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Does vaccine-primed pancreatic cancer offer better candidates for immune-based therapies?

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Cancer immunotherapy is considered to be one of the biggest breakthroughs in cancer therapy in the last decade. However, the success of immunotherapy has so far been limited to a few solid malignancies including melanoma, renal cell carcinoma, non-small-cell lung cancer (NSCLC) and a few hematologic malignancies. In 2011, ipilimumab, a therapeutic monoclonal antibody that blocks CTLA-4, the bona fide immune checkpoint, was US FDA-approved for advanced melanoma [1]. Subsequently, other checkpoint inhibitors including anti-PD-1 and anti-PD-L1 blockade antibodies were also demonstrated to yield an objective response in approximately 20–30% of patients of these malignant diseases; and among the patients who had an objective response, many had a durable response [2–4]. One of anti-PD-1 antibodies (pembrolizumab) was most recently approved by US FDA for unresectable or metastatic melanoma. In addition, sipuleucel-T, a dendritic cell vaccine, has been shown to improve the overall survival of metastatic prostate cancer and subsequently gained FDA approval [5]. Nevertheless, significant objective response and durable responses were not seen in sipuleucel-T-treated pancreatic cancer patients. Melanoma, renal cell carcinoma and NSCLC were unique in their high infiltration of effector lymphocytes in tumor microenvironment (TME) [6]. By contrast, many other solid malignancies including pancreatic cancer are characterized by a highly immunosuppressive TME [7]. Immune tolerance mechanisms within the TME are a major obstacle to effective treatment of these cancers with immunotherapy. Pancreatic cancer and many other malignancies are thus

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considered ‘nonimmunogenic’ neoplasms. This notion has drastically slowed the development and application of immune-based therapies for these diseases.

Immune tolerance mechanisms appear to have been established early in the development of pancreatic cancer [8]. At the stage of invasive adenocarcinoma, not only the TME is skewed toward immunosuppressive cells (e.g., M2 tumor-associated macrophage, myeloid-derived suppressive cells and T-regulatory cells), but the TME also lacks antigen experienced T-effector cells [7]. Several studies showed that pancreatic cancer TME is dominated by innate immune cells and lacks adaptive immune response [7,9]. Thus, the first barrier for an effective immunotherapy strategy to overcome is to activate the adaptive immune response in the TME. Vaccine-based immunotherapies are known to activate antigen-specific T-effector cells in the peripheral lymphocytes. However, it is not known whether vaccine-induced adaptive immune response occurs in the TME. We developed a GM-CSF-secreting pancreatic cancer vaccine, which has so far been one of the most widely tested immunotherapies for pancreatic cancer [10–13]. Our prior work demonstrated clinical activity in a subset of vaccinated patients, and showed that the post-vaccination induction of T-cell responses against mesothelin is associated with longer survival [11,12,14]. However, up to this point, neither our group nor other groups have had the opportunity to conduct in-depth analyses of the effects of an immune-based therapy on the TME of nonmelanoma solid tumors in patients.

Our group has thus conducted a novel neoadjuvant and adjuvant study designed to evaluate postimmunotherapy changes within the TME of primary pancreatic tumors following treatment with our pancreatic cancer GM-CSF-secreting pancreatic cancer vaccine, given either alone or with immune modulating doses of cyclophosphamide to deplete regulatory T cells [15]. Given with the same pancreatic cancer vaccine, it was previously reported that low dose cyclophosphamide enhanced higher avidity T-cell responses that were associated with longer progression-free survival in patients [11]. Provided opportunities to dissect TME in the wholly resected tumors, the neoadjuvant vaccine study revealed novel findings that would not have been demonstrated in prior studies. Pathological examination of tumor tissue resected just 2 weeks following vaccination identified the formation of immunotherapy-induced tertiary lymphoid aggregates, an organized lymphoid structure that was not observed in tumors resected from unvaccinated patients.

Tertiary lymphoid structures have not been previously reported in pancreatic cancer, but have been reported in other cancers at baseline, before therapeutic intervention [16]. Those cancers that demonstrated tertiary lymphoid aggregates at baseline include melanoma and NSCLC, which were also found to respond to the immune checkpoint inhibitors [2–4]. In contrast to primary and secondary lymphoid structure, tertiary lymphoid structures are not pre-existing and are developed only when challenged with antigens [16]. The development of tertiary lymphoid structures is achieved through a lymphoid neogenesis process by importing the lymphocytes from blood and lymph. Thus, the formation of tertiary lymphoid aggregates in pancreatic cancer in response to the vaccine therapy appears to be induced by adaptive immune response that is stimulated by antigens provided by the vaccine.

