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Potential targets for pancreatic cancer immunotherapeutics

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Abstract

Pancreatic adenocarcinoma is the fourth leading cause of cancer death with an overall 5-year survival of less than 5%. As there is ample evidence that pancreatic adenocarcinomas elicit antitumor immune responses, identification of pancreatic cancer-associated antigens has spurred the development of vaccination-based strategies for treatment. While promising results have been observed in animal tumor models, most clinical studies have found only limited success. As most trials were performed in patients with advanced pancreatic cancer, the contribution of immune suppressor mechanisms should be taken into account. In this article, we detail recent work in tumor antigen vaccination and the recently identified mechanisms of immune suppression in pancreatic cancer. We offer our perspective on how to increase the clinical efficacy of vaccines for pancreatic cancer.

Keywords

chimeric antigen receptor; immune suppression; immune therapy; MDSC; pancreatic cancer; T cell; Treg; vaccine

Epidemiology of pancreatic cancer & clinical management

Pancreatic adenocarcinoma, henceforth indicated as pancreatic cancer, accounts for the majority of all pancreatic cancers and has the poorest outcome. Pancreatic cancer is highly aggressive and is the fourth leading cause of cancer death in the USA with over 43,000 estimated new cases in 2010 [1]. The prognosis of these patients is dismal with overall survival less than 5% and a median survival of 4–6 months. At the time of diagnosis, 15–20% of patients present with operable disease whereas approximately 40% are found to have locally advanced, unresectable disease and approximately 45% have metastatic disease. Surgical resection is the only known curative treatment for pancreatic cancer [2]. However, even with complete surgical resection, recurrence is common and a majority of patients recur with distant metastasis. Patients who develop recurrence usually present between 9 and 12 months after resection [3]. Median survival after surgery is 15–20 months with a 5-year survival rate of approximately 20% [3,4]. The median survival of patients with locally advanced, unresectable disease is 10–12 months [2,5]. The significance of this malignancy is

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further illustrated by comparing the estimated 36,800 deaths from pancreatic cancer with the 32,050 and 40,230 deaths from prostate and breast cancer, respectively [1].

The poor prognosis of pancreatic cancer is related to a combination of late detection, as most patients present with locally advanced or metastatic disease, and standards of care that consist of relatively ineffective chemotherapeutic regimens. Gemcitabine is currently approved, and the chemotherapeutic agent of choice, for the treatment of patients with pancreatic cancer, with adjuvant chemoradiation now considered the standard of care in many postoperative pancreatic cancer patients treated in North America. Multiple promising drugs, targeting hallmarks of cancer such as angiogenesis, proliferation and metastasis, have failed to provide any clinically relevant benefit [6].

A role for the immune response in pancreatic cancer

Immune-based treatments for cancer represent a rapidly evolving therapy with great potential. Based on the supposition that the immune system can discriminate tumor from normal self, investigators have pursued immune-based treatments for cancer for over 100 years [7]. Over the past 30 years, a large body of data has been accumulated showing that cancer patients generate B and T cells that recognize antigens expressed on autologous pancreatic tumor cells [8–14]. In addition, evidence has been obtained in animal models showing that mice deficient in genes associated with immunity (e.g., IFN [15] and perforin [16]) are prone to develop cancer. Furthermore, extensive analysis of immune infiltrates in human tumors has demonstrated a positive correlation between prognosis and the presence of a humoral response to pancreatic tumor antigens, such as MUC-1 and mesothelin, and of tumor-infiltrating cytotoxic T cells and Th1 cells [10,11,17,18]. By contrast, in a mouse model in which an activating K-Ras mutation is expressed in the pancreas, preinvasive pancreatic lesions are characterized by the infiltration of immune suppressor cells rather than immune effector cells, suggesting tumor immunity may be blocked from the inception of pancreatic cancer development [19]. All mice with the K-Ras mutation develop pancreatic adenocarcinoma and eventually die of disease. Finally, the finding that antagonism of negative T-cell regulators, such as cytotoxic T-lymphocyte-associated (CTLA) protein-4 and B- and T-lymphocyte attenuator (BTLA), can augment the antitumor immune response confirms that patients mount an immune response to their tumor [20,21]. Despite mounting evidence that an antitumor immune response is elicited in cancer patients, this response is ineffective and does not result in the elimination of tumor. Given that most tumor antigens are self- or mutated self-antigens and that the pancreatic tumor microenvironment is immunosuppressive (discussed at length below), this is not surprising [22]. Of particular note are the findings that both the prevalence of Treg in peripheral blood and tumor, and expression level of programmed death-1 ligand (PD-L1) in tumor are independent predictors of poor survival in pancreatic cancer [23–25]. Tregs that constitute 5–10% of CD4 T cells induce immune tolerance by suppressing host immune responses against self- and nonself-antigens [26–30], thus playing a critical role in tolerance and the immune response to malignancies. These findings strengthen the notion that pancreatic cancers induce antitumor immune responses. Thus, efforts towards improving the clinical efficacy of immune therapy should involve strategies to neutralize or overcome immune suppression.

We discuss in this article preclinical and clinical efforts towards immune therapy of pancreatic cancer.

Vaccination against tumor antigens as a treatment for pancreatic cancer

With the molecular identification of human tumor antigens in the early 1990s [31], the opportunity to specifically sensitize immune cells against tumor antigens became a reality,

leading to a multitude of experimental strategies for immune-based therapy of cancer. While multiple clinical studies have documented evidence of treatment-induced, antigen-specific immune responses, few, if any, protective immune responses have been observed in patients with metastatic disease. Despite this setback, there is renewed optimism for immune therapy, as vaccination postsurgery in patients with no or minimal disease was shown to have an impact in breast and pancreatic cancer [32,33]. In addition, vaccination against tumor antigens is an attractive approach to adjuvant treatment postsurgery, when tumor-induced immune suppression is minimal [34–36].

To be considered an ideal tumor vaccine candidate, expression of the antigen – be it mutated or unaltered self – must be restricted to the tumor or only minimally expressed elsewhere in the body. In the following sections, we have restricted ourselves to discussion of a short list of tumor antigens that fit this criterion for pancreatic cancer (Table 1). As such, the use of immunological adjuvants will not be discussed. We have focused on the most recent examples in pancreatic cancer studies, either murine or human, whenever possible. In this article, some tumor antigen targets are discussed to a greater extent than others, reflecting the quantity of reports in the literature. Table 2 lists a number of clinical trials involving pancreatic cancer patients, representing multiple different vaccine platforms (Figure 1) that will be discussed in the following sections.

Whole-cell vaccines

The simplest vaccine approach that has been applied to cancer is the inoculation of individuals with irradiated tumor cells. This approach has many advantages:

- Specific tumor antigens do not need to be identified or characterized prior to vaccination;
- Immune responses to multiple tumor antigens can be generated, which may protect against tumor escape variants [37];
- Such vaccines are not limited by patient HLA background owing to cross-presentation of tumor antigens after uptake by dendritic cells (DCs) [37,38] – this is particularly advantageous as tumor cell lines are readily available, while the availability of autologous tumor cells may be restricted;
- The tumor cell vaccine platform can be easily modified.

