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Current progress in immunotherapy for pancreatic cancer

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Abstract

Pancreatic cancer remains one of the most lethal cancers with few treatment options. Immune-based strategies to treat pancreatic cancer, such as immune checkpoint inhibitors, therapeutic vaccines, and combination immunotherapies, are showing promise where other approaches have failed. Immune checkpoint inhibitors, including anti-CTLA4, anti-PD-1, and anti-PD-L1 antibodies, are effective as single agents in immune sensitive cancers like melanoma, but lack efficacy in immune insensitive cancers including pancreatic cancer. However, these inhibitors are showing clinical activity, even in traditionally non-immunogenic cancers, when combined with other interventions, including chemotherapy, radiation therapy, and therapeutic vaccines. Therapeutic vaccines given together with immune modulating agents are of particular interest because vaccines are the most efficient way to induce effective anti-tumor T cell responses, which is required for immunotherapies to be effective. In pancreatic cancer, early studies suggest that vaccines can induce T cells that have the potential to recognize and kill pancreatic cancer cells, but the tumor microenvironment inhibits effective T cell trafficking and function. While progress has been made in the development of immunotherapies for pancreatic cancer over the last several years, additional trials are needed to better understand the signals within the tumor microenvironment that are formidable barriers to T cell infiltration and function. Additionally, as more pancreatic specific antigens are identified, immunotherapies will continue to be refined to provide the most significant clinical benefit.

Keywords

vaccine; pancreatic cancer; immunotherapy; immune checkpoint

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1. Introduction

While pancreatic cancer accounts for only 3% of all cancers in the US, it remains the fourth-leading cause of cancer related deaths in the US in 2015. The prevalence of pancreatic cancer is roughly equal between men and women. In 2015, approximately 48,960 people are estimated to be diagnosed with pancreatic, and approximately 40,560 are expected to die from the disease. The relative five-year survival for patients with pancreatic cancer is 26% if the cancer is local at the time of diagnosis and only 2% if the cancer has metastasized at the time of diagnosis (American Cancer Society, 2015). Unfortunately, however, pancreatic cancer is extremely difficult to diagnose at early stages of disease, and most patients are not diagnosed until after their cancer has metastasized. Currently, surgery remains the only potential cure for pancreas cancer, but fewer than 20% of patients are candidates for surgical resection at the time of diagnosis, and approximately 80% of patients who undergo surgical resection with a curative intent will eventually recur and die from the disease (American Cancer Society, 2015). Thus, improved strategies for treating pancreatic cancer are desperately needed.

Pancreatic cancer is highly chemotherapy and radiation therapy resistant, making treatment options extremely limited (Laheru, 2005). Although the tumor microenvironment in pancreatic cancer is highly immunosuppressive, recent advances in immune-based therapies hold promise for treating this deadly disease. Immune-based therapies aim to recruit and activate the host's T cells that recognize tumor-specific antigens (Laheru, 2005). Specifically, during the process of tumorigenesis, tumor antigens become fundamentally different from normal cellular antigens and are often referred to as neoantigens (Pardoll, 2015). These neoantigens are recognized by the host's immune system as foreign and are normally eliminated during the process of immunoediting (Pardoll, 2015). However, tumors develop mechanisms of tolerance to turn off these anti-tumor T cell responses within the tumor microenvironment. This process mimics T cell exhaustion, which occurs during a chronic immune response. Specifically, the upregulation of PDL1 (B7-H1), indoleamine 2,3-dioxygenase (IDO), IL-10, lymphocyte activation gene 3 (LAG3), and transforming growth factor- β (TGF- β) within the tumor microenvironment is correlated with an immunosuppressive microenvironment (Mahoney, 2015). During this chronic immune response, interferon- γ (IFN- γ), expressed by the T cells, causes the tumor cells to upregulate PD-1 and IDO expression, which forms a feedback loop that generates a PD-1 signal and maintains immunosuppression in a dominant manner. This immunosuppressive tumor microenvironment is the primary reason that most single agent immunotherapies have failed to show a clinical benefit (Mahoney, 2015). However, our understanding of these immunoinhibitory pathways have led to the development of immune checkpoint inhibitors to block these immunosuppressive pathways (Mahoney, 2015). Therefore, one area of pancreatic cancer immunotherapy with a high potential has involved the use of immune checkpoint inhibitors, such as anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies. By blocking these inhibitory molecules, these therapies would allow activation of a patient's pre-existing anti-cancer immune response (Mahoney, 2015; Pardoll, 2012). However, because these therapies as single agents only remove the immune suppression, but do not provide a mechanism of immune activation, the results of treating pancreatic cancer with

