

## Review



## Recent Advancements in Pancreatic Cancer Immunotherapy

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### Abstract

The overarching goal of this review is to highlight the current progress of immunotherapy in pancreatic cancer. Despite all the efforts, pancreatic cancer remains a disease that is refractory to almost all therapies and immunotherapy for pancreatic cancer remains in its infancy. In this review, we summarize promising advances and major hurdles in developing immunotherapeutic strategies for pancreatic cancer as presented in recent publications and a clinical trial database. Notably, a combination regimen of chemotherapy followed by immunotherapy appears to be superior to chemotherapy alone, wherein chemotherapeutic agents may play a dual role by reducing overall tumor burden through direct killing of cancerous cells and by indirect release of pro-inflammatory molecules and tumor associated antigens which, when presented in an immunogenic fashion, may function as an in situ “vaccine”. Critically, the timing of administration of standard chemotherapy can markedly impact the induction of antitumor responses. We anticipate that among the gamut of combination immunotherapy and chemo-radiation therapies that are now being evaluated, we will eventually be able to optimize a regimen that can generate long-lasting responses and usher a new weapon in the fight against pancreatic cancer.

**Key Words:** pancreatic cancer; immunotherapy; tumor microenvironment

### Introduction

Immunotherapy has emerged as a pillar (1) in cancer treatment through its gradual acceptance as standard of care for some liquid and solid tumor malignancies, such as: B cell leukemia (2), metastatic melanoma (3, 4) and Non-Small-Cell Lung Cancer (NSCLC) (5, 6),

joining the ranks of conventional modalities such as surgery, chemo/radiation, and targeted therapies. While this era has witnessed remarkable success in immune-based therapies for B cell malignancies as well as more limited effects on solid tumor malignancies such as melanoma and NSCLC, advanced

pancreatic cancer is a disease that remains refractory to almost all the therapies (7, 8). However evidence of success in other solid tumors and a renaissance in our understanding of pancreatic tumor biology has led to translational efforts to pioneer immune-based approaches for the treatment of pancreatic cancer that has been accompanied by significant progress in the field (9).

Here, we present a review of the literature and clinical databases, highlighting promising advances and major hurdles in immunotherapy of pancreatic cancer, in the hope that it may provide insight into potential combination immune-based strategies and a rational therapeutic window within which these approaches may be most effective.

### **Current therapies against pancreatic cancer**

Pancreatic cancer is a devastating and almost uniformly lethal malignancy, notoriously resistant to most therapies. Over 90% of pancreatic cancers present as pancreatic ductal adenocarcinoma (PDAC). In 2015, ~48,960 people in the United States will be diagnosed with this disease (10). Because of the aggressive nature of this cancer, the annual mortality rates closely match the incidence rate, and it is expected that ~40,000 will die from PDAC in the same year, with a 6% 5-year survival rate. For the 20% of patients with disease involving only the pancreas, surgical resection is the primary therapy. In the ~80% of patients with regional disease extension or metastases at presentation, chemotherapy is the primary treatment (11). Among the therapeutic options, polychemotherapy with infusional 5-fluorouracil (5-FU), leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) or gemcitabine plus albumin-bound paclitaxel (nab paclitaxel) are commonly employed (11). Nab-paclitaxel is a cytotoxic agent that binds to overexpressed SPARC (Secreted Protein Acidic and Rich in Cysteine) in cancer cells and stroma in the PDAC tumor microenvironment (TME), thus increasing drug delivery and accumulation in tumor cells. As compared to gemcitabine alone, nab-paclitaxel plus gemcitabine significantly improved overall survival, progression-free survival, and response rate in metastatic PDAC, but rates of peripheral neuropathy and myelosuppression were increased (12). However, because of the inherent chemoresistance of PDAC,

median progression-free survival (PFS) with these intensive regimens is  $\leq 6$  months (12, 13). Approximately 45% of patients who receive such first-line regimens are alive and sufficiently fit to receive second-line therapy (12, 13), but only a few therapies have been approved by the Food and Drug Administration (FDA) for such patients. On October 22, 2015 the U.S. FDA approved Onivyde (irinotecan liposome injection), in combination with fluorouracil and leucovorin, to treat patients with advanced (metastatic) pancreatic cancer who have been previously treated with gemcitabine-based chemotherapy. Onivyde represented a nanotherapeutic version of chemotherapy that directly delivers the agent to the tumor microenvironment and prolongs the active drug uptake. Recent results from these pilot studies demonstrated promise for pancreatic cancer with increased median survival for 2 months (14). Despite off-label use of existing chemotherapeutic agents, survival at 2 years is  $< 10\%$ . Thus, while antitumor benefit has been observed with such regimens, toxicity is substantial and therapeutic options are limited. Novel, less toxic approaches are desperately needed for this lethal cancer, particularly in patients who experience failure of existing first-line therapies. Despite these efforts, the best supportive care and treatment regimens for patients with pancreatic cancer can only prolong survival for a few months, with the goal of achieving palliation rather than cure (15).

### **Tumor microenvironment (TME) is a barrier to immune infiltration in pancreatic cancer**

In the multistep progression of PDAC, the emergence of initiating genetic events within neoplastic cells, such as oncogenic *KRAS* mutations, is subsequently sculpted by additional pressures in the TME, including co-opting of the innate and adaptive arms of the host immune system (16). The importance of the TME in the biology of this disease, as well as a potential substrate for therapy, has become increasingly recognized (17-19). PDAC exists in a complex desmoplastic stroma that provides a structural framework for tumor growth, and conceals the tumor cells from immune surveillance. Tumor-associated stroma comprises a mix of fibroblasts (myofibroblasts

and pancreatic stellate cells) and an abundance of immunosuppressive regulatory T cells (Tregs), myeloid derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and mast cells (20-22) that restrain immunologically-mediated tumor cell killing (23). PDAC cells secrete chemokines that recruit this myriad of immune suppressor cells to the TME, which are then activated either by direct contact, or by cancer cell-derived triggers to selectively release additional tumor-promoting mediators (17, 20). In preclinical models, genetic or pharmacological modulation of either the number, or function, of immune suppressor cells in the TME has been shown to decrease the growth of PDAC (24-26). However, none of these approaches have yet made their way into the clinic, and therefore, single-agent immunotherapy approaches attempted to date, such as checkpoint inhibitors or vaccines, have been at best modestly successful (27-33). In contradistinction to melanoma, PDAC has long been considered a “non-immunogenic” neoplasm, in part due to the formidable barrier of the TME. Converting a non-immunogenic PDAC into an “immunogenic” neoplasm may create a treatment window for judicious application of immunotherapeutic strategies (30, 34, 35).

#### **Current strategies in cancer immunotherapy**

With great strides in the understanding of immunology and cancer biology over the past 20 years, cancer immunotherapy has emerged as a model for successfully translating scientific discovery from the laboratory to the clinical arena. Effective immunotherapies have mediated durable antitumor immune response and cancer regressions, leading to remarkable prolongation in the survival of patients with leukemia, melanoma, lung cancer, liver, bladder, ovarian, renal, and prostate cancers (36). On the heels of these studies, immunotherapy may be poised to revolutionize in similar fashion the treatment of pancreatic cancer. We summarize below the current immunotherapeutic strategies for human cancers in Table 1.

#### **Current clinical trials for pancreatic cancer**

Pancreatic cancer mouse models provide an entry point for the study of immunotherapy in this disease.