For example, tumor cells can be transduced to express immunomodulatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), as was performed by Jaffee *et al.* in a Phase I clinical trial [39]. In their studies, a pancreatic tumor cell vaccine induced a $CD8^+$ T-cell response, specific to mesothelin, regardless of HLA match between the tumor vaccine and recipient – demonstrating that cross-priming had occurred [38,39]. Mesothelin is a particularly promising cancer vaccine target owing to its low level of expression on nontumor tissues and high levels of expression on pancreatic as well as other cancers (i.e., ovarian) [40]. A Phase II trial for this vaccine is ongoing in patients with resectable pancreatic cancer (NCT00389610).

Tumor cell vaccines have also be modified to express epitopes, which increase antibodymediated uptake by DCs. Normally, MUC-1 expressed on tumors is immunogenic owing to overexpression and tumor-restricted hypoglycosylation [41]. The NewLink Genetics Corporation (IA, USA) has developed a whole-cell vaccine expressing MUC-1 modified to express α-gal epitopes, which is the focus of multiple clinical trials (NCT00255827, NCT00614601, NCT00569387 and NCT01072981) [42]. This vaccine takes advantage of anti-α-gal antibodies that are found in most people due to exposure to gastrointestinal flora, resulting in increased uptake of the vaccine in an antibody-dependent

manner [43]. In murine studies, the addition of such α-gal epitopes to a Muc-1^+ pancreatic cancer whole-cell vaccine resulted in increased production of anti-Muc-1 antibodies; enhanced tumor-specific T-cell responses and increased survival after challenge with Muc-1^+ B16 cells in α-gal knockout mice, previously sensitized to α-gal [44]. A study using similarly treated melanoma cells as vaccine resulted in the complete protection against melanoma in mice [45,46].

Autologous DCs have also been used in tumor vaccination when pulsed with tumor lysates or peptides, transfected with whole-tumor mRNA, or transfected with mRNA or cDNA of a specific antigen [47]. Mature DCs have the benefit of expressing high levels of costimulatory molecules in addition to both HLA class I and class II molecules, allowing for direct presentation of tumor antigens to, and enhanced activation of, both $CD8^+$ and $CD4^+$ T cells. For example, Schmidt *et al*. intratumorally vaccinated with whole tumor mRNA transfected DCs and found an antitumor specific immune response and significantly decreased tumor volume in a murine pancreatic cancer model [48]. In melanoma patients, the whole-tumor mRNA approach has been used to generate antitumor $CD4^+$ and $CD8^+$ T-cell responses [49,50]. Apoptotic pancreatic tumor lysates have also been evaluated as a source of antigen and have been demonstrated to elicit stronger antitumor lytic activity when used to stimulate autologous human $CD8^+$ T cells *in vitro* compared with those stimulated with tumor lysate-pulsed DCs [51]. Recently, a peptidepulsed autologous DC vaccine has been US FDA approved for the treatment of asymptomatic metastatic castration-resistant prostate cancer. This vaccine, known as Provenge® (Dendreon Corp., WA, USA) or Sipuleucel-T, consists of autologous, patient-derived DCs pulsed with a fusion protein consisting of the prostate tumor antigen prostatic acid phosphatase and GM-CSF [52]. In a Phase III clinical trial, vaccination resulted in a 3-year survival advantage in vaccinated castration- resistant prostate cancer patients (31.7% survival) compared with placebo (23%). Such a result is encouraging and gives hope that pancreatic cancer-targeted DC vaccines could produce similar effects.

In addition, autologous DCs, virally transduced to express IL-12, have also been used in cancer treatment. One pancreatic cancer patient receiving this treatment had a partial response in studies by Mazzolini *et al.* [53]. As the treatment DCs were not loaded with tumor antigen, cross-presentation of tumor antigens must have occurred.

A variation on the whole-cell approach involves the fusions of cancer cells and DCs, with the resulting cell used as the vaccine. Such vaccines can be made with autologous DCs and autologous tumor, with allogenic DCs and autologous tumor, or with autologous DCs and allogenic tumor [54]. This technique has been used to treat mice in a pancreatic tumor model, resulting in the generation of $CD8^+$ T cells with tumor-specific cytolytic activity and tumor rejection [55].

In cases in which an immunogenic tumor antigen is known, autologous DCs have been transfected with, or virally transduced to express, the mRNA or cDNA of a specific tumor antigen. This technique does not require that the exact immunogenic epitopes of the antigen be identified, as full-length protein is transfected. Such a vaccine consisting of autologous DCs transfected with MUC-1 cDNA was administered to ten patients with advanced breast, pancreatic or papillary cancer in a Phase I/II clinical trial. A MUC-1-specific $CD8^+$ T-cell response was generated in four patients, with a delayed-type hypersensitivity (DTH) response found in three patients [56]. However, all of the pancreatic cancer patients in this study developed progressive disease. Currently, a similar vaccine is in a Phase I/II clinical trial in melanoma patients using DCs transfected with the mRNA of tumor cells along with that of telomerase and survivin (NCT00961844). The use of multiple antigens may protect against tumor escape variants.

For some pancreatic tumor antigens, HLA-restricted immunogenic peptide epitopes have been identified and have been used to pulse DC vaccines. This approach allows the immune system to respond only to the immunologically relevant epitope, although its use is limited to patients expressing the corresponding HLA alleles. In an encouraging study by Lepisto *et al.*, patients with resected pancreatic and biliary tumors were administered MUC-1, peptide-pulsed, autologous DCs in a Phase I/II clinical trial [57]. Although a clear antigen-specific T-cell response was not detectable following vaccination, 25% of the patients were alive at year 4. In similar studies with a telomerase peptide-pulsed DC vaccine, an antigen-specific $CD8^+$ T-cell response was generated in patients who developed various cancers following vaccination. This response was enhanced when DCs were also pulsed with class II telomerase peptides, illustrating the importance of $CD4^+$ T cells in fighting tumor [58]. Pulse can also be performed with peptides from multiple tumor antigens, as was performed in a Phase I clinical study by Carbone *et al.* Patients with various cancers, including pancreatic cancer, immunized with p53 and K-Ras peptide-pulsed PBMCs saw increased survival [59].

Peptide vaccines

Peptides corresponding to immunogenic tumor antigens or antigen epitopes have been administered as cancer vaccines, many of which have been designed to enhance the $CD8^+$ T-cell response – the most important response for elimination of tumor. Peptide vaccines do not require manipulation of patient tissues, whose availability may be limited. A number of peptide vaccines have been successfully used to produce antigen-specific responses in pancreatic cancer patients. In a Phase I study, vaccination with a 100-mer peptide of the MUC-1 extracellular tandem repeat generated a MUC-1-specific T-cell response in some patients with resected or locally advanced pancreatic cancer, with two of the 15 patients alive at 61 months [60]. In a separate Phase I clinical trial using the same 100-mer peptide vaccine, the production of anti-MUC-1 circulating antibodies was detected in patients with inoperable pancreatic or biliary cancer, although no significant impact on survival was discovered [61]. A telomerase-based vaccine, consisting of the human telomerase reverse transcriptase (also known as GV1001) peptide, was found to induce a telomerase-specific immune response in 63% of evaluable patients, as measured by DTH in nonresectable pancreatic cancer. Those with a positive DTH were found to live longer than those that did not have a positive DTH [62]. More recently, a Phase III clinical trial was performed in which the effect of gemcitabine treatment on survival was compared with gemcitabine treatment in combination with GV1001 therapy in unresectable and metastatic pancreatic cancer patients [63]. However, the trial was terminated when no survival benefit was found. In similar studies, VEGF receptor (VEGFR)2–169, a peptide epitope vaccine, has been administered with gemcitabine to patients with advanced pancreatic cancer. A total of 83% of patients receiving the vaccine had an antigen-specific DTH and VEGFR2-specific $CD8^+$ cells were detected in 61% of those vaccinated, with a median overall survival time of 8.7 months [64]. A randomized, placebo-controlled, multicenter, Phase II/III study of this VEGFR2–169 peptide vaccine therapy, combined with gemcitabine, is currently underway in patients with unresectable advanced or recurrent pancreatic cancer [301].