single-agent immune checkpoint inhibitors have been disappointing, particularly for pancreatic cancer (Royal, 2010; Brahmer, 2012). A second area of immune-targeting of pancreatic cancer involves the development of therapeutic vaccines. Specifically, these vaccines are designed to elicit immune responses to tumor-specific antigens (Laheru, 2005). These vaccines have shown great clinical promise, but have recently been found to upregulate immune checkpoints when given as single agents (Lutz, 2014). A final area of pancreatic cancer immunotherapy, which has shown the most promise, involves combining checkpoint inhibitors with therapeutic vaccines (Mahoney, 2015).

2. Immune checkpoint inhibitors

2.1 CTLA-4

Ipilimumab (YERVOY®) is a fully humanized antibody that recognizes CTLA-4 and blocks its interaction with B7-1/B7-2 on antigen presenting cells to enable T cell activation. Ipilimumab was approved by the FDA in 2011 for unresectable or advanced metastatic (stage III or IV) melanoma. Ipilimumab improved median overall survival by 3.7 months (10.1 vs. 6.4 months, $p=0.003$) compared to an investigational vaccine consisting of HLA-A*0201-restricted gp100 peptides administered with incomplete Freud's adjuvant in the pivotal phase III trial (Hodi, 2010). More recently as shown in a phase III study, median progression-free survival was 11.5 months following treatment with ipilimumab in combination with nivolumab (OPDIVO®), an anti-PD-1 antibody, compared to 2.9 months with ipilimumab alone and 6.9 months with nivolumab alone in treatment naive patients with advanced melanoma (Larkin, 2015). In multiple studies comparing the combination of ipilimumab and nivolumab to single immune checkpoint inhibitors, the objective response rate in the groups receiving both ipilimumab and nivolumab was approximately 50% versus approximately 10–20% in the groups receiving single immune checkpoint inhibitors (Larkin, 2015; Postow, 2015; Wolchok, 2013). Additional studies with ipilimumab in combination with nivolumab in metastatic renal cell carcinoma and non-small-cell lung cancer have also shown anti-tumor activity (Antonia, 2014; Hammers, 2014).

Because immunosuppressive cells infiltrate and persist in early pre-invasive pancreatic cancer lesions (Clark, 2007), blocking immunosuppressive signals appears to be essential in enhancing immune-based tumor destruction. In a phase II trial, ipilimumab was administered as a single agent to 27 patients with locally advanced or metastatic pancreatic cancer. Unfortunately, ipilimumab was ineffective as a single agent in these patients and did not prolong survival (Royal, 2010).

2.2 PD-1

Pembrolizumab (KEYTRUDA®) and Nivolumab (OPDIVO®) became the first two FDA-approved PD-1 blocking therapeutic antibodies. Nivolumab is a human IgG4 antibody that recognizes the programmed death receptor-1 (PD-1) and blocks its interaction with PD-L1 (B7-H1) and PD-L2 (B7-DC). Nivolumab was approved by the FDA in 2014 for the treatment of patients with unresectable or metastatic melanoma after demonstrating an overall survival of 72.9% at 1 year and an objective response rate of 40% comparing to an

overall survival of 42.1% at 1 year and an objective response rate of 13.9% in the dacarbazine control group (Robert, 2015; Weber, 2015).

A phase III study comparing nivolumab and docetaxel was also completed in patients with advanced, previously-treated squamous-cell non-small-cell lung cancer. The objective response rate in patients receiving nivolumab was 20% compared to 9% in patients that received docetaxel. The median progression-free survival was 3.5 months with nivolumab treatment compared to 2.8 months in patients that received docetaxel. The median overall survival was 9.2 months in patients treated with nivolumab and 6.0 months in patients treated with docetaxel (Taxotere®). This study concluded that overall survival, response rate, and progression-free survival were all significantly better in patients with advanced, previously-treated squamous-cell non-small-cell lung cancer treated with nivolumab compared to patients treated with docetaxel (Brahmer, 2015). This study led to the FDA approval of nivolumab for the treatment of patients with advanced, previously-treated squamous non-small-cell lung cancer in March 2015 (FDA, 2015).