Syngeneic orthotopic pancreatic cancer allograft mouse models assessing the potency of immune reagents have demonstrated the ability to eliminate small burdens of established tumors, a situation that recapitulates the condition of minimal residual disease commonly found after surgical removal of human tumors (37). In an example of this approach, we have demonstrated that by stabilizing the mast cell membrane, we are able to cure mice with pre-established pancreatic cancer by blocking immunosuppression and unleashing an enhanced endogenous anti-tumor immune response (20, 21).

Genetically engineered mouse models (GEMM) that yield spontaneous PDAC have led to renewed enthusiasm to exploring potential therapies with a mechanistic perspective. A commonly used, well-validated, clinically relevant model, using KrasG12D;Trp53R172H;Pdx1-Cre (KPC) mice (48), can recapitulate the heterogeneity of tumorigenesis and the TME of human PDAC. Similar to the clinical scenario among patients, these mice are largely resistant to conventional therapies allowing the full spectrum of current immunotherapy strategies to be tested in this model; however, with only sporadic success. To date, there is no FDA approved immunotherapeutic regimen for the specific treatment of pancreatic cancer. On the basis of additional studies using novel immune modulatory combinations, in multiple pre-clinical PDAC models, a new wave of clinical trials was launched. In the following Tables 2-16, we surveyed the database of clinicaltrials.gov to identify the most recent immune-based clinical regimens. The key words used in the search included: immune, immunotherapy, vaccine, antibody, antigen, CTLA-4, PD-1, tumor infiltrating lymphocyte (TIL), IDO, CAR-T, mesothelin.

#### **CD40 agonist trials**

As a member of the tumor necrosis factor receptor superfamily, CD40 is expressed on the cell surface of immune cells, in particular B cells, dendritic cells, macrophages and monocytes as well as some normal and malignant cells. Binding of CD154 (the primary ligand for CD40) on T cell surface, CD40 activates T cells by ligand-receptor direct interaction. In addition, this binding indirectly activated T cells by CD154 mediating cytokine release from dendritic cells and

Table 1. Current immunotherapeutic strategies on human cancers

| Category  | Examples  | Description   | Tables             |
|---|---|---|--------------------|
| <b>Vaccines</b>   | Whole cell vaccines,<br>Peptide vaccines,<br>Dendritic cell vaccines (9),<br>Listeria-based vaccines,<br>Recombinant virus-based<br>vaccine | In contrast to traditional prophylactic vaccines against infection, cancer vaccines induce an immune response involving both cellular (T cell), as well as humoral immunity (37, 38). Recent vaccine trials have represented some of the most successful modalities during the past decade for pancreatic cancer patients (38). | 3, 5, 6,<br>14, 15 |
| <b>Antibodies that target tumor antigens</b>  | Monoclonal antibodies,<br>Bi-specific antibodies (e.g. bi-specific T-cell engager, BiTE, and dual-affinity re-targeting, DART) (39)         | Tumor-binding immunoglobulin can kill tumor cells, either directly by fixing complement, or by inducing antibody-dependent cell-mediated cytotoxicity (ADCC), or indirectly, following opsonization and presentation by APCs to elicit T cell responses (40).   | 4, 13              |
| <b>Antibodies that target checkpoint pathways and co-inhibitory/ co-stimulatory molecules</b> | Anti-CTLA-4,<br>Anti-PD-1/Anti-PD-L1  | Ipilimumab (Anti-CTLA-4), pembrolizumab (Anti-PD-1), and nivolumab (Anti-PD-1) (1) target inhibitory molecules involved in regulation of T cell response, leading to boosting of the anti-tumor immune response (41).   | 8, 9               |
| <b>Adoptive cell therapy</b>  | Peripheral blood-derived antigen-specific T cells (7),<br>Tumor-infiltrating lymphocytes (TIL) (8)  | The use of endogenous T cells derived from peripheral blood or tumor infiltrating lymphocyte populations has been successfully used to treat patients with several solid tumors (42).   | 10,                |
| <b>Adoptive cell therapy</b>  | Engineered T cells (43)   | T cells transduced to express chimeric antigen receptors (CAR) can be genetically engineered not only to redirect specificity to desired antigen, but also confer enhanced function, persistence and safety (44-46).  | 11                 |
| <b>Non-specific adjuvants</b>   | Cytokines, such as interleukins (e.g. IL-2, IL-15) or Interferon  | These may be co-administered for facilitate the in vivo function of immune cells and/ or modulate the tumor cells and TME.  | 16                 |
| <b>Targeting on tumor microenvironment</b>  | Anti-CD40, inhibitors to BTK or IDO   | Overcoming the immunosuppressive microenvironment may sensitize combination immunotherapy.  | 2, 7, 12           |

\*Format of the Tables adopted from the previous review (47). All the NCT numbers and their corresponding details are from [clinicaltrials.gov](http://clinicaltrials.gov).

Addition notes for all Tables:

No: Number of patients (estimated number for ongoing trials); PR: partial responses; SD: stable disease; PD: progressive disease; DFS: disease-free survival; PFS: progression-free survival; OS: overall survival; GVAX: Granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting tumor vaccine or GVAX pancreatic cancer vaccine.

macrophages (49). Anti-CD40 agonist antibodies induce tumor regression by both stimulating T cell activation (T-cell-dependent) and macrophage reprogramming (T-cell-independent) (50). It is believe that the T-cell-independent mechanism is related to turnaround of the pro-tumorigenic macrophage and interference of the desmoplastic tumor stroma in PDAC. Several trials (see Table 2) using agonistic CD40 monoclonal antibody CP-870,893 (51) have

demonstrated effective anti-tumor activity in PDAC patients.

#### **GVAX and CRS-207**

GVAX is a vaccine comprised of tumor cells transfected with GM-CSF, which recruits and activates dendritic cells (DCs) *in vivo*. As highly efficient T-cell activator, DCs in the TME is required for stimulating a robust adaptive anti-tumor immune response due to its

Table 2. Anti-CD40 clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References                 | Trial subjects   | Phase | Immune<br>intervention<br>target                                 | No<br>/ | Status / Results / Impact (Median<br>survival)   |
|--|--|-------|--|---------|--|
| <b>NCT00711191</b><br><b>2013</b><br><b>(29)</b> | Chemotherapy-naïve surgically incurable pancreatic cancer (Dose escalation open label) | I     | anti-CD40 agonist monoclonal antibody Gemcitabine                | 22      | 19% had PR, 50% had SD, and 18% had PD. Median PFS was 5.6 months, and the median OS was 7.4 months. Gemcitabine alone achieves a historical tumor response rate of 5.4%, with median PFS of 2.3 months and median OS of 5.7 months. Therefore, combination treatment showed efficacy. |
| <b>NCT02588443</b><br><b>2015</b>                | Newly diagnosed resectable pancreatic carcinoma (Neo-adjuvant)                         | I     | anti-CD40 agonist monoclonal antibody Nab-Paclitaxel Gemcitabine | 10      | Active, not recruiting   |
| <b>NCT02225002</b><br><b>2014</b>                | Solid tumors (Open-label, dose-escalation)   | I     | anti-CD40 agonist monoclonal antibody                            | N       | Completed  |