The most exciting results have come from studies of K-Ras-targeted peptide vaccines. In a pilot vaccine study, pancreatic and colorectal patients were vaccinated with K-Ras peptides containing patient-specific mutations. Three of the five pancreatic cancer patients displayed an antigen-specific immune response to a K-Ras [65]. Disease progression was observed in the two pancreatic cancer patients that did not respond to the vaccine, with the responders having no evidence of disease. Of the pancreatic cancer patients, a mean disease-free survival of 35.2+ months and a mean overall survival of 44.4+ months were observed. In a longer-term study, patients were followed for up 10 years after surgical resection of

pancreatic adenocarcinoma and vaccination with a K-Ras peptide vaccine administered concomitantly with GM-CSF [33]. Remarkably, 20% of patients who received the vaccine were still alive at this time point. Immunological tests showed that a memory T-cell response was still present in 75% of the survivors. Such results with peptide vaccines are highly encouraging.

To increase the immunogenicity of peptide vaccines, some groups have mutated key anchor residues in the peptides such that binding to MHC-I molecules is increased. Therefore, presentation to $CD8^+$ T cells is also increased. This is especially important when vaccinating against tumor (self) epitopes, as they are often weak or only intermediate binders to HLA molecules [66–70]. In a murine model of pancreatic cancer, our group has found this strategy to increase survival when applied to a peptide vaccine derived from murine mesothelin [Hamilton N & Hawkins WG: Unpublished Data]. Similarly, a MUC-1 peptide vaccine modified in this way was shown to enhance production of IFN-γ from patient and normal donor T cells. MUC-1-specific T-cell clones, generated via stimulation with this peptide, could lyse targets pulsed with native Muc-1 epitope as well as $HLA\text{-}A2^+$ $MUC\text{-}1^+$ human tumor cells *in vitro* [71]. Remarkably, one case has been reported in which vaccination with a modified HLA-A2-restricted survivin peptide resulted in remission of liver metastasis in one individual with pancreatic cancer [72].

An alternative method to enhance the presentation of peptides is to link them to antibodies, or other peptides, which target them to DCs. For example, an antimurine DEC205 antibody fused to either human mesothelin or survivin has been shown to enhance the immune response by directing delivery of tumor antigen to activated DCs in murine studies [73,74]. Similarly, the TAT protein from HIV, which facilitates entry into cells, has been fused to antigens in order to enhance uptake by DCs [75]. However, this technique has been used mainly to introduce antigens, such as carcinoembryonic antigen (CEA), into DCs and not as a peptide vaccine alone [76–79].

Another protein-based vaccine approach utilizes an anti-idiotypic vaccine that mimics the tumor antigen, CEA. This murine monoclonal, 3H1, has been successfully used to generate anti-CEA humoral and T-cell responses resulting in the rejection of CEA-expressing tumor in a murine model of colon cancer [80–82]. Two Phase II trials involving 3H1 have been completed in lung and colorectal cancer, although the results have yet to be published (NCT00006470 and NCT00033748).

More recently, an effort has been made to generate personalized peptide vaccines based on the tumor-antigen epitopes that are most immunogenic for a particular patient. Yanagimotto *et al.* applied this approach, in combination with gemcitabine therapy, to pancreatic cancer in a Phase I clinical trial. Prior to vaccination, T cells from patient PBMCs were screened against a panel of tumor antigen-derived peptides. Patients were vaccinated only with the peptides to which they had a response [14]. An increase in tumor antigen-specific T-cell responses was observed from the 13 evaluable patients with no correlation to clinical responses or humoral responses post vaccination, although 11 patients experienced either reduction in tumor size. Median survival time was 7.6 months. In 2010, this group published a similar Phase II study and found a median survival time of 9 months and a 1-year survival of 38% [83].

DNA vaccines

When specific tumor antigens are known, DNA vaccination can be utilized. DNA vaccines can be administered to humans via intramuscular injection with or without electroporation. This technique allows for an immune response to multiple potential epitopes within an antigen to be generated regardless of the recipients' MHC profile [84]. In pancreatic cancer

studies, DNA vaccination has mainly been applied to murine models of cancer, though there are ongoing clinical trials in other cancers (NCT00807781, NCT00859729, NCT00471133 and NCT01064375).

In one such murine pancreatic cancer study, vaccination with either murine or human fulllength survivin DNA generated an antitumor specific response, increased infiltration of tumor with lymphocytes and increased survival [85]. Similar results were found in studies with Muc-1 DNA vaccine when used as a preventative or therapeutic vaccine in a pancreatic cancer model [86]. In a murine ovarian cancer model, a full-length human mesothelin DNA vaccine has also been shown to inhibit growth of human mesothelin-expressing tumors, enhance survival and induce a $CD8^+$ T-cell, $CD4^+$ T-cell and humoral response [87]. Likewise, an oral DNA vaccination with either full-length extracellular domain or regions 1–4 of VEGFR2 resulted in decreased tumor size, intratumoral microvessel and density of liver metastases in murine colon cancer studies. An increase in survival time and VEGFR2 antibody was also observed in serum with both vaccines [88].

DNA vaccination has also been used to express a CEA66, which encodes for CEA linked to a T-helper epitope from tetanus toxin [89]. This vaccine skews the response to a Th1 phenotype and increases IFN-γ production from CEA-specific T cells when boosted with either CEA peptide or with repeat DNA vaccination in mice [90,91]. However, no studies with tumor challenges have been published with the CEA66 vaccine.

Highly engineered molecules, such as HLA-A2 single-chain trimers (SCTs) can also be expressed by DNA vaccination. HLA SCTs consist of the HLA heavy chain, β_2M, and a peptide of interest connected by flexible linker regions (Figure 2). When introduced via DNA vaccination, SCTs bypass antigen processing, leading to direct expression of the encoded HLA/peptide complex on the cell surface [92]. This expression is more stable than that of the endogenous HLA/peptide compound, allowing for increased stimulation of antigen specific $CD8^+$ T cells [93–95]. As such, SCTs are the ideal platform for the presentation of tumor antigens to $CD8^+$ T cells.

In fact, SCT DNA vaccines have been utilized for multiple viral and tumor targets and have been shown in *in vivo* murine studies to be more effective as DNA vaccines than comparable peptide and cDNA vaccines [93,94,96–100]. For example, Hung *et al.* administered such a DNA vaccine encoding a HLA-A2 SCT presenting a human mesothelin peptide to mice that transgenically expressed HLA-A2 in an ovarian cancer model. A mesothelin specific $CD8^+$ T-cell response was generated and tumor-free survival was enhanced compared with vaccination with an Ova-SCT [99]. However, the HLA-A2 mice used were not tolerized to the foreign human mesothelin peptide presented in the SCT, making these data less translationally relevant. More recently, the SCT platform has been improved upon such that peptide binding to the HLA molecule and interaction with CD8 is enhanced. In addition, the pan-T-cell helper epitope (PADRE) has been included in the SCT DNA vaccines [94]. Such a SCT vaccine presenting a West Nile virus peptide showed increased survival in West Nile virus-infected HLA-A2 transgenic mice [100]. This improved SCT platform shows great promise and is currently in development for testing in patients with breast cancer at our institution.

