Immunotherapy and Combination Strategies in Pancreatic Cancer: Current Status and Emerging Trends

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Current Treatment Options for Pancreatic Cancer

Pancreatic cancer is one of the most aggressive solid tumors with no effective therapeutic options for long-term tumor control so far. The vast majority of pancreatic cancer patients are diagnosed at an advanced stage, and treatment options are limited as the cancer is highly refractory to conventional therapies. 2 intensified chemotherapy regimens, FOLFIRINOX (leucovorin (LV), 5-fluorouracil (5-FU), irinotecan, oxaliplatin) and gemcitabine plus albumin-bound paclitaxel (nab-paclitaxel), have emerged as the standard of care for metastatic pancreatic cancer. Both regimens showed improved overall and progression-free survival when compared to gemcitabine alone in phase III randomized controlled trials \cite{1, 2}. Nevertheless, median progression-free and overall survival remain poor (less than 6 and 12 months, respectively), and only up to 30% of patients show a response to either regimen. Another new treatment option for metastatic pancreatic cancer patients previously treated with gemcitabine-based therapy is the combination of nanoliposomal irinotecan (nal-IRI) with 5-FU/LV. The NAPOLI-1 trial showed that, after 313 events, nal-IRI + 5-FU/LV significantly improved median overall survival compared to 5-FU/LV alone (6.1 vs. 4.2 months) \cite{3}. However, there is still an imperative need for alternative treatment options to achieve more effective and durable clinical responses.

Challenges and Opportunities for Immunotherapy in Pancreatic Cancer

Over the last decade, a paradigm shift has been brought to the clinic with therapeutic regimens targeting immune cells instead of cancer cells in order to enhance antitumor immunity. The immune system is capable of recognizing and eliminating tumor cells. How...
ever, aggressive tumor cells have evolved in a manner that allows them to escape and even suppress antitumor immune response. Cancer immunotherapy attempts to harness the power and specificity of the immune system for the treatment of malignancy. The challenge in immunotherapy is to apply advances in cellular and molecular immunology to the development of strategies that effectively and safely augment antitumor responses [4].

There are several barriers to applying immunotherapy in pancreatic cancer. First, the mutational load in pancreatic cancer is very low compared with other cancers such as melanoma and lung cancers [5, 6]. It is generally believed that cancers with a higher mutational load are associated with an increased number of neoantigens which allow recognition by the immune system [7]. The efficacy of immunotherapy in pancreatic cancer might be hampered by an insufficient cumulative mutational load inducing neoantigen expression. Besides, pancreatic cancer is characterized by a highly immunosuppressive microenvironment in which dense desmoplasia with prominent infiltration of tumor-promoting tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) is present [8], interfering with T-cell infiltration into the tumor microenvironment. As a result, sufficient and effective T-cell responses cannot be elicited against pancreatic tumors [9].

Despite inadequate T-cell responses in the microenvironment of pancreatic cancer, the presence of tumor-infiltrating T cells is a critical factor for good prognosis, and specifically higher levels of CD4+ and CD8+ tumor-infiltrating T cells are associated with a better prognosis [10]. In a genetically engineered Pdx1Cre;KrasG12D;Tp53R172H (KPC) mouse model, immunosuppression was observed to occur already in the early stages of tumorigenesis; thus, tumor cells might have been shielded from immune pressure and preserved their sensitivity to antitumor responses [8]. Recently, Balachandran et al. [11] demonstrated that tumors with both a high number of neoantigens and abundant CD8+ T-cell infiltrates, but neither alone, stratified pancreatic cancer patients according to longer survival. A novel approach was introduced to model tumor response to immunotherapy by identifying a subset of high-quality neoantigens present in tumors of long-term survivors of pancreatic cancer. By shifting the focus away from the quantity of neoantigens towards the quality of expressed neoantigens, the authors underscored the significance of immunotherapy in malignancies such as pancreatic cancer in which low mutational loads are typically observed.