ability to present tumor-associated antigens to T cells and maintain the antigen-specific T cell potency (38). CRS-207 (52) is a live-attenuated recombinant *Listeria monocytogenes* bacterium engineered to express human mesothelin that was overexpressed in pancreatic cancerous ducts but not in pancreatitis and normal tissues. CRS-207 is capable of both delivering mesothelin antigen into both class I and II antigen-processing pathways to induce tumor-specific T cell response and triggering cytokine release to boost natural killer (NK) cell activation. As a whole-cell vaccine platform, GVAX and CRS-207 form a potent sequential 2-vaccine program. On the basis of safety, feasibility, and immune activation (53) with GVAX plus CRS-207, other regimens, such as chemo/radiotherapy, checkpoint control antibodies were introduced to the trials to maximize the efficacy. GVAX in conjunction with immunomodulatory cyclophosphamide (54) has been applied to many preclinical tumor models and several human cancers as the low dose cyclophosphamide serves as a vaccine enhancer in disrupting immune tolerance through depleting regulatory T cell in situ and promoting dendritic cell maturation. Indeed, heterologous prime/boost with GVAX and CRS-207 in pancreatic

patients conditioned with cyclophosphamide extended survival with minimal toxicity (28). Recent trials involving this combinational vaccine approach are listed in Table 3. Chemotherapy, such as Folfirinox, or radiation therapy, such as high-energy X rays, work in different ways to kill tumor cells and are being tested in these trials with GVAX/CRS-207/Cyclophosphamide combination therapy. In addition, checkpoint control antibodies, such as anti-PD-1 and anti-CTLA-4, are the most current immunotherapeutic regimens for boosting pre-existent anticancer immunity initiated by GVAX/CRS-207/Cyclophosphamide combination therapy.

#### G17DT

Transgenic, hypergastrinemic InsGas mice (56) were developed to express human CCK2 receptor for investigating the role of gastrin/CCK in pancreatic exocrine cells. Because gastrin and its receptors are co-expressed on human pancreatic adenocarcinoma, this mouse model validated the mitogenic potency of gastrin peptides. When it was discovered that intramuscular injection of gastric antigens can generate an immune response, clinical trials were

Table 3. GVAX clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References                 | Trial subjects  | Phase | Immune<br>intervention /<br>target  | No  | Status / Results / Impact<br>(Median survival)  |
|--|---|-------|---|-----|---|
| <b>NCT00305760</b><br><b>2015</b><br><b>(55)</b> | Metastatic or locally advanced pancreatic cancer  | II    | GVAX<br>Cyclophosphamide (Cy)<br>Cetuximab  | 60  | The median DFS is 17.3 months with median survival of 24.8 months. The postimmunotherapy induction of mesothelin-specific CD8 <sup>+</sup> T cells in HLA-A1 <sup>+</sup> and HLA-A2 <sup>+</sup> patients correlates with DFS. |
| <b>NCT02451982</b><br><b>2015</b>                | Surgically resectable pancreatic cancer (Neoadjuvant/adjuvant)  | I/II  | GVAX<br>PD-1 Antibody<br>CY   | 50  | Not yet recruiting  |
| <b>NCT00727441</b><br><b>2015</b>                | Patients undergoing chemotherapy and radiation therapy for stage I or stage II pancreatic cancer that can be removed by surgery |       | GVAX<br>CY  | 87  | Active, not recruiting  |
| <b>NCT00084383</b><br><b>2013</b>                | Resected stage I or stage II adenocarcinoma (cancer) of the pancreas  | II    | GVAX<br>Adjuvant<br>Chemoradiotherapy   | 60  | Completed   |
| <b>NCT01417000</b><br><b>2015</b>                | Pancreatic cancer (Safety and efficacy)   | II    | GVAX<br>CRS-207   | 93  | Active, not recruiting  |
| <b>NCT02243371</b><br><b>2015</b><br><b>(28)</b> | Previously treated metastatic adenocarcinoma of the pancreas  | II    | GVAX<br>CRS-207<br>PD-1 Blockade<br>Antibody<br>(Nivolumab)   | 94  | Recruiting  |
| <b>NCT02004262</b><br><b>2015</b>                | Pancreatic cancer (Safety and efficacy)   | II    | GVAX<br>CRS-207   | 240 | Active, not recruiting  |
| <b>NCT01595321</b><br><b>2015</b>                | Resected adenocarcinoma of the pancreas   |       | GVAX<br>Low Dose Cy<br>Fractionated<br>Stereotactic<br>Body Radiation<br>Therapy (SBRT)<br>FOLFIRINOX | 19  | Active, not recruiting  |
| <b>NCT01896869</b><br><b>2015</b>                | Metastatic pancreatic cancer (Multicenter study)  | II    | GVAX<br>CTLA-4 antibody<br>FOLFIRINOX   | 92  | Recruiting  |

initiated to exploit the use of this target for eliciting an antitumor response. G17DT, an antigastrin immunogen, can produce neutralizing antibodies against the tumor growth factors amidated and glycine-extended form of gastrin-17 (57). Rapid and

sustained antibody production after immunization with G17DT can generate a robust anti-tumor response, thus, G17DT may represent a new therapeutic option for responders with pancreatic cancer (Table 4).

Table 4. Gastrin immunogen clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References                 | Trial subjects  | Phase | Immune<br>intervention<br>/ target         | No  | Status / Results / Impact<br>(Median survival)  |
|--|---|-------|--|-----|---|
| <b>NCT02118077</b><br><b>2014</b><br><b>(57)</b> | Advanced pancreatic cancer (Prospective, randomized, double-blind, placebo-controlled, group sequential trial)  | III   | G17DT<br>Gemcitabine<br>5-<br>fluorouracil | 154 | Patients developing anti-G17DT responses (73.8%) survived longer than non-responders or those on placebo (176 vs. 63 vs. 83 days) |
| <b>NCT00044031</b><br><b>2014</b>                | Previously untreated subjects with locally advanced (nonresectable stage II and III), recurrent disease following primary resection, or metastatic (stage IV) adenocarcinoma of the pancreas (Protocol No. PC4) (Prospective, randomized, controlled, double-blind, multi-national, multi-center) | III   | G17DT<br>Gemcitabine                       | 394 | Completed   |
| <b>NCT02098239</b><br><b>2014</b>                | Advanced pancreatic cancer (Open, multiple dose, single centre)   | II    | G17DT                                      | 41  | Completed   |
| <b>NCT02098291</b><br><b>2014</b>                | Advanced Pancreatic Carcinoma (Open, single-center, safety and tolerability)  | II    | G17DT                                      | 34  | Completed   |

### CEA/MUC-1

CEA (carcinoembryonic antigen, an oncofetal antigen) and MUC-1 (mucin, a highly glycosylated protein) are expressed at high levels on most human pancreatic cancers. T cells can recognize HLA-restricted epitopes derived from CEA and non-HLA-restricted epitopes encoded by MUC-1 (58, 59). A vaccine, engineered with poxviruses targeting both CEA and MUC-1, can generate polyclonal T cells, which produce antigen-specific anti-tumor effects and appear to prevent tumor escape during antigen loss. This vaccination protocol has proven to be safe and well tolerated (60) with some significant response, as indicated in Table 5.

### Algenpantucel-L

A cell-based allogeneic pancreatic cancer vaccine, algenpantucel-L, targets alpha-1,3 galactosyl epitopes to induce better antitumor immune priming on the concept of the hyperacute rejection response. This

vaccine was engineered to express alpha-Gal (murine alpha-1,3-galactosyltransferase gene) in two human pancreatic ductal adenocarcinoma cell lines (HAPa-1 and HAPa-2). Since alpha-Gal is inactive in normal human cells, Algenpantucel-L xenotransplantation will produce high titer of anti-alpha-Gal antibodies that trigger hyperacute rejection presenting as complement-mediated tumor cell lysis and antibody-dependent cell-mediated cytotoxicity (61). Through the subsequent opsonization and phagocytosis of the whole tumor cells, APCs will process and present the entire library of TAAs to cytotoxic effector T cells to fight the patient's own cancer. Algenpantucel-L has demonstrated survival benefit for pancreatic cancer patients in a Phase II trial for patients with surgically resected disease. The results shown in Table 6 reflect improved survival in patients when compared with a historical control (62) in phase II studies which remain to be proven in randomized Phase III trials (currently accruing).