Vaccines with microorganisms

In addition to more traditional (i.e., peptide-based) vaccine modalities, other groups have utilized various properties of microorganisms or viruses to enhance vaccination. Microorganisms can be used essentially as an antigen delivery system, but also have the added benefit of acting as an adjuvant by stimulating an innate immune response though engagement of pattern recognition receptors, such as Toll-like receptors (TLRs). A number

of groups have used virus to deliver pancreatic cancer associated antigens and have tested them in murine models and/or clinical trials. For example, one group has used virus as a scaffold to display MUC-1, administration of which resulted in a robust immune response and a delayed growth of MUC-1-expressing tumors in mice transgenic for human MUC-1 [101].

However, most virally based vaccines are designed to deliver transgenes that enable infected cells to produce the tumor antigen of interest. Adenovirus has been used in this way to deliver CEA and was found to decrease tumor volume in mice bearing CEA-expressing tumors [102]. In human clinical trials, a pox virus-based approach to deliver tumor antigens, as well as costimulatory molecules, has been used. Kaufman *et al*. have completed a Phase I study of pancreatic cancer patients vaccinated via a vaccinia virus (PANVAC-V) expressing a HLA-A2-restricted CEA peptide, a MUC-1 peptide and three costimulatory molecules (B7.1, ICAM-1 and LFA-3 – also known as TRICOM) with GM-CSF administered as an adjuvant. An additional vaccination with a fowlpox virus (PANVAC-F) expressing the same molecules was also administered [103]. An antigen-specific antibody response was discovered in all ten patients and antigen-specific T-cell responses were found in five out of eight patients tested, with increased survival seen in those who generated an immune response.

Similarly, Morse *et al*. have generated an alphavirus vector expressing CEA that is packaged in a virus-like particle termed CEA(6D) VRP (AVX701) [104]. The alphavirus was chosen as it preferentially infects DCs [105]. Although only one pancreatic cancer patient was included in this study, the liver metastasis of that individual remarkably resolved after vaccination. A lentiviral system has also been generated that specifically targets DCs and has been shown to inhibit tumor growth in a murine tumor model. However, this virus has not been used to vaccinate against pancreatic tumor antigens, nor has it been utilized in a pancreatic cancer model or clinical studies [106].

Heat-killed yeast, transfected with tumor antigens, have been shown to activate DCs when used as vaccines in murine models [107]. Remondo *et al.* showed that *Saccharomyces cerevisiae* (SC), expressing a CEA transgene, activated human DCs, which then successfully activated CEA-specific T-cell clones. The T-cell clones were able to lyse human CEA^+ pancreatic tumor lines [108]. In murine studies, vaccination with SC-CEA resulted in the generation of CEA-specific T cells, reduced tumor volume and increased survival when vaccinated hCEA-transgenic mice were challenged with a CEA-expressing tumor [109,110]. When compared with a CEA-pox vaccine platform, similar levels of CEA-specific T cells were generated in response to SC-CEA [110]. However, T-cell receptor (TCR) repertoires differed in their specificity and avidity. The authors suggest taking advantage of this diversity by vaccinating with both modalities, although this has not been performed in humans as of yet. In a murine carcinogen-induced lung tumor model, SC has been used to vaccinate against K-Ras, resulting in tumor regression [111].

Bacteria can also be used in vaccination, with the most popular being the intracellular facultative bacteria *Listeria monocytogenes* (LM) and *Salmonella enterica* serovar Typhimurium (ST) [112]. Bacteria have the advantages of being inexpensive to generate, are easily attenuated and can be killed with antibiotics if needed. LM infects phagocytic cells and has the unique ability to escape the phagosome and live intracellularly. Therefore, live-attenuated LM can deliver a protein, mRNA or cDNA of interest directly to the cytosol of antigen-presenting cells such as macrophages. This platform has been applied to human mesothelin, resulting in a mesothelin-specific $CD8^+$ and $CD4^+$ T-cell response in mice and in cynomolgus monkeys, which had a therapeutic effect in tumor-bearing mice [40]. More recently, a Phase I clinical trial found this LM-based mesothelin-expressing vaccine,

CRS-207, to be well tolerated [113]. LM vaccines expressing Her-2/neu, p53 and VEGFR2 have been applied to murine breast cancer models, showing antigen-specific T-cell responses and decreased tumor growth [114–117].

Similar to LM, ST infects phagocytic cells but evades lysis by blocking the fusion of the phagosome and lysosome. Most work has focused on developing oral ST-based DNA vaccines. One such vaccine delivered CEA DNA and was shown to prevent growth of lung carcinoma in CEA transgenic mice [118]. Similarly, a ST-CEA/CD40 DNA vaccine resulted in a complete rejection of a CEA-expressing MC38 colon carcinoma in murine studies [119]. In addition, the type III secretion system of ST can be utilized to deliver protein antigen directly into the cytosol of antigen-presenting cells. When this strategy was used to vaccinate against survivin in a murine model, an antitumor immune response against CT26-derived tumors was observed [120].

Monoclonal antibody treatment

The humoral immune response to tumor antigens, such as mesothelin, has been found to be a favorable prognostic factor for pancreatic cancer [10,17,121,122]. The anticancer effect of such antibodies is thought to be primarily mediated via antibody-dependent cell cytotoxicity (ADCC), although complement-mediated lysis may contribute [108,123–126]. A number of groups have generated monoclonal antibodies to cell surface tumor antigens, such as mesothelin, MUC-1, CEA and Her-2/neu. In addition to ADCC, these antibodies can be linked to immunotoxins resulting in direct killing of the tumor cell after internalization or linked to a radioisotope and used as radiopharmaceuticals. We restrict our discussion to antibodies directed at antigens that have been extensively studied as vaccine targets (Table 3). As such, antibodies targeted to blocking angiogensis, such as bevacizumab, will not be discussed.

A number of antibodies to mesothelin have been studied in both mouse and humans. For example, SS1P is a murine single-chain Fv, specific for human mesothelin, which has been linked to *Pseudomonas* exotoxin A (PE), which results in cell death via inhibition of EF2 when internalized [127]. In Phase I clinical studies SS1P was found to be well tolerated and administration of a version of SS1P with releasable PEGylation resulted in complete regression of a mesothelin-expressing human carcinoma in mice with only a single dose [127–129]. A mouse–human chimeric IgG1k monoclonal antibody, MORAb-009, utilizes the same Fv as SS1P and was shown to elicit ADCC on mesothelin-positive cells. When combined with gemcitabine, MORAb-009 reduced tumor growth in nude mice compared with either treatment alone and had no adverse effects in nonhuman primates [130]. Two fully human, antihuman mesothelin antibodies, M912 and HN1, have been developed, which bind mesothelin- positive cells and result in their lysis via ADCC [131,132]. Similar to SS1P, HN1 has been fused to truncated PE-A immunotoxin, although its binding site on mesothelin probably binds a distinct but overlapping epitope to that of SS1P [132].