Additionally, a phase III, randomized trial (NCT01673867) was recently completed comparing nivolumab versus docetaxel in advanced non-squamous cell, non-small-cell lung cancer (Paz-Ares, 2015). The median overall survival and objective response rate for patients receiving nivolumab were significantly improved compared to patients that received docetaxel. In pancreatic cancer, nivolumab is currently being studied as a monotherapy or in combination with other agents (see discussion below), such as ipilimumab (NCT01928394).

Pembrolizumab was the first anti-PD-1 antibody approved in 2014 for the treatment of advanced, metastatic melanoma after pembrolizumab was demonstrated to result in 38% objective response in advanced melanomas and durable response in the majority of these responding patients (Hamid, 2013). In addition, the KEYNOTE-002 study showed that pembrolizumab significantly improved the 6-month progression-free survival to 34% in the pembrolizumab 2 mg/kg group and 38% in the 10 mg/kg group compared to 16% in the chemotherapy group (Ribas, 2015). In a randomized, controlled, phase III study, 834 patients with advanced melanoma were treated with either pembrolizumab every 2 or 3 weeks or ipilimumab every 3 weeks. The objective response rate was improved with pembrolizumab administered every 2 weeks (33.7%) and every 3 weeks (32.9%), as compared with ipilimumab (11.9%) (Robert, 2015). Thus, anti-PD-1 antibody therapy, as exemplified by pembrolizumab, results in a significantly higher response rate and longer survival compared to ipilimumab.

In addition, a study (KEYNOTE-001) of non-small-cell lung cancer patients who received pembrolizumab showed anti-tumor activity of pembrolizumab with a 19.4% objective response rate in this malignant disease (Garon, 2015). Moreover, pembrolizumab has been shown to be highly effective in gastrointestinal cancers as a single agent in patients with mismatch repair deficiencies. Specifically, because somatic mutations have the potential to create “non-self” immunogenic neoantigens, patients with mismatch repair defects, which promote somatic mutations, may have increased intratumoral effector T cell responses to the neoantigens. In a phase II study, 41 patients with progressive metastatic carcinoma with or without mismatch repair deficiency were given pembrolizumab every 14 days. Whole-

exome sequencing of tumors from these patients revealed a mean of 1782 somatic mutations per tumor in mismatch repair deficient tumors and only 73 somatic mutations in mismatch repair proficient tumors. Not surprisingly, high somatic mutation loads, which are associated with high immune activities presumably aroused by mutated neoantigens, correlated with prolonged progression-free survival ($p=0.02$). The immune-related objective response rate was 40% (4 out of 10 patients) in colorectal cancer patients with mismatch repair deficiencies compared to 0% (0 out of 18 patients) in patients with mismatch repair proficient tumors. Additionally, patients with mismatch repair deficient cancers had a progression-free survival of 5.4 months compared to 2.2 months for patients with mismatch proficient cancers (Le, 2015a). Due to the impressive clinical response seen in this trial, this study is currently being expanded to include 50 more patients with mismatch repair deficient cancers, including pancreas cancer (NCT01876511). Nevertheless, mismatch repair deficiency is only present in approximately 10–20% of gastrointestinal malignancies (Koopman, 2009) and less than 5% of pancreatic cancers (Goggins, 1998); thus, effective immune-based therapeutic strategies are highly demanded for the majority of malignancies that are naturally immune quiescent.

An additional immune checkpoint target is one of the ligands for PD-1, PD-L1. Roche/Genentech have developed an anti-PD-L1 antibody, MPDL3280A, which has been tested in multiple cancers such as melanoma, non-small-cell lung cancer, non-squamous cell lung cancer, squamous cell lung cancer, and renal cell carcinoma. In the study by Herbst and colleagues (2014), patients with incurable cancers received MPDL3280A intravenously every 3 weeks. The overall response rate across all tumor types in this study was 36%, with the highest response being observed in melanoma. Not surprising, this study found that the higher the PD-L1 expression in the tumor, the better the response to the MPDL3280A treatment. This trial (NCT01375842) is currently on-going and recruiting patients with various solid cancers.

A phase I study conducted by Brahmer and colleagues (2012) evaluated Bristol-Myers-Squibb's anti-PD-L1 antibody, BMS-936559, in patients with advanced cancer. In this study, a total of 207 patients with non-small-cell lung cancer, melanoma, colorectal cancer, renal cell cancer, ovarian cancer, pancreatic cancer, gastric cancer, or breast cancer were treated for 2 to 111 weeks (median duration 12 weeks) with BMS-936559. Tumor regression was observed between 6 to 17% of patients and prolonged disease stabilization (>24 weeks) was observed in 12 to 41% of patients, again with melanoma patients having the highest response rate (Brahmer, 2012).