Table 5. CEA/MUC-1 clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects  | Phase | Immune intervention / target  | No | Status / Results / Impact (Median survival)  |
|----------------------------------|---|-------|---|----|--|
| <b>2007<br/>(60)</b>             | Advanced pancreatic cancer (Open-label)   | I     | CEA<br>MUC-1  | 10 | A significant increase in OS was noted in responders compared with non-responders (15.1 vs. 3.9 months). |
| <b>NCT00669734<br/>2015</b>      | Unresectable pancreas cancer: Intratumoral Recombinant Fowlpox PANVAC (PANVAC-F) plus subcutaneous recombinant vaccinia PANVAC (PANVAC-V), PANVAC-F and rH-GM-CSF | I     | Falimarev (CEA & MUC-1)<br>Inalimarev (CEA & MUC-1)<br>Sargramostim (rH-GM-CSF) | 14 | This study is ongoing, but not recruiting participants.  |

### Bruton's Tyrosine Kinase inhibitor

The first clinically approved use of ibrutinib, a novel BTK inhibitor developed by Pharmacyclics, has led to decreased mouse tumor growth and improved responsiveness to gemcitabine in orthotopic pancreatic cancer mouse models (64). In another

preclinical study, ibrutinib improved the survival of both genetically engineered PDAC models, as well as mice bearing patient-derived PDAC xenografts (65). Ibrutinib therapy also reduced the stromal desmoplasia in preclinical models, and improved delivery of chemotherapeutics to the tumor milieu.

Table 6. Algenpantucel-L clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References     | Trial subjects   | Phase | Immune intervention / target           | No  | Status / Results / Impact (Median survival)         |
|--------------------------------------|--|-------|--|-----|---|
| <b>NCT01836432<br/>2015</b>          | Borderline resectable or locally advanced unresectable pancreatic cancer | III   | HyperAcute®-Pancreas (algenpantucel-L) | 280 | Recruiting  |
| <b>NCT02405585<br/>2015</b>          | Borderline resectable pancreatic cancer                                  | II    | HyperAcute®-Pancreas (algenpantucel-L) | 48  | Recruiting  |
| <b>NCT01072981<br/>2015<br/>(63)</b> | Surgically resected pancreatic cancer                                    | III   | HyperAcute®-Pancreas (algenpantucel-L) | 722 | Active, not recruiting                              |
| <b>NCT00569387<br/>2015<br/>(63)</b> | Surgically resected pancreatic cancer                                    | II    | HyperAcute®-Pancreas (algenpantucel-L) | 73  | 12-month DFS was 62 %, and the 12-month OS was 86 % |
| <b>NCT00614601<br/>2015</b>          | Surgically resected pancreatic cancer (Low dose)                         | II    | HyperAcute®-Pancreas (algenpantucel-L) | 9   | Terminated  |
| <b>NCT00255827<br/>2015</b>          | Surgically resected pancreatic cancer                                    | I/II  | HyperAcute®-Pancreas (algenpantucel-L) | 7   | Completed   |



Table 7. ACP-196 clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References  | Trial subjects  | Phase | Immune<br>intervention /<br>target       | No  | Status /<br>Impact<br>survival) | Results /<br>(Median<br>survival) |
|-----------------------------------|---|-------|--|-----|---------------------------------|-----------------------------------|
| <b>NCT02362048</b><br><b>2015</b> | Advanced or metastatic pancreatic cancer (KEYNOTE144) | II    | ACP-196<br>pembrolizumab                 | 76  | Recruiting                      |                                   |
| <b>NCT02570711</b><br><b>2015</b> | Previously untreated metastatic pancreatic cancer     | II    | ACP-196<br>Nab-paclitaxel<br>gemcitabine | 120 | Recruiting                      |                                   |

Inhibiting BTK has the desired effect of inhibiting mast cell degranulation and cytokine secretion, a result that our group and others have shown, mediating tumor suppression in PDAC (20-22). However, Ibrutinib suffers from multiple off-target effects that limit its application in solid tumors where enhancing effector T-cell function is a crucial intent (2). Ibrutinib's potency on ITK (IL2-inducible T-cell kinase) and TXK (Tyrosine-protein Kinase) may explain why it interferes with cell-mediated anti-tumor activities and immune-mediated killing in the TME.

Acerta Pharma has developed ACP-196, an orally bioavailable, 2<sup>nd</sup> generation inhibitor of Bruton's tyrosine kinase (BTK). BTK is a non-receptor enzyme in the Tec kinase family that is expressed by cells of hematopoietic origin, including B cells, myeloid cells, and mast cells, where it regulates multiple cellular processes, including proliferation, differentiation, apoptosis, and cell migration (66-68). In addition, BTK-dependent activation of mast cells, myeloid cells and other immunocytes in the peritumoral inflammatory stroma has been shown to sustain the complex microenvironment needed for solid tumor maintenance (69-71). Overall, BTK inhibition interferes with the cross-talk between neoplastic cells and the TME, such as immune regulatory cells recruitment and infiltration into tumor, suggesting disruption of intrinsic and extrinsic survival signals (70, 72). Importantly, unlike Ibrutinib, ACP-196 did not inhibit ITK or TXK, which are both involved in Teff function (73-75). Two ongoing trials at MD Anderson Cancer Center (Table 7) will evaluate whether the combination therapy of BTK inhibition and checkpoint blockade leads to greater benefit compared with monotherapy.

### Immune checkpoint control antibodies

The activation and inhibition of the immune system are controlled through signals from a panel of ligands and receptors of co-stimulatory and co-inhibitory molecules, which have been recognized as immune checkpoint control pathways. Anti-CTLA-4, anti-PD-1, and anti-PD-L1 antibodies have been applied in cancer immunotherapy for interfering with the inhibitory signaling in T cells (76). By rescuing anti-tumor immune responses, these antibodies can mediate durable cancer regressions and long-term survival (77).

#### CTLA-4

CTLA-4 (cytotoxic T lymphocyte antigen 4) (77-79), a costimulatory molecule similar to CD28, is expressed on activated T cells and also bind to the ligands CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells. CD28 molecule is constitutively expressed on T cell surface and stimulating T cell activation through binding to the ligands, whereas CTLA-4 can transmit an inhibitory signal due to compete for CD28 binding to CD80/86. Anti-CTLA-4 antibody, via blocking the high affinity binding of CTLA-4 to CD80/86, allows CD28 to interact with CD80/86. In patients with melanoma (80), anti-CTLA-4 can magnify anti-tumor response induced by the neoepitopes from tumor mutations. By releasing the "brake" on T cell activation (81), CTLA-4 blockade expands immunological memory and boosts long-lasting immunogenicity. Ipilimumab anti-CTLA-4 IgG1 antibody gained FDA-approval for second-line treatment of patients with metastatic melanoma. Trials incorporating combination regimens in Table 8 are being tested in patients with pancreatic cancer. Tremelimumab (82) anti-CTLA-4 IgG2 antibody

