IMMUNOTHERAPY FOR PANCREATIC CANCER — SCIENCE DRIVING CLINICAL PROGRESS

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Abstract | The identification of key signalling pathways involved in immune-system regulation, along with the development of early pancreatic tumours in mouse models have provided new opportunities for pancreatic cancer treatment and prevention. Immunotherapy for pancreatic cancer is one approach that is at a crucial crossroads, as therapeutics that are designed to target pancreatic-cancer-associated antigens and regulatory signalling molecules are entering clinical trials.

Pancreatic cancer is the fourth leading cause of cancer mortality in both men and women. Approximately 32,000 Americans each year will develop and also die from this disease. Despite aggressive surgical and medical management, the mean life expectancy is approximately 15–18 months for patients with local and regional disease, and 3–6 months for patients with metastatic disease[1,2]. Early detection methods are under development but do not yet exist in practice for pancreatic cancer. Therefore, most patients present with advanced disease that cannot be cured by surgery (pancreaticoduodenectomy).

Pancreatic cancer cells present an enormous challenge, as they are naturally resistant to current chemotherapy and radiation therapy. In addition, known pancreatic cancer antigens have generated relatively weak immune responses. This is probably due to a combination of mutations in oncogenes such as *KRAS* and tumour-suppressor genes such as *TP53*, *CDKN2A*, *DPC4* (deleted in pancreas cancer 4), *BRCA2* and *ERBB2* (also known as HER2/neu), as well as overexpression of growth factors such as transforming growth factor-α (TGFα), interleukin-1 (IL-1), IL-6 and IL-8, tumour-necrosis factor-α (TNFα), or vascular endothelial growth factor (VEGF), their receptors, or constitutive expression of multidrug-resistant genes[2–5]. Alternative therapeutic approaches are therefore urgently needed for this disease.

Immune-based therapies aim to recruit and activate T cells that recognize tumour-specific antigens. In addition, recombinant monoclonal antibodies are being designed to target tumour-specific antigens — these would kill tumour cells either by direct lysis or through delivery of a conjugated cytotoxic agent. Both approaches are attractive for the treatment of pancreatic cancer for several reasons. First, these immune-based therapies act through a mechanism that is distinct from chemotherapy or radiation therapy, and represent a non-cross-resistant treatment with an entirely different spectrum of toxicities. Second, through the genetic recombination of their respective receptors, the B cells and T cells of the immune system are capable of recognizing a diverse array of potential tumour antigens. In addition, both T and B cells can distinguish small antigenic differences between normal and transformed cells, providing specificity while minimizing toxicity. New insights into the mechanisms by which T cells are successfully activated and by which tumours evade immune recognition are driving the development of new combinatorial immunotherapy approaches. In addition, recent advances in gene-expression analysis have allowed for the identification of new pancreatic targets, including candidate tumour antigens that might serve as T-cell and antibody targets. These advances now make it possible to exploit the immune system in the fight against pancreatic cancer (FIG. 1).

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Summary

- Pancreatic cancer represents a significant challenge, as the tumour cells are naturally resistant to current chemotherapy and radiation therapy.
- Mechanisms of immune escape both at the local and systemic level are recognized. Such mechanisms will probably need to be circumvented to fully develop an effective pancreatic cancer vaccine.
- So far, monoclonal antibodies to vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) have been tested in combination with chemotherapy in patients with advanced pancreatic cancer.
- Several pancreatic vaccine approaches have been tested including peptide-based and gene-modified whole-cell vaccine approaches, both in patients with resected pancreatic cancer who are at risk for recurrence and in patients with advanced disease.
- New immunotherapy targets have been identified and the discovery of a relevant pancreatic cancer animal model should lead to more efficient and rapid testing and development of vaccine strategies.
- It is clear that the most effective strategy will require a combined approach incorporating the best targeted interventions taken from each respective modality.

IMMUNE TOLERANCE
A general term describing the state by which the immune system is rendered non-reactive towards self or non-self antigens.

HLA CLASS I
A set of major histocompatibility complex (MHC)-encoded polypeptides involved in immune recognition.

ANTIGEN-PRESENTING CELL
A specific type of immune cell that is able to carry antigen in a form that can stimulate lymphocytes.

NATURAL KILLER CELL
A specific type of lymphocyte that has the intrinsic ability to recognize and lyse virally infected cells and some tumour cells.

DENDRITIC-CELL MATURATION
The process by which specific antigen-presenting cells that are present in draining lymph nodes or the spleen are activated on encountering antigen. Once maturation is complete, these cells can efficiently activate T cells.