Medimmune/AstraZeneca have also developed an anti-PD-L1 antibody, MEDI4736. In the phase I/II trial (NCT01693562) of MEDI4736, 10–20 patients per cancer type (non-small-cell lung cancer, melanoma, gastroesophageal, hepatocellular carcinoma, pancreatic, squamous cell carcinoma of the head and neck, and triple negative breast cancer) were initially enrolled. The patient population was then expanded when clinical activity was observed. MEDI4736 was administered intravenously every 2 weeks for 12 months, with re-treatment being initiated if the patient progressed after 12 months of treatment. Anti-tumor activity was observed in multiple tumor types, including melanoma, head and neck, and gastroesophageal cancer (Segal, 2014). MEDI4736 is currently in a phase III clinical trial for

non-small-cell lung cancer, which is anticipated to enroll a total of 702 patients across 100 sites globally (NCT02352948).

2.3 Checkpoint inhibitor sensitive and insensitive diseases

Although immune checkpoint inhibitors have successfully achieved durable responses in many different types of malignant diseases, they are only effective in a fraction of patients in each type of malignant disease (Table 1). Therefore, the use of immune checkpoint inhibitors is not limited to specific malignant disease types but is more likely limited to malignant diseases with specific immunobiologic characteristics. PD-L1 expression has been suggested to predict the response to anti-PD-1/PD-L1 antibody therapies. However, no consensus on a reliable PD-L1 staining assay has been made. A more prominent immunobiologic characteristic of immune checkpoint inhibitor sensitive malignant diseases is abundant effector T cell infiltration. This is better characterized in melanoma treated with immune checkpoint inhibitors (Taube, 2014), but is also seen in other cancer types (Lipson, 2013). Essentially all the checkpoint inhibitor sensitive tumors are abundantly infiltrated with CD8+ T cells and can be classified as “immune active” tumors. “Immune quiescent” tumors, which lack infiltration of effector T cells that are the targets of immune checkpoint inhibitors, are almost always resistant to single agent checkpoint inhibitor treatment. All pancreatic and colorectal cancers, except those with mismatch repair deficiencies, are considered to be immune quiescent tumors and are insensitive to therapeutic single agent checkpoint inhibitors.

3. Therapeutic vaccines

Because pancreatic cancer is immune quiescent and naturally resistant to radiation and chemotherapy, alternative treatment options are desperately needed. One alternative treatment that has shown significant promise is the use of therapeutic vaccines. Specifically, these vaccines involve administering pancreatic tumor antigens to stimulate the patient’s own immune system to recognize the distinct, small antigenic differences between tumor cells and normal pancreas cells. Because the patient’s own immune system is recruited to fight the cancer, this treatment is specific and results in minimal toxicities. Currently, there are two major types of therapeutic vaccines being tested in the treatment of pancreatic cancer, whole-cell vaccines and antigen-specific, vector-based vaccines (Table 2).

3.1 Whole-cell vaccines

Two major whole-cell vaccines have shown significant promise in the treatment of pancreatic cancer. First, Jaffee and colleagues (2001) developed an allogenic granulocyte-macrophage colony-stimulating factor (GM-CSF) whole cell pancreatic tumor vaccine (GVAX) by stably transfecting two different human tumor cell lines with the human cytokine GM-CSF, which is a potent cytokine capable of mobilizing monocytes, eosinophils, and lymphocytes into the tumor. These cells are then irradiated and administered to patients intradermally. In the phase I study, 14 patients with stage 1, 2, or 3 pancreatic ductal adenocarcinoma (PDA) received multiple vaccinations of between 1×10^7 and 50×10^7 irradiated vaccine cells following pancreaticoduodenectomy. In this study, 3 patients who

received higher numbers of vaccine cells and developed delayed-type hypersensitivity (DTH) remained disease-free for greater than 10 years (Jaffee, 2001).

After the phase I study showed that GVAX was well tolerated and resulted in only minor toxicities with the most common being erythema, induration, and mild pain at the vaccination sites that were self-limiting, a phase II study was conducted with 60 patients with resected PDA. Patients received 5 vaccinations of 5×10^8 irradiated, GM-CSF secreting vaccine cells following surgical resection, in addition to 5-FU-based chemoradiation. The median disease-free survival and median overall survival in these patients was 17.3 months and 24.8 months, respectively. An important observation in this study was that the vaccine induced mesothelin-specific CD8+ T cells responses in HLA-A1+ and HLA-A2+ patients, which correlated with disease-free survival (Lutz, 2011).