Table 8. CTLA-4 antibody clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References                 | Trial subjects  | Phase | Immune intervention / target   | No  | Status / Results / Impact<br>(Median survival)  |
|--|---|-------|--|-----|---|
| <b>NCT01473940</b><br><b>2014</b>                | Stage III-IV or recurrent pancreatic cancer that cannot be removed by surgery | I     | CTLA-4 antibody (Ipilimumab)<br>Gemcitabine                                      | 28  | Recruiting  |
| <b>NCT00112580</b><br><b>2009</b><br><b>(83)</b> | Stage IV pancreatic cancer that cannot be removed by surgery                  | II    | CTLA-4 antibody (Ipilimumab)   | 82  | Single agent Ipilimumab at 3.0 mg/kg/dose is ineffective for the treatment of advanced pancreas cancer. |
| <b>NCT00836407</b><br><b>2013</b>                | Locally advanced, unresectable or metastatic pancreatic cancer                | I     | CTLA-4 antibody (Ipilimumab)<br>Pancreatic Cancer Vaccine                        | 30  | Completed   |
| <b>NCT01896869</b><br><b>2015</b>                | See Table 3   |       |  |     |   |
| <b>NCT02311361</b><br><b>2014</b>                | Unresectable pancreatic cancer  | I     | CTLA-4 antibody (Tremelimumab)<br>PD-L1 antibody (MEDI4736)<br>Radiation Therapy | 60  | Recruiting<br>GEMM model (41)   |
| <b>NCT02558894</b><br><b>2016</b>                | Metastatic Pancreatic Ductal Carcinoma  | II    | CTLA-4 antibody (Tremelimumab)<br>PD-L1 antibody (MEDI4736)                      | 130 | Recruiting  |
| <b>NCT02639026</b><br><b>2015</b>                | Pancreatic Cancers  | I     | CTLA-4 antibody (Tremelimumab)<br>PD-L1 antibody (MEDI4736)<br>Radiation Therapy | 30  | Recruiting  |
| <b>NCT02527434</b><br><b>2016</b>                | Advanced Solid Tumors (including pancreatic ductal adenocarcinoma)            | II    | CTLA-4 antibody (Tremelimumab)<br>PD-L1 antibody (MEDI4736)                      | 76  | Recruiting  |

achieved survival benefit for 9 patients with pancreatic cancer when combined with gemcitabine in a phase I trial, and its subsequent combination regimens were under the trials in Table 8.

#### PD-1/PD-L1

PD-1 (84) is an inhibitory receptor that down-regulates T-cell activation when bound to a PD-L1 or PD-L2 ligand on tumor cells. Notably, for patients with melanoma with positive expression of the PD-1 ligand, anti-PD-1 immunotherapy has been associated with a

better response (85). Nivolumab (86), a fully humanized immunoglobulin G4 (IgG4) mAb targeting human PD-1 with high affinity, has been approved by FDA for treatment on melanoma and lung cancer. There was no difference in the overall median survival rate for nivolumab or nivolumab and ipilimumab combined, but among patients with PD-1 ligand-negative tumors, progression-free survival was longer with the combination therapy of nivolumab and ipilimumab than with nivolumab alone (3). In light of these promising results in melanoma, PD-1/PD-L1

blockades are being evaluated in several clinical trials (see Table 9) involving combination/chemotherapy

regimens for the treatment of patients with pancreatic cancer.

Table 9. PD-1/PD-L1 clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects  | Phase | Immune intervention / target  | No  | Status / Results /<br>Impact (Median<br>survival) |
|----------------------------------|---|-------|---|-----|---|
| <b>NCT01313416<br/>2015</b>      | Resected pancreatic cancer  | II    | PD-1 antibody (CT-011, Pidilizumab)<br>Gemcitabine  | 29  | Suspended   |
| <b>NCT02451982<br/>2015</b>      | See Table 3   |       |   |     |   |
| <b>NCT02309177<br/>2015</b>      | Pancreatic Cancer (Safety)  | I     | PD-1 antibody (Nivolumab)<br>Nab-Paclitaxel Plus or Minus<br>Gemcitabine  | 138 | Recruiting  |
| <b>NCT02243371<br/>2015</b>      | See Table 3   |       |   |     |   |
| <b>NCT02546531<br/>2015</b>      | Advanced cancer   | I     | PD-1 antibody (Pembrolizumab)<br>Defactinib<br>Gemcitabine  | 50  | Not yet recruiting                                |
| <b>NCT02452424<br/>2015</b>      | Advanced melanoma and other solid tumors  | I/II  | PLX3397: a selective colony-stimulating factor 1 receptor (CSF1R) kinase inhibitor<br>PD-1 antibody (Pembrolizumab) | 400 | Recruiting  |
| <b>NCT02303990<br/>2015</b>      | Advanced and metastatic cancers (RADVAX: stratified phase I)  | I     | PD-1 antibody (Pembrolizumab)<br>Hypofractionated Radiotherapy  | 70  | Recruiting  |
| <b>NCT02305186<br/>2015</b>      | Resectable or borderline resectable pancreatic cancer (Safety and immunological effect)                 | I/II  | PD-1 antibody (Pembrolizumab)   | 56  | Recruiting  |
| <b>NCT02331251<br/>2015</b>      | Advanced cancer (PembroPlus)  | I/II  | PD-1 antibody (Pembrolizumab)<br>Chemotherapy   | 90  | Recruiting  |
| <b>NCT02432963<br/>2015</b>      | Vaccine Therapy and Pembrolizumab in Treating Patients With Solid Tumors That Have Failed Prior Therapy | I     | Modified Vaccinia Virus Ankara Vaccine Expressing p53<br>PD-1 antibody (Pembrolizumab)                              | 12  | Not yet recruiting                                |
| <b>NCT02009449<br/>2015</b>      | Advanced solid tumors   | I     | pegylated recombinant human IL-10 (PEG-rHuIL-10, AM0010)<br>PD-1 antibody (Pembrolizumab)                           | 135 | Recruiting  |
| <b>NCT02268825<br/>2015</b>      | Advanced GI cancers   | I/IIA | PD-1 antibody (MK-3475)<br>Chemotherapy   | 128 | Recruiting  |
| <b>NCT02528357<br/>2016</b>      | Advanced solid tumors   | I     | OX40 antibody<br>PD-1 antibody  | 264 | Recruiting  |
| <b>NCT02221960<br/>2016</b>      | Advanced solid tumors   | I     | OX40 antibody<br>PD-1 antibody  | 224 | Recruiting  |

Table 10. Tumor infiltrating lymphocyte (TIL) clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects         | Phase | Immune intervention / target  | No   | Status / Results / Impact<br>(Median survival)  |
|----------------------------------|------------------------|-------|---|------|---|
| <b>NCT01174121</b>               | Metastatic cancer      | II    | Young TIL with IL-2   | 260  | Recruiting  |
| <b>NCT00003780<br/>2013</b>      | Cancer of the pancreas | II    | TIL therapy<br>Chemotherapy   | 150  | Active, not recruiting<br>(The information of recruitment status has not been verified recently.) |
| <b>NCT00019084<br/>2013</b>      | Advanced cancer        | II    | Mutant p53 or Ras peptide vaccine<br>sargramostim<br>Therapeutic lymphocytes<br>TIL with IL-2<br>autologous | null | Completed   |

### Adoptive T cell therapy

Adoptive T-cell therapy (42) involves the ex vivo enrichment and expansion of tumor-reactive T cells for infusion. As an immune-based approach, adoptive therapy has become an increasingly attractive modality for the treatment of patients with cancer due to its potential for high specificity, non-cross resistance with conventional therapies, and the promise of long-term immunoprotection. Adoptive cellular therapy encompasses the use of tumor reactive T cells derived from an endogenous source (tumor infiltrating lymphocytes, TIL or peripheral blood) or a genetically modified lymphocyte population engineered to recognize tumor antigen through expression of the cognate chimeric antibody or T-cell receptor (CAR/TCR).