Many anti-CEA antibodies have been generated, with the bulk of studies in radioimmunotherapy, which will not be covered in the scope of this article. However, there are a number of anti-CEA antibodies that have been used for immunotherapy. One such antibody is hPRIA3, a humanized anti-CEA antibody, originally developed in mice, which binds membrane-bound CEA [133,134]. hPRIA3 has been shown in *in vitro* studies to bind to, and enhance, CEA-specific cytotoxicity of CEA^+ human colorectal cancer cell lines when incubated with human PBMCs and NK cells, which can be increased when the Fc portion of the antibody is glycosylated [135,136]. A second anti-CEA monoclonal, hMN-14 (labetuzumab), has also been shown to induce ADCC *in vitro* with CEA^+ colon tumor cells and inhibited growth of lung metastases in nude mice [137]. A Phase I/II trial with hMN-14

in pancreatic cancer patients has been completed but the results have not been published (NCT00041639).

PankoMab™ (Glycotope, Germany) is a murine antihuman MUC-1 antibody that binds to a carbohydrate induced conformational tumor epitope of MUC-1, greatly increasing its tumor specificity [138]. PankoMab can induce ADCC of MUC-1 positive cells and can also induce death following internalization by inhibition of RNA polymerase when linked to β-amanitin. The humanized version of PankoMab has been shown to react to the tumor expressed MUC-1 in multiple human carcinomas, although no clinical trials have been published [139].

An anti-Her-2/neu antibody, know as Herceptin® (Genetech Inc., CA, USA) or trastuzumab, has been used with some success to treat pancreatic cancer in murine models. Treatment with trastuzumab prolonged survival and reduced liver metastasis in nude mice orthotopically challenged with human pancreatic tumor cell lines that expressed Her-2/neu at low levels. The pancreatic lines were sensitive to ADCC lysis by trastuzumab *in vitro* [140]. Similar results were found when nude mice, challenged with Her-2/neu^{high}-expressing human pancreatic cancer cell lines, were treated with both trastuzumab and 5-fluorouracil [141]. The combination of treatment significantly inhibited tumor growth compared with either treatment alone. When combined with matuzumab, an anti-EGFR antibody, trastuzumab treatment resulted in inhibited tumor growth in a nude mouse model of pancreatic cancer [142]. This combined treatment was more than four-times more effective than treatment with either antibody alone. This group also found that the combined antibody treatment was more effective than gemcitabine treatment in orthotopic nude mice studies [143]. A number of clinical trials involving trastuzumab are listed in Table 3.

Passive T-cell therapy

In recent years, much work has focused on adoptive tumor immunotherapy in which the patient's own T cells are expanded and reinfused into the patient. One method results in the selective expansion of T cells endogenously expressing TCRs specific for the tumor antigen of interest. For example, in a murine ovarian cancer model, Hung *et al.* vaccinated mice with a murine mesothelin DNA prior to antigen-specific T-cell expansion *ex vivo*. The mesothelin-specific T-cell clones were transferred to tumor-challenged mice, resulting in decreased tumor volume and increased survival [144]. Similarly, in a clinical study, MUC-1-specific autologous T cells, isolated from patient PBMCs, were expanded by incubation with a MUC-1-presenting cell line prior to administration to pancreatic cancer patients. The mean survival time for unresectable patients in this study was 5 months [145]. However, patients with resectable pancreatic cancer had 1-, 2- and 3-year survival rates of 83.3, 32.4 and 19.4%, respectively, and a mean survival time of 17.8 months. In a similar study by the same group, Kondo *et al.* isolated adherent cells from patient PBMCs to generate mature DCs that were then pulsed with MUC-1 peptide. The pulsed DCs were administered, along with autologous expanded MUC-1-specific T cells, to patients with unresectable or recurrent pancreatic cancer. Remarkably, a complete response was observed in one patient with lung metastases and the mean survival time of the whole group was 9.8 months, suggesting that the addition of pulsed DCs may have improved the outcome [146].

Chimeric antigen receptors

An alternative to antigen-specific expansion is the transduction of patient T cells, usually via lentivirus, with a chimeric antigen receptor (CAR) specific for a tumor antigen. Such receptors are transmembrane proteins typically comprise an antibody-derived single-chain variable fragment (scFv) specific for a tumor antigen fused to a hinge region, a spacer, a membrane spanning element and signaling domain [147,148]. Often the intracellular

signaling domain contains the signaling motifs from multiple molecules, such as 41BB, OX40, CD28 and TCRζ chain. This allows for both TCR and costimulatory signaling cascades to be initiated, leading to T-cell activation. The resulting T cells recognize the tumor antigen in its native form and do not rely on presentation of antigen by MHC I. This is highly advantageous, as tumors often downregulate MHC-I expression.

Chimeric antigen receptors have been created with specificity for mesothelin, CEA, MUC-1 and Her-2/neu. Carpenito *et al.* have generated a CAR that recognizes mesothelin via the SS1 scFv and utilizes the intracellular signaling domains of CD3ζ, CD28 and CD137 [147]. When human T cells expressing this receptor were transferred to tumor bearing *NOD/SCID/IL2rγ*$^{-/-}$ mice, a reduced tumor volume was observed. Similar results were generated when T cells expressing CARs, possessing CD28 and TCRζ intracellular motifs and a specificity for human CEAs were administered to CEA^{+} tumor-challenged mice [149]. In addition, Shirasu *et al.* generated a CEA-specific CAR utilizing the human monoclonal antibody C2–45 and cytoplasmic motifs of CD28 and CD3ζ [150]. When expressed in Jurkat cells, the CEA-CAR initiated signaling cascades similar to those expected from TCR-mediated activation.

A CAR with specificity to MUC-1 has also been generated using the SM3 antibody with intracellular signaling motifs of CD28, OX40 and CD3ζ [151]. T cells expressing this CAR proliferated and produced cytokine in response to antigen challenge, killed MUC-1^{+} tumor cells *in vitro* and delayed growth of Muc-1 tumors in mice. Bakhtiari *et al.* developed a similar anti-MUC-1 CAR with the intracellular signaling motifs CD28 and CD3ζ [152]. When expressed in Jurkat cells, MUC-1-specific stimulation included cytokine production and killing of a MUC-1^{+} cell line.

T cells expressing a Her-2/neu-specific CAR with CD28 and CD3ζ chain signaling motifs has been shown to produce cytokines and to lyse a Her-2/neu^{+} breast cancer tumor line following antigen stimulation [153]. Mice challenged with breast cancer tumors had long-term tumor-free survival after administration of these T cells and were found to have developed a memory response that protected from rechallenge with tumor. Likewise, T cells expressing a Herceptinbased CAR with signaling motifs from CD28, CD3ζ and 41BB was shown to lyse Her-2/neu^{+} tumor *in vitro* and suppressed tumor growth in an *in vivo* murine model [154]. A number of Phase I clinical trial involving Her-2/neu CAR-expressing T cells are currently recruiting breast cancer patients (NCT00889954, NCT00902044 and NCT01109095).

Although this process results in a large population of antigen-specific T cells, adoptive immunotherapy is a very expensive and time consuming process in comparison with the vaccine and antibody therapies discussed in this article. There are no published studies utilizing CAR expressing T cells in pancreatic cancer or in murine models of pancreatic cancer.

Pancreatic cancers induce an immunosuppressive tumor microenvironment

Despite all the progress in vaccine development and increased understanding of basic immune mechanisms, the clinical application of therapeutic cancer vaccines has been characterized by many setbacks. Recent vaccines for melanoma (e.g., Canvaxin™ [CancerVax, CA, USA] and Melacine® [Corixa, WA, USA]), renal cell carcinoma (Oncophage® [Antigenics Inc., MA, USA]) and prostate cancer (G-VAX), tested in Phase III clinical trials, all failed to show clinical efficacy [7,155]. This has increased awareness of gaps in our knowledge regarding tumor-induced immune suppression.