For metastatic PDA, Laheru and colleagues (2008) compared vaccine alone with vaccine administered in sequence with cyclophosphamide (Cy). Several preclinical studies have previously shown that immune modulating doses of Cy can enhance vaccine induced anti-tumor immune responses by inhibiting immune suppressive CD4+/CD25+ regulatory T cells (Tregs) (Berd, 1986; Ercolini, 2005; Holmberg, 2001; Thomas, 2004). Laheru and colleagues (2008) demonstrated that administering Cy one day prior to vaccine results in enhanced immune responses with minimal toxicity.

To study whether vaccine-based immunotherapy can modify the immune quiescent microenvironment of pancreatic cancer, GVAX was given alone or in combination with Cy in the preoperative setting for surgically resectable PDAs. Examination of tumor infiltrating immune cells revealed vaccine-induced intratumoral tertiary lymphoid aggregates. Multiple immune regulatory gene expression signatures were identified in these lymphoid aggregates, including those showing a suppressed Treg pathway and an enhanced Th17 pathway within these aggregates was associated with improved survival, enhanced post-vaccination mesothelin-specific T-cell responses, and increased intratumoral effector T cells to Tregs ratios. This study was the first to demonstrate that immune-based therapies can convert non-immunogenic tumors into immunogenic tumors (Lutz, 2014). This study also suggested that vaccine therapy alone would not be optimal for the majority of the patients and that further combining vaccine therapy with immune modulating agents would further optimize this immunotherapy for pancreatic cancer. Importantly, this study found that aggregate formation resulted in the upregulation of immune checkpoint signals, such as PD-1 and PD-L1, indicating that priming the patients with the GVAX therapy may overcome the lack of response of pancreatic cancer patients to single-agent immune checkpoint inhibitor treatments (Lutz, 2014).

The second major whole-cell vaccine demonstrating clinical promise in pancreatic cancer is algenpantucel-L, an irradiated, live combination of two human allogeneic pancreatic cancer cell lines expressing murine α -1,3-galactosyl transferase (α GT), which directs the synthesis of α -galactosyl (α Gal) epitopes on surface proteins and glycolipids on the cell lines. alenpantucelA-L induces complement-mediated lysis and antibody-dependent cell-mediated toxicity toward algenpantucel-L cells to generate hyperacute rejection of allografts in humans. Hardacre and colleagues (2013) conducted a phase II study of algenpantucel-L with

5-FU and gemcitabine adjuvant chemoradiotherapy in 70 patients with resected pancreatic cancer. The 12-month disease-free survival and 12-month overall survival were 62% and 86%, respectively. A multi-institutional, phase III study is being conducted and has completed accrual (NCT01072981).

3.2 Antigen-specific vaccines

Advances in technology have enabled the identification of new pancreatic tumor antigens. Peptide vaccines against these antigens are being developed in the treatment of pancreatic cancer. A major peptide vaccine currently under development for the treatment of pancreatic cancer is the Kras peptide vaccine. Mutant Kras is found in approximately 90% of patients with pancreatic cancer and is specific to tumor cells (Gjertsen, 1995; Gjertsen, 1996; Gjertsen, 2001; Abou-Alfa, 2011). However, one major challenge of peptide vaccines is the HLA-type restriction that has limited vaccine use to HLA-type matched patients. Another major challenge is that immune evasion occurs more frequently when the peptide vaccine's anti-tumor activity depends on an immune response to a single epitope. A third challenge is that peptide- or protein-based vaccines would need to be combined with adequate immune adjuvants or be carried by a vector to elicit a strong immune response. Therefore, immune dominant antigens that can convey robust immune response to multiple epitopes are being evaluated, and new vaccine vector systems are being developed (Kast, 2002).