### Tumor Infiltrating Lymphocyte (TIL)

To date, only TIL therapies have been reported for the treatment of pancreatic cancer, as shown in Table 10, although efforts are underway in our lab and others to explore the use of MS/MS and RNAseq analysis of patient tumor immunopeptidome to develop T cell therapy targeting autologous tumor neo-epitopes for pancreatic cancer.

### CAR-T cells

Adoptive therapy with T cells transduced to express chimeric antigen receptors (CAR) consisting of fusion

proteins joining antibody variable regions to T-cell signaling chains. The antibody moiety confers antigen specificity and activates the T cell signaling generally in tandem with the cytoplasmic signaling domains of CD28, 4-1BB and other costimulatory molecules (44-46). CAR-T cell represents an important modality with the promise of off-the-shelf strategies for the treatment of pancreatic cancer. The following clinical trials in Table 11 are being evaluated for use in several solid tumor malignancies including pancreatic cancer (46).

### **IDO inhibitor**

Indoleamine 2,3-dioxygenase (IDO), which is overexpressed in metastatic PDAC (87), contributes to the immune suppressive tumor microenvironment by starving T lymphocytes of tryptophan (87). Active IDO enzyme can catalyze L-tryptophan (L-Trp) into kynurenine (Kyn) pathway metabolites. In PDAC, L-Trp was completely consumed by tumor cell-derived IDO, resulting in the significant reduction of L-Trp concentration in TME. In response to L-Trp deficiency, tumor infiltrating CD8+ T cells will be subjected to cell cycle arrest and anergy through mTOR (mammalian target of rapamycin) and PKC signaling pathways. IDO inhibitors may restore L-Trp level and overcome the immune suppressive TME in vivo (88). Clinical grade IDO inhibitors are currently undergoing evaluation in

Table 11. CAR-T cells clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects  | Phase | Immune intervention /<br>target  | No  | Status / Results /<br>Impact (Median<br>survival) |
|----------------------------------|---|-------|--|-----|---|
| <b>NCT02349724<br/>2015</b>      | CEA positive cancer   | I     | Anti-CEA-CAR T   | 75  | Recruiting  |
| <b>NCT01583686<br/>2015</b>      | Metastatic cancer   | I/II  | Anti-mesothelin CAR<br>Aldesleukin (IL-2)  | 136 | Recruiting  |
| <b>NCT01897415<br/>2015</b>      | Pancreatic cancer   | I     | Autologous T cells<br>transfected with chimeric<br>anti-mesothelin<br>immunoreceptor SS1 | 10  | Completed   |
| <b>NCT02587689<br/>2015</b>      | MUC1 positive advanced<br>refractory solid tumor                          | I/II  | anti-MUC1 CAR T Cells  | 20  | Recruiting  |
| <b>NCT02580747<br/>2015</b>      | Relapsed and/or<br>chemotherapy refractory<br>advanced malignancies       | I     | anti-meso-CAR vector<br>transduced T cells   | 20  | Recruiting  |
| <b>NCT02465983<br/>2015</b>      | Metastatic pancreatic cancer  | I     | CART-meso-19 T cells   | 12  | Recruiting  |
| <b>NCT02541370<br/>2015</b>      | Relapsed and/or<br>chemotherapy refractory<br>advanced malignancies       | I     | anti-CD133-CAR vector-<br>transduced T cells   | 20  | Recruiting  |
| <b>NCT02416466<br/>2015</b>      | CAR-T Hepatic Artery<br>Infusions and Sir-Spheres for<br>Liver Metastases | I     | anti-CEA CAR-T cells   | 6   | Recruiting  |
| <b>NCT02159716<br/>2014</b>      | Mesothelin expressing<br>cancers  | I     | CART-meso  | 24  | Recruiting  |

solid tumors, including pancreatic cancers listed in Table 12.

### Mesothelin

Mesothelin is a tumor antigen upregulated on most human pancreatic cancer. Although the normal function of membrane-bound mesothelin is still unknown, the soluble mesothelin (89) can be detected in the peripheral blood of patients with pancreatic cancer, intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm, but not in healthy individuals. Mesothelin specific antibody and T-cell response in those patients indicated antimesothelin immunity and offered potential therapy against pancreatic cancer. In addition to mesothelin vaccine trials using GVAX and CRS-207 in combination (28), antibodies (90) against mesothelin

provide an alternative for pancreatic cancer immunotherapy, as shown in Table 13.

### Dendritic cell

As a professional antigen-presenting cell, dendritic cells (DCs) play an essential role in the anti-tumor immune response with their capacity to capture, process, and present antigens to T cells (91). DCs are extremely efficient for vaccination by priming naïve CD8+ T cells into cytotoxic T cells as well as cytokine release (92). In addition to the vaccine related studies listed in the above Tables, we list a broad spectrum of DC vaccine trials in Table 14. In addition to shaping the specificity of response against tumor antigens through DC vaccination, these trials also focus on modifying the function and activation of dendritic cells. In the immune-privileged microenvironment of pancreatic

Table 12. IDO inhibitor clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects                      | Phase | Immune intervention /<br>target                                 | No  | Status / Results /<br>Impact (Median<br>survival) |
|----------------------------------|-------------------------------------|-------|---|-----|---|
| <b>NCT02077881<br/>2015</b>      | Metastatic pancreatic cancer        | I/II  | IDO Inhibitor (Indoximod)<br>Gemcitabine and Nab-<br>Paclitaxel | 98  | Recruiting  |
| <b>NCT00739609<br/>2012</b>      | Relapsed or refractory solid tumors | I     | IDO Inhibitor (1-methyl-D-tryptophan)                           | 17  | Terminated  |
| <b>NCT02318277<br/>2015</b>      | Selected advanced solid tumors      | I/II  | PD-L1 antibody (MEDI4736)<br>IDO Inhibitor (INCB024360)         | 157 | Recruiting  |

cancer, insufficiency of dendritic cells is the main roadblock for T cell activation, a typical malfunction observed in many other cancer types (93). Dendritic cell expansion and maturation may overcome the resistance to checkpoint immunotherapy and reprogram the TME to an immunogenic microenvironment (93).

### Ras

The oncogenic Ras mutation is an essential and initiating event in pancreatic tumorigenesis (48). Preclinical studies (94) demonstrated that Kras mutation is required for PDAC maintenance. Targeting oncogenic/mutant Ras may provide a novel therapeutic avenue for pancreatic cancer, and the

ongoing trials that are associated with Ras targeting are summarized in Table 15. Notably, the long-term follow-up (over 10 years) of twenty-three patients who were vaccinated after surgical removal of pancreatic adenocarcinoma (22 pancreaticoduodenectomies, one distal resection), in two Phase I/II clinical trials (95, 96), showed great promise on correlation of sustained immunological T-cell reactivity and prognosis. The mechanism of the vaccine was attributed to the long synthetic mutant ras peptides designed primarily to trigger T-helper responses. Seventeen of 20 evaluable patients (85%) responded immunologically to the vaccine. Median survival for all patients was 27.5 months and 28 months for immune responders. The 5-year survival