T_{Reg} CELLS
$CD4^+CD25^+$ T cells that are known to be important in the suppression of self-reactive T cells (peripheral tolerance).

CTLA4
CTLA4 is a member of the immunoglobulin superfamily and is a co-stimulatory molecule expressed by activated T cells. It is similar to CD28, and both molecules bind to B7-1 and B7-2 on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal.

B7 FAMILY
B-lymphocyte-activation antigens expressed by antigen-presenting cells. B7 proteins provide regulatory signals for T lymphocytes as a consequence of binding to the CD28 and CTLA4 ligands of T cells.

Immune surveillance and tumour evasion

The extraordinary features of the immune system make it possible to discern self from non-self. However, most human cancers, and pancreatic cancer in particular, are known to be poorly immunogenic, as crucial somatic genetic mutations can generate pancreatic cancer proteins that are essentially altered self proteins. Furthermore, promising immunotherapeutic approaches that have been used for relatively immunogenic cancers such as melanoma have met with variable success[6]. These observations have revealed that for tumours to form and progress, they must develop local and/or systemic mechanisms that subsequently allow them to escape the normal surveillance mechanisms of the intact immune system. Immune-based therapies must therefore incorporate at least one agent against a pancreatic cancer target as well as one or more agents that will modify both local and systemic mechanisms of pancreatic-cancer-induced IMMUNE TOLERANCE (TABLE 1).

It is now clear that both local characteristics of the tumour microenvironment as well as systemic factors are important for the immune evasion of tumours. For example, T-cell recognition of pancreatic tumours might be inhibited or suppressed due to the downregulation of human leukocyte antigen (HLA) CLASS I tumour-antigen complexes on tumour cells by a range of intracellular mechanisms[4,7] — upregulation of immune-inhibition molecules[8–17], loss of immune-regulation signals[15–30], defects in immune-cell tumour localization[31–51] and loss of co-stimulatory molecules[52–57] (TABLE 1). Such alterations within a tumour cell would not be unexpected, as they have unstable genomes. The local inflammatory reaction is also an important triggering event in the recruitment of professional ANTIGEN-PRESENTING CELLS (APCs) and effector cells, such as T cells and NATURAL KILLER (NK) CELLS, to the tumour site. However, pancreatic tumour cells express a range of proteins that inhibit pro-inflammatory cytokines and DENDRITIC CELL (DC) MATURATION[58–60] (TABLE 1).

In addition, the numbers of $CD4^+CD25^+$ T regulatory (T_{Reg}) CELLS — a subset of T cells that are known to be important in the suppression of self-reactive T cells (peripheral tolerance) — accumulate in pancreatic tumours[61–63]. Although these cells are thought to be activated during the immunization process, T_{Reg} cells seem to localize to tumour sites. Tumour production of the chemokine CCL22 probably attracts the T_{Reg} cells by interacting with the CCR4 receptor that is expressed by these cells[64].

Other important elements in regulating the T-cell recognition of pancreatic tumours are the inhibitory pathways, known as 'immunological checkpoints'. Immunological checkpoints serve two purposes. One is to help generate and maintain self-tolerance, by eliminating T cells that are specific for self-antigens. The other is to restrain the amplitude of normal T-cell responses so that they do not 'overshoot' in their natural response to foreign pathogens. The prototypical immunological checkpoint is mediated by the cytotoxic-T-lymphocyte-associated protein 4 (CTLA4) counter-regulatory receptor that is expressed by T cells when they become activated[15,23]. CTLA4 binds two B7-FAMILY members on the surface APCs — B7.1 (also known as CD80) and B7.2 (also known as CD86) — with roughly 20-fold higher affinity than the T-cell surface protein CD28 binds these molecules. CD28 is a co-stimulatory receptor that is constitutively expressed on naive T cells. Because of its higher affinity, CTLA4 out-competes CD28 for B7.1/B7.2 binding, resulting in the downmodulation of T-cell responses[20].

A range of B7-family members interact with co-stimulatory and counter-regulatory inhibitory receptors on T cells. Two recently discovered B7-family members, B7-H1 (also known as PD-L1) and B7-DC (also known as PD-L2) also seem to interact with T-cell co-stimulatory and counter-regulatory inhibitory receptors[18,29,30]. PD-L1, which is upregulated on T cells when they become activated, seems to control a counter-regulatory immunological checkpoint when it binds PD-1 (REFS 26,28,29). Activating receptors for B7-DC and B7-H1 have not yet been definitively identified. B7-DC is expressed on DCs, and is likely to have a co-stimulatory role in increasing activation of naive or resting T cells. In contrast to B7.1, B7.2 and B7-DC, B7-H1 is also expressed on several peripheral tissues and on many tumours, including pancreatic tumours[30].