Mesothelin-specific immune responses were observed in patients with increased disease-free survival, in patients who received GVAX; therefore, mesothelin became a candidate for protein specific vaccines. A recombinant live-attenuated, double-deleted *Listeria monocytogenes* engineered to secrete tumor antigens into the cytosol of infected antigen presenting cells for processing and presentation by the antigen presenting cells, was developed (CRS-207) and previously found to induce both innate and adaptive immunity to antigens (Brockstedt, 2004; Le, 2012). CRS-207 was tested in sequential combination with GVAX and administered to patients with metastatic pancreatic cancer. In this phase II trial, Cy/GVAX + CRS-207 was compared to Cy/GVAX alone. Overall survival for all patients receiving Cy/GVAX + CRS-207 was 6.1 months compared to 3.9 months for those receiving only Cy/GVAX. In patients that received at least 3 doses of Cy/GVAX, overall survival was 9.7 months for patients that also received CRS-207 compared to 4.6 months for patients that only received Cy/GVAX (Le, 2015b). A listeria-based vaccine for Annexin A2, a novel pancreatic cancer antigen shown to be involved in metastasis of PDA (Zheng, 2011; Foley 2015), is currently underway (Zheng, 2012).

3.3 Neoantigen-based vaccines

Accumulating evidence has supported the immunogenicity of genomic mutations in neoplasms. Mutated genes, if expressed in neoplasms, result in neoepitopes, which can be processed and recognized by T cells (Segal, 2008). Indeed, neoplasms with higher mutation rates are more abundantly infiltrated with effector T cells. However, the patient's immune system must be tolerant to these neoepitopes because it has allowed the neoplasm to grow. Therefore, an effective immunotherapy must break this tolerance. The major tolerance mechanism appears to be the immune checkpoints, because PD-L1 becomes highly expressed on these neoplasms during tumor development and PD-1 becomes upregulated on

T cells. Therefore, as expected, these types of neoplasms are more sensitive to immune checkpoint inhibitor treatments (Schumacher, 2015).

Pancreatic cancers carry genomic mutations, although the frequency of the mutations in pancreatic cancer are not as high as that of melanoma or non-small-cell lung cancer. Nevertheless, pancreatic cancer is believed to express neoepitopes, making a vaccine strategy targeting these neoepitopes possible. Therefore, the future development of pancreatic cancer vaccines should consider targeting neoantigens. Theoretically, a neoantigen-based vaccine should be more immunogenic because the neoepitopes would less likely to evade the immune system. However, neoantigen-based vaccines may also activate immune checkpoints. Thus, combining neoantigen-targeting vaccines with immune checkpoint inhibitors is necessary to achieve an optimal anti-tumor immune response.

4. Combinatorial immunotherapy

While immune checkpoint inhibitors alone have shown promise in other types of cancers, immune checkpoint inhibitors are not effective as single agents in the treatment of pancreatic cancers (Brahmer, 2012). The tumor microenvironment in pancreas cancer is predominately infiltrated with immune suppressive cells and is sparsely infiltrated with immune responsive cells (Clark, 2009; von Bernstorff, 2001). Therefore, the tumor microenvironment would first need to be primed with effector T cells before immune checkpoint inhibitors could play their roles. Vaccine-based therapies are the most efficient way to induce effector T cell infiltration into the tumors.

Preclinical research supports the concept of synergy between cancer vaccines and immune checkpoint blockade in non-immunogenic tumors. Specifically, Soares and colleagues (2015) have shown that PD-L1 is weakly expressed in untreated pancreatic tumors from both humans and mice, but treatment with GVAX significantly upregulates PD-L1 expression on the membrane of pancreatic tumor cells. This data supports the clinical observations made by Lutz and colleagues (2014). The combination of GVAX and anti-PD-1 therapy significantly improved survival of tumor-bearing mice compared to PD-1 monotherapy or GVAX alone. Furthermore, PD-1 blockade increased effector T cell infiltrates into the tumor microenvironment and tumor-specific interferon- γ production (Soares, 2015). This preclinical study supports the combination of GVAX and immune checkpoint inhibitors in the treatment of pancreatic cancer.

Combinational immunotherapy approaches with therapeutic vaccines and immune checkpoint inhibitors are beginning to show promise clinically in the treatment of pancreatic cancer (Le, 2013). In a pilot study, ipilimumab alone (Arm 1) or in combination with GVAX (Arm 2) was evaluated in 30 patients with previously treated, advanced PDA in a Phase Ib study. Patients were given induction doses every 3 weeks for a total of 4 doses and then received maintenance doses every 12 weeks. GVAX was given prior to ipilimumab to patients in Arm 2. Objective responses were observed in 20% of patients receiving the combination of ipilimumab and GVAX in Arm 2, whereas none of the 15 patients in Arm 1 responded to single agent ipilimumab. The median overall survival for patients in Arm 1 was 3.6 months compared to 5.7 months in Arm 2. In patients with an overall survival of greater

than 4.3 months, there was an increase in the number of mesothelin-specific T cells as well as an enhancement of the T cell repertoire (Le, 2013).