Table 13. Mesothelin related clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects   | Phase | Immune intervention /<br>target                                    | No  | Status / Results /<br>Impact (Median<br>survival) |
|----------------------------------|--|-------|--|-----|---|
| <b>NCT00325494<br/>2014</b>      | Pancreatic cancer  | I     | a chimeric monoclonal antibody that targets mesothelin (MORAb-009) | 24  | Completed   |
| <b>NCT01413451<br/>2013</b>      | High mesothelin cancers  | 0     | Amatuximab (MORAb-009)   | 7   | Terminated  |
| <b>NCT02341625<br/>2015</b>      | Selected advanced solid tumors   | I/IIa | anti-mesothelin antibody-drug conjugate BMS-986148                 | 204 | Recruiting  |
| <b>NCT02485119<br/>2015</b>      | Japanese subjects with advanced malignancies (Dose escalation, intravenous infusion) | I     | anti-mesothelin antibody-drug conjugate BAY94-9343                 | 15  | Recruiting  |

Table 14. Dendritic cell related clinical trials\* against pancreatic cancer

| NCT Number<br>Year          | Trial subjects  | Phase | Immune intervention/<br>target  | No | Status/Results/Impact<br>(Median survival) |
|-----------------------------|---|-------|---|----|--|
| <b>NCT00868114<br/>2015</b> | Unresectable pancreatic cancer (Direct tumor injection)   | II    | KLH-pulsed autologous dendritic cell vaccine  | 35 | Active, not recruiting                     |
| <b>NCT00795977<br/>2011</b> | Pancreatic cancer (Preoperative intratumor injection)   | I/II  | dendritic cells, OK-432   | 20 | Active, not recruiting                     |
| <b>NCT01677962<br/>2015</b> | Pancreatic adenocarcinoma   | I     | Poly-ICLC dendritic cell  | 12 | Active, not recruiting                     |
| <b>NCT01410968<br/>2015</b> | Metastatic, locally advanced, unresectable, or recurrent pancreatic adenocarcinoma (Feasibility and safety) | I     | vacc. w/ Poly-ICLC & peptide-pulsed dendritic cells   | 12 | Active, not recruiting                     |
| <b>NCT00547144<br/>2008</b> | Unresectable pancreatic cancer (Intratumor DC)  | I/II  | Gemcitabine Dendritic Cell XRT  | 2  | Completed                                  |
| <b>NCT02548169<br/>2015</b> | Pancreatic cancer   | I     | DC Vaccine<br>Chemotherapy  | 20 | Recruiting                                 |
| <b>NCT00843830<br/>2010</b> | Metastatic pancreatic carcinoma (Intra-metastasis administration)   | I     | Autologous KLH-pulsed Dendritic cell vaccination<br>Tumoral radiation therapy                           | 4  | Terminated                                 |
| <b>NCT01897636<br/>2013</b> | Unresectable pancreatic cancer (Efficacy and safety)  |       | EUS-guided fine-needle injection of DCs vaccinations  | 30 | Not yet recruiting                         |
| <b>NCT00600002<br/>2013</b> | Resectable pancreatic adenocarcinoma  | I     | GM-CSF  | 30 | Active, not recruiting                     |
| <b>NCT00726037<br/>2012</b> | Metastatic pancreatic cancer (Efficacy of regulatory T-cell suppression)                                    | II    | Ontak (an engineered protein combining Interleukin-2 and Diphtheria toxin)                              | 7  | Terminated                                 |
| <b>NCT00128622<br/>2009</b> | Metastatic cancer   | I     | denileukin diftiox recombinant fowlpox-CEA(6D)/TRICOM vaccine<br>therapeutic autologous dendritic cells | 24 | Completed                                  |
| <b>NCT00004604<br/>2002</b> | Metastatic cancer   | I     | CEA RNA-pulsed DC cancer vaccine  | 24 | Completed                                  |
| <b>NCT00027534<br/>2007</b> | Advanced or metastatic cancer   | I     | TRICOM-CEA(6D)  | 14 | Completed                                  |
| <b>NCT00003434<br/>2002</b> | Stage I, Stage II, or Stage III surgically resected pancreatic cancer                                       | I     | carcinoembryonic antigen peptide 1<br>hepatitis B antigen peptide                                       | 8  | Terminated                                 |
| <b>NCT01882946<br/>2015</b> | Solid tumors (Safety and efficacy)  | I/II  | DCVax-Direct  | 60 | Active, not recruiting                     |
| <b>NCT00648102<br/>2009</b> | Incurable, locally advanced or metastatic breast, colorectal, pancreatic, bladder or ovarian Cancer         | I     | CDX-1307  | 36 | Completed                                  |
| <b>NCT01781520<br/>2015</b> | Unresectable locally advanced pancreatic cancer   | I/II  | S-1 plus DC-CIK   | 30 | Recruiting                                 |

was significantly improved to 22% and 29%, respectively. Strikingly, 10-year survival was 20% (four

patients out of 20 evaluable) versus zero (0/87) in a cohort of non-vaccinated patient treated in the same

Table 15. Ras related clinical trials\* against pancreatic cancer

| NCT Number<br>Year<br>References | Trial subjects   | Phase | Immune intervention / target   | No   | Status<br>Results<br>Impact<br>(Median<br>survival) |
|----------------------------------|--|-------|--|------|---|
| <b>NCT00019006<br/>2005</b>      | Colon, pancreatic, or lung cancer                                    | I     | Detox-B adjuvant ras peptide cancer vaccine  | null | Completed   |
| <b>NCT00837135<br/>2009</b>      | Pancreatic cancer  | I     | GI-4000 Vaccine (a vaccine composed of yeast that is made to carry the same proteins called "mutated Ras proteins")<br>GI-4000 Vaccine + Activated T Cells | 32   | Withdrawn   |
| <b>NCT00006387<br/>2013</b>      | Advanced pancreatic or colorectal cancer                             | I     | QS21 ras peptide cancer vaccine  | 7    | Completed   |
| <b>NCT00019331<br/>2007</b>      | Metastatic solid tumors  | II    | aldesleukin<br>ras peptide cancer vaccine<br>sargramostim<br>DetoxPC   | null | Completed   |
| <b>NCT00300950<br/>2015</b>      | Non-metastatic, post-resection pancreas cancer (Safety and efficacy) | II    | GI-4000<br>Gemcitabine   | 176  | Completed   |
| <b>NCT00005594<br/>2010</b>      | Advanced cancer of the pancreas                                      | II    | ISIS 2503 (an Antisense Inhibitor of H-Ras)  | 4    | Completed   |

period. Three patients presented a memory response up to 9 years after vaccination. The encouraging milestone of long-term immune response together with 10-year survival following surgical resection indicates that vaccination against oncogenic/mutant K-ras may boost the effect of surgery and represent an adjuvant alternative for future treatment (97).

#### Combination of chemotherapy and immunotherapy

Chemotherapy with gemcitabine has been the standard first-line treatment for patients with unresectable locally advanced or metastatic pancreatic cancer for 18 years (99). Since 2013, chemotherapy with nab-paclitaxel plus gemcitabine has significantly improved overall survival, progression-free survival, and response rate in patients with metastatic pancreatic adenocarcinoma as compared with gemcitabine alone (12). Unfortunately, the improved median survival is only 8.5 months. The strength of combination with chemotherapy and immunotherapy is being tested in animal models.

It has been recognized that an optimized approach combining anti-cancer vaccination with chemotherapy and/or radiation therapy is urgently needed. The use of vaccines is also a compelling approach for maintenance therapy of patients who are grossly disease-free, or whose disease is at least stabilized, upon chemotherapy and/or radiation therapy. Prime-boost vaccination strategies, in particular those that use *Listeria* vaccines for boosting, are a promising approach for maintenance therapy (100).