Likely as a result of adaptive immune response to the vaccine treatment, PD-L1 expression is induced in the lymphoid aggregates by the pancreatic cancer vaccine treatment [15]. At baseline, a small percentage of pancreatic cancers express low levels of membranous PD-L1 on the epithelial tumor cells or stromal cells. By contrast, vaccine therapy induced the expression of membranous PD-L1 moderately on the epithelial tumor cells, but more significantly induced the infiltration of PD-L1 positive cells in all the intratumoral lymphoid aggregates [15]. PD-L1 expression may be regulated by oncogenic pathways. However, in most cancers, PD-L1 is induced by cytokines such as IFN- γ as a result of adaptive immune response [17]. In melanoma, NSCLC and renal cell carcinoma, PD-L1 expression was observed in tumor cells in approximately 53–89% of untreated patients' tumors and in tumor infiltrating immune cells in approximately 50–100% of tumors [6]. PD-L1 expression in both tumor cells and tumor infiltrating immune cells is associated with PD-1 expression in tumor-infiltrating lymphocytes, which was also associated with more abundant infiltration of immune cells and the presence of lymphoid aggregates in these untreated patients' tumors. The high prevalence of PD-L1 expression in these malignancies may explain their relatively high response rates to anti-PD-1 or anti-PD-L1 therapies [2–4]. By contrast, pancreatic cancer demonstrated a minimal response to anti-PD-1/PD-L1 therapies, which is likely due to low PD-L1 expression in pancreatic cancer. However, vaccine therapy, by inducing PD-L1 expression in TME, may have primed the pancreatic cancers for anti-PD-1/PD-L1 therapies.

The combination of anti-PD-1/PD-L1 therapies and vaccine therapy was tested by our group in the preclinical model of pancreatic cancer [18]. Interestingly, anti-PD-1/PD-L1 therapies alone did not significantly alter the TME of murine pancreatic tumors. By contrast, vaccine therapies moderately enhanced the infiltration of effector T cells into TME. However, by combining with the vaccine therapies, antitumor effector T-cell response in the TME was significantly enhanced by anti-PD-1/PD-L1 therapies. As a result, the combination of vaccine and anti-PD-1/PD-L1 therapies has increased the cure rate of tumor-bearing mice compared with either single therapy alone. This yet unpublished follow-up study suggests that vaccine therapy is more critical in priming pancreatic cancer TME with effector T-cell infiltration, and that the combination of anti-PD-1/PD-L1 therapies, by overcoming adaptive immune resistance, is more critical in inducing an effective antitumor immune response. A clinical trial that is supported by this preclinical study will soon be initiated as part of the Stand Up To Cancer (SU2C) project.

Besides anti-PD-1/PD-L1 therapies, vaccine therapy may prime pancreatic cancer for other immune-based therapies. Gene microarray analysis of microdissected lymphoid aggregates from the pancreatic cancer patients treated with the neoadjuvant vaccine therapy identified gene signatures in five pathways, including the TH17/T-regulatory cell, NF- κ B, integrin/adhesion, chemokine/chemokine receptor and ubiquitin-proteasome pathways, which are associated with improved post-vaccination immune responses and longer survival [15]. Therefore, lymphoid aggregates can express both effector activating and effector downregulatory immune signatures. Thus, vaccine therapy potentially primes pancreatic cancer to other immune-based therapies or targeted therapies that enhance effector activating immune signatures or that inhibit effector downregulatory immune signatures.

Vaccine therapy is obviously not the only therapy that can prime the cancer for immunotherapies. Epigenetic therapies including DNA demethylating agents or histone deacetylation inhibitors have also been suggested to prime NSCLC for anti-PD-1 therapies [19]. Although the exact mechanisms are unknown, a broad immune stimulatory role for DNA demethylating drugs in multiple cancers including colorectal cancer, breast cancer, ovarian cancer, NSCLC and a number of hematologic malignancies has been proposed [19,20]. DNA demethylating agents were found to induce the expression of cancer testis antigens that are not expressed in most of cancers at baseline and thus are not tolerated by the patients' immune system. In addition, DNA demethylating treatments upregulate immunomodulatory pathways including interferon signaling, antigen presentation and certain cytokines/chemokines [19,20]. PD-L1 expression also appeared to be induced by DNA demethylating treatments [19]. Thus, clinical trials testing the role of epigenetic therapy in priming the cancer for anti-PD-1 therapies are being conducted. Nevertheless, other modalities of treatments that generate an inflammatory response may also have roles in priming the TME for immunotherapies. Moreover, one type of immunotherapy may also prime the TME for another type of immunotherapy. One good example is that the combinatorial therapy of anti-CTLA-4 antibody and anti-PD-1 antibody resulted in higher response rate and durable response. This concept has been tested in the preclinical model and subsequently supported by the clinical trial of the combination of ipilimumab and nivolumab for advanced melanomas [21]. Thus, even for those malignancies that are relatively sensitive to immune checkpoint inhibitors, the priming mechanism through a combinational immunotherapy approach would potentially convert those immunotherapy nonresponding patients to responders.

In conclusion, vaccine therapy is able to reprogram the TME of pancreatic cancer and potentially that of many other malignancies. By reprogramming the TME, vaccine therapy may have primed these malignancies for more effective immunotherapies. Such a treatment strategy may also be applied to other combinational modalities of cancer therapies.

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