We, and others, have shown that the tumor microenvironment in pancreatic cancer preferentially favors the recruitment of immune suppressor, rather than immune effector, cell types (Figure 3) [30,156]. Pancreatic cancer cells themselves actively contribute to immune suppression through production of immune suppressive cytokines (e.g., TGF-β [157]) and by expressing surface molecules that mediate immune suppression (e.g., FasL [158], PD-L1 [25] and possibly BTLA [159]). In addition, tumor-associated macrophages (TAMs), tumor-associated fibroblasts, plasmacytoid dendritic cells (pDCs) expressing the tryptophan-converting enzyme 2,3-indoleamine dioxygenase (IDO) [160], Treg and soluble factors produced by suppressor cells all contribute to pancreatic cancer-induced immune suppression. Finally, recent studies have described a heterogeneous population of immature cells arising from the myeloid lineage, termed myeloid-derived suppressor cells (MDSCs) [161]. MDSCs express CD11b and myelomonocytic/macrophage markers such as CD14 and CD33, or granulocytic/neutrophilic markers such as CD15. In some reports, the immature status of MDSCs was demonstrated by the absence of markers of terminal differentiation ($Lin^{-}DR^{lo/-}$) [162]. As such, MDSCs differ from TAMs and pDCs, which are fully differentiated lineages of cells. The accumulation of MDSCs in patients with advanced cancers, including pancreatic cancer, were demonstrated to be closely related to the extent of disease and correlated well with disease stage [163].

Instrumental in shaping the immunosuppressive environment are the tumor cells and tumor-associated fibroblasts, in particular through secretion of chemokines and other cytokines [164]. Chemokines induce motility of endothelial cells and tumor cells. In addition, leukocyte subpopulations recruited to the tumor by chemokines are skewed in the tumor environment towards cells that support tumor growth, promoting angiogenesis, tumor invasion and metastasis formation while suppressing immune effector cells. For example, we recently observed that Treg cells in the tumor microenvironment of human pancreatic adenocarcinoma express CCR5 and the pancreatic tumor cells produce greater than tenfold higher levels of the CCR5 ligands, CCL3, CCL4 and CCL5 [165].

There is clear evidence of cross-talk between the various suppressor cell subsets. For example, MDSCs can promote Treg recruitment and maintenance through TGF-β-dependent and -independent mechanisms. IDO production by tumor-associated DCs and macrophages has also been implicated in Treg activation and expansion. IDO expression can be induced in DCs by reverse signaling through B7–1 and B7–2 (CD80 and CD86, respectively) and CTLA-4 expressed on Tregs. In turn, IDO activates Tregs and induce differentiation of naive CD4 T cells towards Tregs. By converting tryptophan into *N*-formyl kynurenine, effector T cells that are dependent on tryptophan for proliferation, are inhibited [166,167]. TAMs and MDSCs also skew the CD4 T-cell subset composition towards a Th2 phenotype through cytokines such as IL-10. The interplay between the various subsets of suppressor cells and cytokines creates a powerful barrier against immune attack.

Enhancing vaccine efficacy by targeting immune suppression

As we make progress in defining the molecular pathways of immune suppression, it becomes more and more relevant to integrate strategies that activate antitumor immunity with those that neutralize or overcome tumor-induced immune suppression (Figure 4) [155]. Just as immune activation strategies should be multifaceted to take advantage of both innate and adaptive immune effector mechanisms, targeting immune suppression may need to be equally comprehensive in order to have an effect. Preclinical studies in animal tumor models have provided proof-of-concept that targeting immune suppression may have an impact on cancer vaccines. For example, a promising antibody treatment involves the use of anti-CTLA-4 [168]. Aimed at preventing the downregulation of activated T cells, anti-CTLA-4 monotherapy has shown encouraging results in a small percentage of patients with advanced

cancers [169]. In a recently completed Phase III trial, anti-CTLA-4 antibody with or without gp-100 peptide vaccine significantly prolonged survival of patients with metastatic melanoma. Interestingly, anti-CTLA-4 did not improve the gp-100 peptide vaccine efficacy. The reason for the lack of additive effect is not clear to the investigators of the study but may be due to minimal clinical activity of the gp-100 vaccine [170]. Although disappointing, the clinical benefit of anti-CTLA-4 was statistically significant with or without gp-100 vaccine, supporting the idea that in patients with an existing endogenous antitumor immune response, the use of anti-CTLA-4 may enhance this response and induce a clinically meaningful response. At the same time, it is unlikely that anti-CTLA-4 monotherapy will lead to long-term cures in the majority of patients. In this regard, a recently completed Phase II trial in patients with locally advanced or metastatic pancreatic cancer (NCT00836407) showed that anti-CTLA-4 monotherapy is ineffective in this patient population [171]. Likewise, the most commonly used strategy for neutralization of the PDL1-PD1 pathway is via antibodies that either block signalling through PD1 or that neutralize PDL1. The first clinical studies have been performed with PD-1-blocking antibodies, both in solid tumors (MDX-1106)[172] and in hematologic malignancies (CT-011)[173]. An experimental drug, AMP-224, consisting of a PD-L2/Ig fusion protein that binds to PD-1 on T cells and prevents the inhibitory signal [302] has not yet been tested clinically. Finally, the depletion of MDSCs in murine models has been associated with improved host immune responses, resulting in delayed tumor growth, improved survival and increased efficacy of vaccine therapy. Elimination of MDSCs has been shown to improve antitumor responses, restore CTL and NK function, decrease tumor angiogenesis and enhance immunotherapy [174–176].

Rather than describe specific strategies in detail, we will offer some comments on this topic:

- It can be concluded from our current knowledge of immune suppressor mechanisms in cancers that multiple mechanisms are at play, which act in concert. Some of these, for example the production of certain cytokines such as IL-10, are not restricted to an individual cell type. Thus, successful intervention may require a multipronged attack;
- There is heterogeneity among different histological types of tumor with regard to which suppressor mechanisms are present or dominant, and perhaps even within tumors of the same clinical stage and histologic type. This has obvious implications for the type of strategy that will be used;
- Vaccination against self-antigens has been associated with induction Tregs and MDSCs. For example, Nishikawa *et al*. have reported that immunization of mice with plasmids encoding tumor antigens resulted in marked enhancement of *in vivo* tumor growth in a murine pulmonary metastasis model [177]. Subsequent analysis of their findings revealed that depletion of Tregs reversed this effect and adoptive transfer restored it, suggesting that vaccination promoted the proliferation of autoantigen-reactive Tregs. Similarly, T-cell clones derived from tumor-infiltrating lymphocytes were recently described, which recognized the melanoma antigen, LAGE-1 with characteristics of Tregs [178];
- Finally, we reiterate that tumor vaccines may be most effective in a setting of minimal disease (i.e., postsurgery, at a time when tumor-induced immune suppression is likely to also be minimal). In this setting, vaccines that induce immunological memory through tumor-specific CD4 T cells and IgG antibodies may be most effective.

However, as discussed previously, most patients with pancreatic cancer have advanced disease at the time of diagnosis and are not candidates for surgery.