In preclinical studies, combination therapy of chemotherapy followed by immunotherapy is superior to chemotherapy alone. In these *in vivo* tests, chemotherapeutic agents played a dual role. On one hand, they reduced overall tumor burden by direct killing of cancer cells; on the other hand, they indirectly lead to the release of pro-inflammatory molecules and tumor associated antigens (101), which may be functioning as a "vaccine" by presumably releasing tumor antigens in an immunogenic fashion. This may potentially explain the improved efficacy of



Table 16. Other clinical trials\* with immunotherapeutic regimens against pancreatic cancer

| NCT Number<br>Year<br>References                 | Trial subjects                                     | Phase | Immune<br>intervention /<br>target                            | No  | Status / Results / Impact<br>(Median survival)   |
|--|--|-------|---|-----|--|
| <b>NCT00540579</b><br><b>2013</b><br><b>(98)</b> | Untreated advanced carcinoma of the pancreas       | I/II  | Pomalidomide: an immunomodulating drug (IMiD).<br>Gemcitabine | 23  | PRs were 15%. SDs after two cycles were 70%. CA 19-9 decreased by >50% in 43% of patients. |
| <b>NCT00389610</b><br><b>2015</b>                | Pancreatic cancer that has been removed by surgery | II    | allogenic GM-CSF tumor cell vaccine                           | 56  | Active, not recruiting   |
| <b>NCT00720785</b><br><b>2015</b>                | Pancreatic Neoplasms                               | I     | NK cells<br>+CliniMACs CD3<br>and CD56 systems                | 61  | Recruiting   |
| <b>NCT01801852</b><br><b>2015</b>                | Pancreatic Cancer                                  | I     | NKT cells   | 300 | Recruiting   |

CD40 agonists when given after, but not before, chemotherapy (102). This synergistic efficacy is also observed with combined chemotherapy using anti-CD47 antibody (103). Notably, there might be a limited window of time when chemotherapy drugs can be effective, yet without blunting anti-tumor immunity (103).

In contrast, chemotherapy administered after immunotherapy had detrimental effects on development of beneficial antitumor memory immune responses. Chemotherapy administered after anti-CD47 treatment did not result in faster tumor regression than anti-CD47 alone. Chemotherapy can suppress the immune system and kill recently activated immune cells including memory cells. The ultimate consequence of this sequence is to destroy the therapeutic effects of immunotherapy (103). In addition, recent clinical studies have experimented using the sequential administration of anti-cancer vaccines and chemotherapy/radiation; however, the immunosuppressive effects of these standard cytotoxic regimens may compromise the efficacy of immunotherapy. Thus, the timing of administration of standard chemotherapy markedly impacted the induction of antitumor responses by immune-based therapy.

#### **Combination of radiation therapy (radiotherapy) and immunotherapy**

The most recent advancement of radiation technology, including intensity modulated radiation therapy, stereotactic radiosurgery, proton therapy, antibody-radionuclide conjugates, has been applied to cancer therapy for 20 years (104). Radiotherapy is based on the ionizing energy causing DNA damage to induce direct tumor cell death, such as apoptosis, mitotic catastrophe, or necrosis, along with the cascade events in tumor microenvironment (TME) (105). The best efficacy of radiotherapy will not only rely on the direct tumor cell death, but also the contribution of intrinsic anti-tumor immune response. The damage-associated molecular patterns in TME trigger immune sensors such as toll-like receptors (TLR) (104) which serve as the link between radiation and immune properties. The damaged tumor cells up-regulated tumor-associated antigens (TAA), recruited dendritic cell migration and maturation, and induced T effector cells to TME for removing the residual tumor cells. One of the cascade events in TME is abscopal effect, a poorly understood phenomenon that radiation can induce tumor regression outside the exposure area of radiation. Recent progress in immunology allowed researcher to demonstrate that abscopal effect is immune mediated and T cell dependent (106) peripheral clonal expansion, warranting further immune intervention in this setting of cancer therapy. Indeed, combination of immunotherapy with radiation therapy has improved cancer treatment through synergy (107). Trials (104) with TLR agonists

and radiation therapy have been tested in patients with lymphoma, breast cancer, prostate cancer, etc. The superiority (108, 109) of radiation and anti-CTLA-4 and anti-PD-1 therapy is confirmed in preclinical melanoma models, although the mechanism of TCR repertoire diversity and tumor heterogeneity remains unknown. In the near future, it might be possible that radiation can turn pancreatic cancer from an immune privileged tumor microenvironment to an immune active state. Radioimmunotherapy (110) for pancreatic cancer may emerge as a novel regimen (111) complementing to the multimodality tumor directed therapy.

### Perspectives and Conclusion

The principal goal of this review is to highlight current progress of immunotherapy in pancreatic cancer. The seminal advantages that immunotherapy bring to the treatment of pancreatic cancers are: (1) The ability to broaden and encompass greater numbers of patients eligible for treatment and to achieve a personalized approach to medicine by using the autologous cancer antigens as a means of generating a populations antigen-specific effector cells. (2) A means to trigger T cell memory and retain long-term remission. (3) A means to modulate the immune microenvironment for example through targeted therapies to mitigate desmoplasia, a physical barrier where pancreatic cancer stem cells may be sequestered. (4) The development of novel Immune adjuvants defined through mouse models, that may reverse the noxious immune suppressive tumor microenvironment, a functional barrier of pancreatic cancer. (5) Immune reagents that potentiate the penetrance of chemotherapeutic reagents and improve drug delivery. It is hoped that multiple possible avenues to developing a deeper understanding of immune resistance, T cell persistence, mechanisms of immune checkpoint inhibition and vaccination strategies, will allow us to chart a strategy that exploits the potential of the immune system to eradicate the refractory disease and maintain a durable clinical response in patients with pancreatic cancer.

### Abbreviations:

5-FU, fluorouracil  
ADCC, antibody-dependent cell-mediated cytotoxicity

BiTE, bi-specific T-cell engager  
BTK, Bruton's tyrosine kinase  
CAR, chimeric antigen receptors  
CEA, carcinoembryonic antigen  
CTLA-4, cytotoxic T lymphocyte antigen 4  
Cy, cyclophosphamide  
DART, dual-affinity re-targeting  
DCs, dendritic cells  
DFS, disease-free survival  
FDA, Food and Drug Administration  
GEMM, genetically engineered mouse models  
GM-CSF, granulocyte-macrophage colony-stimulating factor  
GVAX, granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting tumor vaccine or GVAX pancreatic cancer vaccine.  
IDO, indoleamine 2,3-dioxygenase  
IMiD, immunomodulating drug  
IPMN, intraductal papillary mucinous neoplasm  
ITK, IL2-inducible T-cell kinase  
KPC, KrasG12D;Trp53R172H;Pdx1-Cre  
Kyn, kynurenine  
L-Trp, L-tryptophan  
MDSC, myeloid derived suppressor cells  
mTOR, mammalian target of rapamycin  
MUC-1, mucin  
NSCLC, Non-Small-Cell Lung Cancer  
OS, overall survival  
PD-1, programmed cell death protein 1  
PD-L1, programmed death-ligand 1  
PD, progressive disease  
PDAC, pancreatic ductal adenocarcinoma  
PFS, progression-free survival  
PR, partial responses  
SBRT, Stereotactic Body Radiation Therapy  
SD, stable disease  
SPARC, secreted protein acidic and rich in cysteine  
TAA, tumor-associated antigens  
TCR, T-cell receptor  
Teff, effector T cells  
TIL, tumor-infiltrating lymphocyte  
TLR, toll-like receptors  
TMAs, tumor-associated macrophages  
TME, tumor microenvironment  
Tregs, regulatory T cells  
TXK, tyrosine-protein kinase

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