More than 70% of patients receiving ipilimumab experienced high grade autoimmune-related adverse events compared to approximately 12% or less of patients receiving anti-PD-1 antibodies (Gangadhar, 2014). Therefore, combining therapeutic vaccines with anti-PD-1 antibodies may be safer and more feasible in the treatment of pancreatic cancer. To this end, a phase I/II study with GVAX and nivolumab entitled, “A randomized study of a GM-CSF secreting allogenic pancreatic cancer vaccine (GVAX) with or without a PD-1 blockade antibody (nivolumab) for the neoadjuvant and adjuvant treatment of patients with surgically resectable adenocarcinoma of the pancreas” is about to begin recruiting patients (NCT02451982). Additionally, “A randomized, phase II study of the safety, efficacy, and immune response of GVAX pancreas vaccine (with cyclophosphamide) and CRS-207 with or without nivolumab in patients with previously treated metastatic pancreatic adenocarcinoma” (NCT02243371) is currently open and actively recruiting patients.

5. Prospective

Pancreatic cancer remains a difficult cancer to treat with very few treatment regimens showing substantial improvement in survival. Currently, the only potential cure for PDA is surgical resection. However, most patients with local disease eventually recur after surgical resection of their tumor. Immunotherapy has the potential to treat minimal residual disease and prevent recurrence with minimal toxicity to patients, and studies in patients with metastatic and nonresectable disease have begun to show the utility of immunotherapy in these patients (Laheru, 2008; Le, 2015b).

Combination therapies should be the emphasis of immunotherapy research to better understand the ideal combination needed to treat each unique tumor in the majority of patients who do not respond to single agent checkpoint inhibitors (Table 1). A promising vaccine therapy approach is to target neoantigens, which would be less likely to be subjected to immune evasion. In addition to using vaccine-based therapies to prime the tumor microenvironment with effector cells, other therapeutic strategies including epigenetic modifiers, stroma modulators, radiotherapy, and T cell transfer therapies may also prime pancreatic cancer for immune checkpoint inhibitor therapies (Foley, 2013; Neureiter, 2014; Rucki, 2014; Wolfgang, 2013).

Both vaccine therapy and immune checkpoint inhibitors work on T cells. However, T cells are not the only determinant cell population. Macrophages and MDSCs are known to be important for pancreatic cancer development (Clark, 2007; Stromnes, 2014; Zheng, 2013), and thus, may also play an important role in anti-tumor immune responses. In the future, combination therapy approaches should include therapies that target multiple types of tumor infiltrating immune cells.

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Highlights

- Pancreatic cancer remains one of the most lethal cancers with few treatment options
- Immune-based strategies are showing promise where other approaches have failed
- Immunotherapies include immune checkpoint inhibitors and therapeutic vaccines
- Combining immune checkpoint inhibitors with therapeutic vaccines is most effective

Table 1

The objective response rates of “immune active” tumors and “immune quiescent” tumors to immune checkpoint inhibitors.

Classification	Tumor type	Objective Response Rates	References
Immune active tumors	Melanomas (with a combinational treatment of nivolumab and ipilimumab)	53%	Wolchok et al., 2013
	Non-small cell lung cancer (Squamous cell type)	20%	Brahmer et al., 2015
	Non-small cell lung cancer (Adenocarcinoma)	19.2%	Paz-Ares et al., 2015
	Gastroesophageal adenocarcinoma (PD-L1+ tumor)	22%	Muro et al., 2015
	Colorectal carcinoma (MSI tumors)	40%	Le et al., 2015a
	Renal cell cancer	20–30%	Motzer et al., 2015; McDermott et al., 2015
	Squamous cell carcinoma of the head and neck	18.2%	Seiwert et al., 2015
	Hepatocellular carcinoma	23%	El-Khoueiry et al., 2015
	Hodgkin's Lymphoma	87%	Ansell et al., 2015
Immune quiescent tumors	Pancreatic adenocarcinoma	~0%	Brahmer et al., 2012
	Colorectal carcinoma (MSS tumors)	0%	Le et al., 2015a
	Non-responsive melanoma, NSCLC, GA, RCC, SCCHN, HCC, etc.	0%	See above

NSCLC, non-small cell lung cancer; SCCHN, squamous cell carcinoma of the head and neck; HCC, hepatocellular carcinoma; GA, gastroesophageal adenocarcinoma; RCC, renal cell carcinoma; MSI, microsatellite instable; MSS, microsatellite stable.