Conclusion

There is ample evidence to suggest that pancreatic cancer induces an antitumor immune response. There is also ample evidence that this response can be enhanced by various immune-based strategies. However, successful clinical application may require a better understanding of how pancreatic cancers evade immune recognition and integration of specific strategies that target immune suppressor mechanisms.

Future perspective

When reviewing the current status of FDA-approved immune-based interventions for cancer, it is relevant to ask whether there is a future for immune therapy for the treatment and prevention of cancer. With the recent FDA approval of Provenge being a prime example [52,179,180] we consider the future to be optimistic for several reasons. First, our understanding of immune mechanisms has accelerated over the past decade and will probably continue. This includes specific knowledge on how to overcome tolerance to self (tumor) antigens, implementation of immunological adjuvants and strategies to induce immunological memory. Combined with the growing emphasis on defining molecular pathways of immune suppression and negative regulatory pathways (CTLA-4, PD-1 and others), the challenge going forward in the near future is to integrate everything we have learned into new generation vaccines. Furthermore, we expect the rapidly evolving techniques, developed to sequence cancer genomes, to have unprecedented consequences for the treatment and prevention of cancer. What was considered a monumental task just several years ago will become a routine and straightforward assay in the next few years. The first such efforts have already been successfully completed in pancreatic cancer [181,182]. These studies not only demonstrated the presence of multiple mutations that can be targeted; they also showed that many of these mutations are already present in primary pancreatic cancers, and suggest vaccination after primary resection may protect against recurrence. The main implication for immune therapy will be that personalized vaccines will no longer be science fiction, but state of the art. The main advantage will be that vaccines can be tailored towards unique mutations found only in the patient's cancer [183], or, alternatively, applied to certain subtypes of tumors. Such mutations have been identified in the past [184], but in the next 4–6 years we expect the sequencing of a patient's cancer (and normal cells) to be part of the diagnosis. From an immunological standpoint, the immune response to mutated (nonself) antigens will be much more robust, as tolerance to such antigens should be minimal.

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301. The International Clinical Trials Registry offers a registration of all interventional clinical trials conforming to WHO standards International Clinical Trials registry ID number. JPRN-UMIN000001664 http://apps.who.int/trialsearch/trial.aspx?trialid=JPRN-UMIN000001664

302. Amplimmune Inc. is a company that is involved in developing immune-based biologics to treat patients in the fields of cancer, autoimmunity, transplantation and infectious disease www.amplimmune.com

Executive summary

Epidemiology of pancreatic cancer & clinical management

- Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the USA.
- No effective therapy exists for pancreatic adenocarcinoma owing to its late discovery and strong resistance to conventional therapy.

A role for the immune response in pancreatic cancer

- Pancreatic cancer elicits an adaptive antitumor immune response.
- Both the prevalence of Tregs and expression levels of PD-L1 in the tumor are prognostic factors.

Vaccination against tumor antigens as a treatment for pancreatic cancer

- Vaccination against pancreatic cancer antigens is an attractive approach to adjuvant treatment postsurgery.
- The ideal cancer antigen not only elicits a strong immune response but is exclusively expressed on tumor cells.

Whole-cell vaccines, peptide vaccines, DNA vaccines & vaccines with microorganisms

- Pancreatic cancer-associated antigens that are candidates for immune targeting include Her2/neu, Muc-1, CEA, mesothelin, telomerase and survivin.
- Multiple vaccine platforms have been used successfully for induction of antigen-specific immune responses in animal tumor models and in *in vitro* studies.

Monoclonal antibody treatment

- Adoptive transfer of antibodies to pancreatic cancer surface antigens such as mesothelin has been used for therapy with promising results in animal tumor models.
- The antibodies may induce antibody-dependent cell cytotoxicity or complement-dependent lysis, or block tumor growth, for example when directed to growth factor receptors such as EGF and Her2/neu.

Passive T-cell therapy

- Promising clinical results were obtained through adoptive transfer of patient-derived T cells expanded *ex vivo*.

Chimeric antigen receptors

- A variation of passive T-cell therapy involves the genetic engineering and expansion of T cells expressing a chimeric receptor consisting of a single-chain variable fragment antibody chain specific for a pancreas cancer-associated antigen fused to a transmembrane signaling domain essential for T-cell activation such as CD28 and TCRζ.
- This strategy has not yet been tested in patients with pancreatic cancer.

Pancreatic cancers induce an immunosuppressive tumor microenvironment

- The tumor environment in pancreatic cancer is highly immunosuppressive through increased prevalence of multiple types of suppressor cells.

Enhancing vaccine efficacy by targeting immune suppression

- It is key for the treatment of patients with established disease to integrate immunization strategies with those that neutralize immune suppression.
- Alternatively, vaccines may be most efficacious when administered as an adjuvant therapy, when immune suppression is minimal.

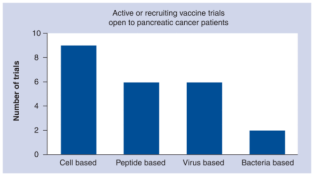

Figure 1. Overview of current immunotherapy trials in pancreatic cancer

All active trials listed on Clinical Trials.gov were categorized based on the type of vaccine used.

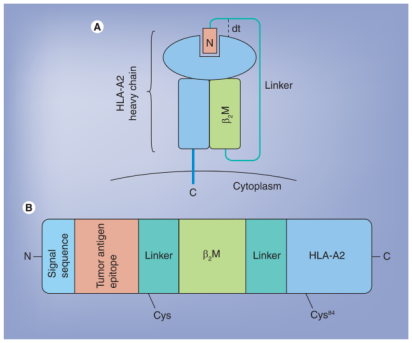

Figure 2. Structure of the disulfide trap single-chain trimer

Representation of a fully folded dtSCT as expressed on the cell surface **(A)**. Note the presence of the *Y84C* mutation, creating the disulfide trap. SCTs that contain this mutation are more stably expressed on the cell surface. dtSCTs are encoded by a single DNA sequence that, when expressed, produces a peptide–MHC class I complex **(B)**.

β_2M: HLA heavy chain; dt: Disulfide trap; SCT: Single-chain trimer.

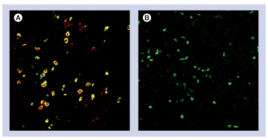

Figure 3. Immunohistology of human pancreatic adenocarcinoma

(A) Tissue slides were stained with antibodies for CD11b (red), and CD15 (green), and shows dual positive cells (yellow), indicative of MDSC. **(B)** Intracellular Foxp3 staining as a marker of Treg (green). No immune infiltrate was detectable in normal human pancreas tissue (data not shown).

MDSC: Myeloid-derived suppressor cell.

Images courtesy of David C Linehan and Jonathan B Mitchem, Washington University, Department of Surgery.