**Emergence of Checkpoint Therapy in Cancers**

Although various checkpoint molecules have been reported, unprecedented clinical success was mainly observed for therapies targeting 2 specific checkpoint molecules of T-cell response: cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1). CTLA-4 blocks the indispensable CD28 co-stimulation by competing and depleting B7-1 and B7-2, ligands of CD28, on antigen-presenting cells. On the other hand, PD-1 interferes with T-cell receptor-mediated signaling pathways and blocks T-cell response by binding to its ligands, programmed death-ligand (PD-L1) and PD-L2, which are present on various tumors cells [12].

Monoclonal antibodies targeting CTLA-4 or PD-1 have demonstrated durable clinical responses and prolonged overall survival in patients with melanoma, a highly immunogenic cancer. In 2011, ipilimumab (anti-CTLA-4) was approved by the Food and Drug Administration (FDA) for treatment of melanoma. Single-agent PD-1/PD-L1 inhibitors have demonstrated impressive clinical benefit in many cancers such as non-small cell lung cancer (NSCLC), renal cell carcinoma, bladder cancer, and Hodgkin’s lymphoma [12–15]. These results led to the FDA approval of the PD-1 inhibitors pembrolizumab and nivolumab in melanoma [16–18] and the approval of pembrolizumab and nivolumab as well as the PD-L1 inhibitor atezolizumab in NSCLC [13, 17–19].

**Current Status and Emerging Trends for Checkpoint Therapy in Pancreatic Cancer**

**Monotherapy Targeting Checkpoint Molecules**

Efficacies of CTLA-4 and PD-L1 inhibitors are currently being explored in pancreatic cancer [17, 20]. However, early clinical trials exploring single therapy with anti-CTLA-4 or anti-PD-1/PD-L1 alone were largely ineffective in pancreatic cancer [17, 20, 21]. In a phase II study, ipilimumab as a single-agent treatment was found to be safe but failed to induce tumor response in patients with advanced pancreatic cancer [21]. Among 27 patients, only 1 patient demonstrated a delayed response after initial progression. Similarly, single-agent BMS-936559, an anti-PD-L1 monoclonal antibody, did not show any activity in 14 patients with advanced pancreatic cancer in a phase I study [17].

**Combination Strategies for Checkpoint Immunotherapy**

Due to the lack of signal for immune response with the use of single checkpoint inhibitors, their combination with additional therapies such as chemotherapy and cancer vaccines, which can change the tumor microenvironment from ‘cold’ to ‘inflamed’, represents an important strategy to sensitize tumor cells to immune checkpoint therapies. Table 1 shows some ongoing or completed clinical trials for PD-1/PD-L1 and CTLA-4 immunotherapy and the corresponding combination strategies.

**Combination with Chemotherapy**

Gemcitabine is one of the backbone chemotherapeutic agents for pancreatic cancer treatment. The combination of gemcitabine and immune checkpoint blockade has been evaluated for potential synergy. A phase I clinical study evaluated the combination of gemcitabine and anti-CTLA-4 antibody (tremelimumab) in metastatic pancreatic cancer patients. Among 28 patients, 2 achieved a partial response (PR) and 7 showed stable disease (SD) for > 10 weeks [22]. In another ongoing phase Ib study of unresectable pancreatic cancer, preliminary results showed that among 11 patients treatment with ipilimumab and gemcitabine resulted in 2 PR and 5...
Emerging Trends for Immunotherapy in Pancreatic Cancer

Targeting TAMs

As described earlier, an important barrier to the success of immunotherapy in pancreatic cancer is the highly immunosuppressive tumor microenvironment which is enriched with tumor-infiltrating myeloid cells such as MDSCs and TAMs. Increasing evidence suggests that dysregulated polarization of TAM, the most abundant immune cell population in the tumor microenvironment, facilitates tumor growth and metastasis [9, 30, 31]. TAMs are a heterogeneous population of myeloid cells that differentiate in the tumor microenvironment and become sensitized to tumor-derived suppressive signals [32]. They act to subdue local immune responses by presenting antigens inefficiently and inhibiting lymphocyte infiltration and function [33]. In pancreatic cancer patients, increased numbers of tumor-promoting TAMs are associated with significantly shorter patient survival [34–37]. Therefore, the suppressive cytokine milieu and mechanisms of tolerance promoted by TAMs must be overcome for efficient tumor clearance by adaptive immune responses.