Table 2 Summary of selected clinical trials of cancer vaccine and immunotherapy with cohorts of pancreatic cancer patients.

Vaccine Type	Investigator	Phase	Stage	Therapy	Clinical and Immunological Outcomes
Immune checkpoint inhibitors	Royal (2010)	II	27 patients with locally advanced PDA	Ipilimumab	No responders by response evaluation criteria.
	Brahmer (2012)	I	14 patients with advanced PDA	Nivolumab	No objective responses were observed.
Therapeutic vaccines	Segal (2014)/NCT01693562	I/II	10–20 patients with PDA	MED14736	Anti-tumor activity was preliminarily reported for some PDA patients at the 2014 ASCO annual meeting.
	Jaffee (2001)	I	14 patients with resected PDA	GM-CSF vaccine (GVAX) with chemoradiotherapy	3 patients developed DTH and remained disease-free for greater than 10 years.
	Lutz (2011)	II	60 patients with resected PDA	GM-CSF vaccine (GVAX) with chemoradiotherapy (5FU)	Median disease-free survival was 17.3 months and median overall survival was 24.8 months. Vaccine induced mesothelin-specific CD8+ T cell responses were observed in HLA-A1+ and HLA-A2+ patients, which correlated with DFS.
	Laheru (2008)	II	50 patients with advanced PDA	GM-CSF vaccine (GVAX) with cyclophosphamide (Cy)	Median survival in patients given Cy 1 day before vaccine was 4.3 months (2.3 months without Cy). Mesothelin-specific CD8+ T cell responses in HLA class I patients were enhanced with Cy.
	Lutz (2014)	pilot	54 patients with resected PDA	GM-CSF vaccine (GVAX) with cyclophosphamide (Cy)	Vaccine-induced intratumoral tertiary lymphoid aggregates were observed. Enhanced survival and mesothelin-specific T cell responses were observed in patients with a suppressed Treg pathway and an enhanced Th17 pathway. Upregulation of PD-1 and PD-L1 in the tumor was observed after vaccination.
	Hardacre (2013)	II	70 patients with resected PDA	Algenpantucel-L with 5-FU and gemcitabine	Disease-free survival was 62% at 1 year. Overall survival was 86% at 1 year.
	Gjertsen (1996)	I/II	5 patients with histologically confirmed PDA	Mutated Kras peptide	2 patients with an immune response showed longer survival.
	Gjertsen (2001)	I/II	48 patients, 10 with surgically resected PDA and 38 with advanced PDA	Mutated Kras peptide with GM-CSF	Peptide specific immunity was induced in 58% of evaluable patients. Active CD4+T cells specific for mutant Kras were found in the tumor post-vaccination. Patients with advanced PDA were able to develop an immune response to the peptide vaccine, which correlated with prolonged

Vaccine Type	Investigator	Phase	Stage	Therapy	Clinical and Immunological Outcomes
	About-Alfa (2011)	I	24 patients with resected PDA	Mutated Kras peptide	Median recurrence free survival was 8.6 months, while median overall survival was 20.3 months. Only 1 patient (11%) had detectable immune responses specific to the patient's Kras mutation, which was assessed as a DTH. 3 patients (13%) displayed non-specific DTH responses.
	Le (2012)	I	7 patients with PDA	CRS-207	Vaccine-induced immune responses against listeria were observed in 3 of 7 PDA patients who had an overall survival of 15 months. Induction of mesothelin-specific immune responses did not correlate with overall survival in this small study.
Combinatorial immunotherapy	Le (2013)	Ib	15 previously treated patients with advanced PDA	Ipilimumab + GM-CSF vaccine (GVAX)	Median overall survival was 5.7 months and 1 year overall survival was 27%. Patients with an overall survival > 4.3 months had an increase in mesothelin-specific T cells and enhancement of the T cell repertoire.
	Le (2015b)	II	61 previously treated patients with advanced PDA	GM-CSF vaccine (GVAX) with cyclophosphamide (Cy) + CRS-207	Median overall survival was 9.7 months. An increase in mesothelin-specific CD8+ T cell responses were observed at 20 weeks. Robust mesothelin-specific CD8+ T cell responses was associated with longer overall survival.