PD-L1. Although PD-L1 expression may be regulated by oncogenic pathways, PD-L1 is also induced by cytokines produced by infiltrating immune cells, such as IFNγ. In immunotherapy-naïve patients with melanoma, NSCLC and renal cell carcinoma, PD-L1 expression has been observed in approximately 53–89% of tumors and by infiltrating

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**Figure 1.** Model explaining the inefficacy of single agent immunotherapy for pancreatic cancer. At baseline, pancreatic tumors are predominantly infiltrated with immunosuppressive regulatory cells, such as T regulatory cells (Treg), and few effector T cells (T cell); express low levels of PD-L1 on tumor cells; and are infiltrated with few to no PD-L1-expressing innate immune cells (PD-L1+ immune cell). In this non-immunized and inactive state, treatment with immune checkpoint inhibitors alone, such as anti-PD-1/PD-L1, is hampered by the lack of effector T cells to act on. Treatment with the GVAX vaccine (Vaccine) combined with low dose cyclophosphamide (Cy) converts the pancreatic tumor microenvironment from a relatively inactive to an active state by inducing the infiltration of effector T cells and the formation of intratumoral tertiary lymphoid aggregates (Lymphoid aggregate). However, cytokines produced by the immune cells that are induced to traffic the tumor, such as IFNγ, induce the upregulation of immunosuppressive mechanisms, such as the upregulation of PD-1 and PD-L1 expression. These countering immunosuppressive mechanisms limit the activity and efficacy of vaccination, but also prime the pancreatic tumor microenvironment for immune modulators, such as checkpoint-inhibitors. Thus, optimal activity and antitumor efficacy of either of these single approaches is dependent on the other.
immune cells in approximately 50–100% of tumors. The expression of PD-L1 in tumors is associated with more abundant immune cell infiltration and the presence of lymphoid aggregates. The naturally high prevalence of PD-L1 in these tumor types may explain their relatively high response rates to anti-PD-1 or anti-PD-L1 therapies, whereas the low PD-1/PD-L1 levels expression by PDAC may explain why these agents have been less effective against PDAC. However, by inducing T cell infiltration and PD-L1 expression in the TME, GVAX may prime PDACs for anti-PD-1/PD-L1 therapies.

This study demonstrates that GVAX can convert a ‘non-immunogenic’ neoplasm into an ‘immunogenic’ neoplasm by inducing infiltration of T cells and development of tertiary lymphoid structures. However, this conversion coincides with the upregulation of immunosuppressive regulatory mechanisms. These data may explain why GVAX and checkpoint-inhibitors given alone have failed against PDAC, but importantly, also suggest that vaccine-primed PDAC patients may be better candidates for checkpoint immunotherapy than vaccine-naive patients (Fig. 1). In support of this notion, we recently showed that the combination of GVAX with ipilimumab induces objective responses in patients with metastatic PDAC that are not observed with either single therapy alone. These data support a new approach for evaluating checkpoint inhibitors in ‘non-immunogenic’ cancers, like PDAC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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