CSF-1 (M-CSF) is a cytokine supporting the differentiation, proliferation, and function of monocytes/macrophages [38]. In tumors, CSF-1 promotes immune suppression by promoting the expansion and differentiation of MDSCs and tumor-promoting TAMs that express its cognate receptor, CSF-1R [31, 39]. Targeting CSF-1R was found to reprogram and decrease tumor-promoting TAMs, relieve immunosuppression, and also improve response to chemotherapeutic and checkpoint therapies in mouse pancreatic cancer models [40, 41]. CD40, a costimulatory molecule expressed on antigen-presenting cells, has been targeted to boost antitumor immunity [42]. Upon CD40 ligation, antigen-presenting cells are licensed for maturation and activation, allowing effective antigen-presentation and T-cell activation. In patients with unresectable pancreatic cancer, CD40 agonist (CP-870,893) led to encouraging clinical response when combined with gemcitabine [42, 43]. It is noteworthy that tumor infiltration by macrophages played a substantial role in depleting stroma and tumor cells [43]. A recent study demonstrated that concurrent

<table>
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Table 1. Clinical trials testing checkpoint immunotherapies and combination treatments

SD [23, 24]. For PD-1/PD-L1, in a mouse model of pancreatic cancer, combining gemcitabine with either anti-PD-1 or anti-PD-L1 antibody enhanced tumor infiltration with CD8+ T cells and resulted in complete responses in the treated animals [25]. Positive PD-L1 expression in resected pancreatic cancer was correlated with worse overall survival [25]. A clinical pilot study of the combination of gemcitabine and anti-PD-1 antibody is now closed to enrollment (NCT01313416), and results are being awaited.

Combination with Cancer Vaccines

Currently, the most extensively studied vaccine for pancreatic cancer is GVAX, a whole-cell vaccine composed of irradiated and allogeneic pancreatic tumor cells genetically engineered to secrete granulocyte-macrophage colony-stimulating factor which stimulates dendritic cell activation and T-cell priming. GVAX induced expansion of pancreatic cancer-specific CD8+ T cells in a phase II study [26]. Furthermore, GVAX and low-dose cyclophosphamide (an alkylating agent depleting regulatory T cells) induced T-cell infiltration and the formation of intratumoral tertiary lymphoid aggregates, suggesting that GVAX might turn pancreatic cancers into immunogenic tumors [27]. In a phase Ib study, GVAX was used in combination with ipilimumab in 30 advanced pancreatic cancer patients previously treated with gemcitabine-based chemotherapy. Combination therapy led to improved survival (27 vs. 7% at 1 year) when compared to ipilimumab alone. Longer survival was associated with an increase in mesothelin-specific T cells and a larger T-cell repertoire, indicating a positive role of T-cell response [28]. In a mouse model, the combination of GVAX and anti-PD-1 antibody led to better survival than anti-PD-1 antibody alone, and the activity was correlated with increased CD8+ T cells and elevated interferon gamma (IFN-γ) production in the tumor microenvironment [29]. Recently, a randomized clinical study (NCT02451982) was initiated to evaluate GVAX with or without anti-PD-1 antibody (nivolumab) in patients with resectable pancreatic cancer; however, the study was terminated due to drug supply issues.
CSF-1R blockade and CD40 agonism lead to profound changes in the composition of immune infiltrates, causing a decrease in immunosuppressive cells and a shift toward a more inflammatory milieu in preclinical tumor models. Importantly, increased survival was demonstrated compared with monotherapy treatment in mice [44]. These studies show that combined therapeutic approaches may remove inhibitory immune cells and simultaneously sustain endogenous antitumor immune responses for efficient tumor suppression.