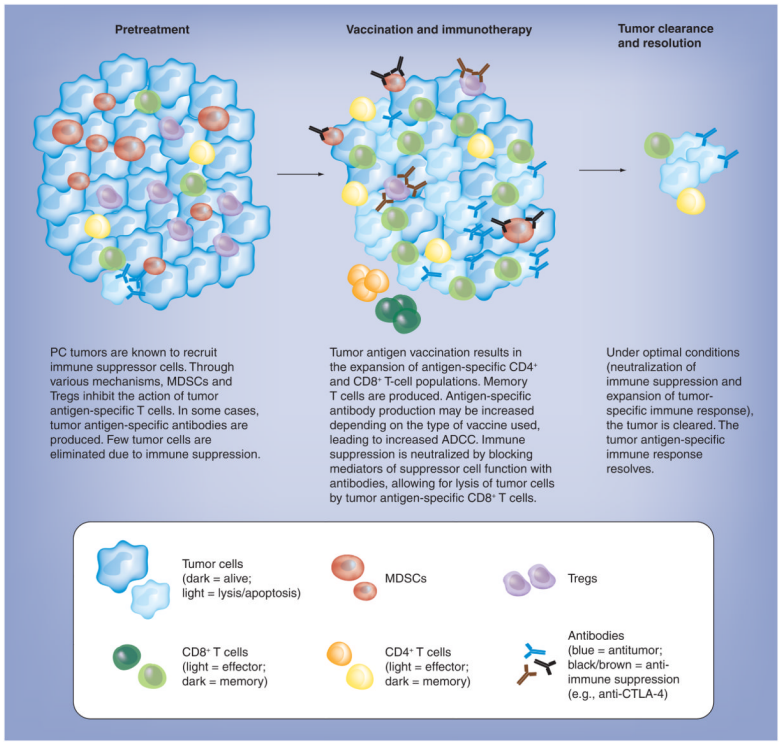

Figure 4. Depiction of ideal therapy strategy

Prior to therapy, the immunological milieu is characterized by high levels of immune suppression and low levels of immune responders, as well as immunological memory. The ideal immune therapy not only augments the antigen-specific immune response, but also reduces immune suppression. Key players such as NK cells, macrophages, B cells, complement and cytokines have been omitted to increase clarity.

ADCC: Antibody-dependent cell cytotoxicity;

MDSC: Myeloid-derived suppressor cell; PC: Pancreatic cancer.

Table 1

Overview of candidate pancreas cancer-associated antigens for immune targeting.

Antigen	Location	Expression in tumor	Prevalence (%)	Description	Ref.
CEA	Cell surface (GPI-linked)	Overexpressed	30–100	Glycoprotein, normally expressed only on oncofetal tissues. Functions as cell-adhesion molecule. First tumor antigen to be described	[185–187]
Her2-neu	Transmembrane	Overexpressed	>50	A receptor tyrosine kinase, member of the EGF−receptor family, involved in cell growth and differentiation	[188–190]
K-Ras	Intracellular	Mutated self	90	Mutated form of ras, a GTPase important for cell proliferation, differentiation and survival	[191,192]
Mesothelin	Cell surface (GPI-linked)	Overexpressed	~100	GPI-linked glycoprotein normally expressed on the surface of mesothelial cells lining the pleura, peritoneum and pericardium at low levels. Binding partner of CA125/MUC16	[40,193–196]
MUC-1	Transmembrane	Overexpressed, hypo-glycosylation	90	Type 1 transmembrane glycoprotein, expressed on apical surface of ductal and glandular epithelial cells at low levels. Extracellular domain has a polypeptide core with multiple tandem repeats of 20 amino acids	[197,198]
p53	Intracellular	Mutated self	50–70	Tumor suppressor that regulates cell cycle. Normally inhibits survivin at the transcription level and can initiate apoptosis if DNA damage is unrepairable	[199,200]
Survivin	Intracellular	Overexpressed	80	Member of IAP family. Inhibits caspase activation; is found in most human tumors and fetal tissue, but is completely absent in terminally differentiated cells	[201–204]
Telomerase	Intracellular	Overexpressed	95	Ribonucleoprotein that is responsible for RNAdependent synthesis of telomeric DNA. TERT is its catalytic subunit	[205,206]
VEGFR2	Transmembrane	Overexpressed	64	A tyrosine kinase and member of platelet derived growth factor family. Receptor for VEGF with functions in blood vessel development	[207,208]

CEA: Carcinoembryonic antigen; GPI: Glycosylphosphatidylinositol; IAP: Inhibitor of apoptosis protein; MUC: Mucin; TERT: Telomerase reverse transcriptase; VEGFR: VEGF receptor.

Table 2

Selection of current vaccine/immunotherapy clinical trials open to pancreatic cancer patients.

Treatment	ClinicalTrial.gov ID	Status	Phase
CEA RNA-pulsed DC	NCT00004604	Active, not recruiting	I
Muc-1 α-gal epitope (HyperAcute®)	NCT00569387	Active, not recruiting	II
Muc-1 α-gal epitope (HyperAcute®)	NCT00614601	Active, not recruiting	II
Muc-1 α-gal epitope (HyperAcute®)	NCT01072981	Recruiting patients	III
Pancreatic tumor cell line/GM-CSF	NCT00389610	Active, not recruiting	II
Pancreatic tumor cell line/GM-CSF	NCT01088789	Recruiting	II
Pancreatic tumor cell line/GM-CSF (GVAX)	NCT00727441	Recruiting	I
Pancreatic tumor cell line/GM-CSF ± ipilimumab	NCT00836407	Recruiting	I
Pancreatic tumor cell line/GM-CSF and cetuximab	NCT00305760	Active, not recruiting	II
CEA peptide	NCT00203892	Active, not recruiting	II
Muc-1 peptide	NCT00008099	Active, not recruiting	I/II
Survivin peptide	NCT00108875	Recruiting patients	I/II
Telomerase peptide (GV1001)	NCT00425360	Recruiting patients	III
VEGFR1/VEGFR2 peptide	NCT00639925	Active, not recruiting	I/II
VEGFR1/VEGFR2 peptide	NCT00655785	Active, not recruiting	I/II
ALVAC-CEA, Vaccinia-CEA, GM-CSF, aldesleukin	NCT00003125	Active, not recruiting	II
PANVAC™-VF (CEA, MUC-1, TRICOM)	NCT00088660	Active, not recruiting	III
Fowlpox-CEA (6D), TRICOM, autologous DCs, denileukin diftitox	NCT00128622	Active, not recruiting	I
PANVAC-V, PANVAC-F, GM-CSF (CEA, MUC-1, TRICOM)	NCT00669734	Recruiting	I
Vaccinia Virus Ankara Vaccine (p53)	NCT01191684	Recruiting	I
SC-K-ras (GI-4000)	NCT00300950	Active, not recruiting	II
SC-CEA (GI-6207)	NCT00924092	Recruiting patients	I

ALVAC: Canary pox virus; CEA: Carcinoembryonic antigen; DC: Dendritic cell; GM-CSF: Granulocyte–macrophage colony-stimulating factor; MUC: Mucin; PANVAC-F: Vaccine containing fowlpox virus; PANVAC-V: Vaccine containing vaccinia virus; SC: *Saccharomyces cerevisiae*; TRICOM: Three costimulatory molecules (B7.1, ICAM-1 and LFA-3); VEGFR: VEGF receptor.

Table 3

Selection of current monoclonal therapy clinical trials open to pancreatic cancer patients.

Treatment	ClinicalTrials.gov ID
MORAb-009 (anti-mesothelin)	NCT01018784, NCT00711191
Trastuzumab (anti-Her2/neu)	NCT00003797
Cetuximab (anti-EGFR)	NCT00923299, NCT00042939, NCT00448838, NCT00305760, NCT00075686, NCT00408564, NCT00467116
Panitumumab (anti-EGFR)	NCT00004879, NCT00601627, NCT00550836
Nimotuzumab (anti-EGFR)	NCT00561990
Trastuzumab and cetuximab	NCT00599833

EGFR: EGF-receptor.