Recent findings revealed that TAMs also played an important role in modulating therapies targeting the PD1-PDL1 axis. Arlauckas et al. [45] showed that macrophages removed anti-PD1 antibodies from T cells, thereby lowering the therapeutic efficacy, which represents a potential TAM-mediated mechanism of checkpoint inhibition resistance. Targeting TAM inhibition with checkpoint inhibition might represent a promising therapeutic strategy to overcome primary or secondary resistance to checkpoint inhibitors. A recent report by Gordon et al. presented a more complex interaction between TAMs and the PD1/PD-L1 pathway. The group showed that both mouse and human TAMs express PD-1, which increased with stage of colorectal cancer. TAM PD-1 expression correlates negatively with phagocytic potency against tumor cells, and blockade of PD-1/PD-L1 in vivo increases macrophage phagocytosis, reduces tumor growth, and lengthens the survival of mice in mouse models of cancer in a macrophage-dependent fashion [45]. This suggests that PD-1/PD-L1 therapies may also function via a direct effect on macrophages.

Irradiation combined with myeloid targeting is another therapeutic avenue with great clinical potential. Local irradiation of tumor cells induces release of tumor antigens, thereby facilitating specific immune responses and causing severe damage to the tumor vasculature [46]. Following irradiation, TAMs as the primary tumor-resident population of phagocytes promote early endothelial regeneration and restoration of the vasculature by secreting vascular endothelial growth factor and other proangiogenic mediators [47]. Accumulating preclinical evidence suggests that targeting TAMs can lead to decreased angiogenesis, tumor progression, and metastasis. A recent study on pharmacological macrophage depletion by liposomal clodronate in KPC models also demonstrated prominent reduction in metastasis formation, angiogenesis, and regulatory T cell levels [48]. Taken together, the above studies and findings set the stage for assessing combined approaches targeting both innate and adaptive immune responses to overcome resistance to established antitumor therapies in pancreatic cancer.

**Conclusion and Future Perspectives**

Given the limited efficacy of single checkpoint therapies against CLTA-4 or PD-1/PD-L1 in pancreatic cancer, the focus of ongoing studies and future directions is on constructing novel combination therapies in which additional treatment modalities may unleash durable antitumor immune responses by enhancing tumor-specific T-cell response and/or harnessing the immunosuppressive microenvironment (fig. 1). Increasing efforts are being made to improve both checkpoint blockade efficacy and patient outcome prediction, and more novel treatment modalities are foreseen in the near future. In this context, the role of the host microbiome in influencing the outcome of immunotherapy has sparked tremendous interest in the field. 2 elegant studies addressed this question by demonstrating the gut microbiome as the driver of checkpoint blockade immunotherapy in preclinical tumor models. Vetizou et al. [49] and Sivan et al. [50] demonstrated that gut microbiota influenced the outcome of anti-PD-1 and anti-CTLA-4 therapies by enhancing dendritic cell activation and subsequent antitumor T-cell responses. This innovative concept unveils the crucial role of innate immunity in shaping antitumor responses, and explains the heterogeneity of antitumor immunity observed in patients.

Intriguingly, the current focus has shifted to neoantigen characterization. As described earlier, the recent report by Balachandran et al. [11] regarding neoantigen quality in pancreatic cancer has opened a new avenue for investigating tumor progression in the context of immunosuppression by not only the classical approach of neoantigen-HLA binding but also the long-overlooked T-cell receptor recognition. Besides, advances in neoantigen discovery methods could dramatically improve vaccine design, which might ultimately unlock the potential of personalized neoantigen vaccines. Future efforts can be anticipated to identify immunogenic hotspots for directed neoantigen targeting and to harness neoantigen-specific immunity to treat currently checkpoint blockade-resistant malignancies such as pancreatic cancer.

**Disclosure Statement**

The authors did not provide a disclosure statement.
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