Another new B7-family member, B7-H4, seems to mediate a predominantly inhibitory function in the immune system[14]. Recent data indicate that pancreatic tumours also express B7-H4 (D.L. and E.M.J., manuscript in preparation), and both B7-H1 and B7-H4 probably protect tumours from immune-system attack. Preclinical studies have already demonstrated that it is possible to downregulate B7-H1 signalling in mice, improving the antitumour response to vaccination[18]. Monoclonal antibodies that downregulate B7-H1 and B7-H4 are currently in clinical development. These antibodies will probably begin clinical testing in patients with pancreatic cancer within 2 to 3 years (FIG. 2).

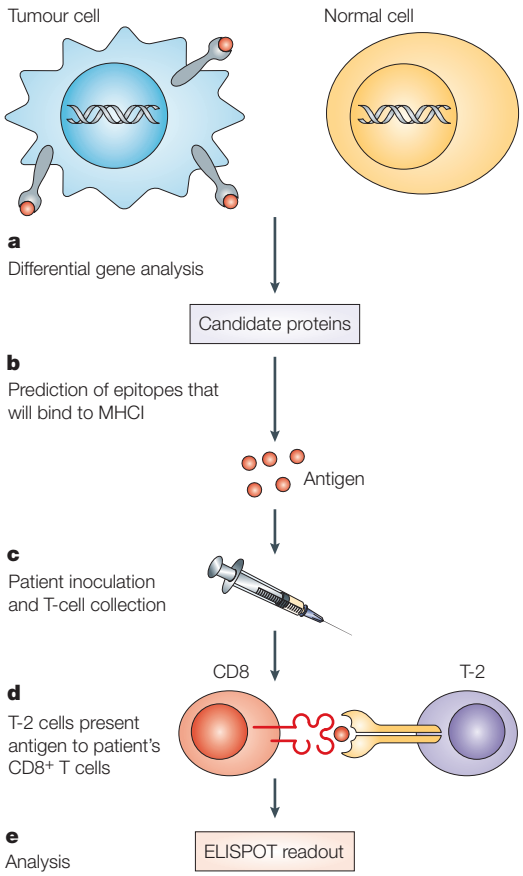

Figure 1 | **Tumour-antigen identification using serial analysis of gene expression.** Several methods have been used to identify pancreatic cancer antigens, but an 'indirect' antigen-discovery method — serial analysis of gene expression (SAGE) — has been particularly promising. SAGE uses differential gene-display technology to identify genes that are more strongly expressed by tumour cells relative to the normal cells of origin (**a**). The most relevant of these candidate proteins can then be identified based on other important considerations. These include identifying proteins that were non-mutated, were thought to be of biological importance to tumour growth and disease progression, and were not expressed or minimally expressed in normal tissue. Computer algorithms (BIMAS and SYFPEITHI[107,108]) can then be reliably used to predict peptides from these candidates that bind to the appropriate human leukocyte antigen (HLA) molecules (**b**). Individual patients are vaccinated with a whole-tumour-cell vaccine and $CD8^+$ T cells are collected from the peripheral blood of these patients before and after vaccination (**c**). Antigen-presenting T2 cells, which have been engineered such that they will transiently present an exogenously expressed HLA-restricted antigen of interest, are then used to present these candidate peptides to the un-manipulated $CD8^+$ T cells of a patient (**d**). Antigen-specific $CD8^+$ T cells can then be quantified using an enzyme-linked immunosorbent spot (ELISPOT) readout (**e**). This allows the potential of the antigen for use in immunotherapy-based treatments to be assessed.

Targeting signalling molecules

By the time that patients are diagnosed with pancreatic cancer, the tumour has typically progressed and invaded adjacent structures. Perineural invasion, metastasis to lymph nodes and liver, and an intense DESMOPLASTIC STROMAL REACTION are commonly observed. A range of signalling pathways, including epidermal growth factor receptor (EGFR) and the PI3K–AKT–mTOR–S6K cascades, are known to mediate pancreatic tumour growth and progression[65]. In addition, new blood-vessel formation (angiogenesis) is required for the growth of primary pancreatic tumours and is essential for metastasis. In pancreatic tumours, this process is probably regulated by fibroblast growth factor, platelet-derived endothelial-cell growth factor and VEGF family members. In fact, several pancreatic-cancer-associated genes have been linked to angiogenesis. DPC4 upregulates VEGF expression, and mutated KRAS expression is associated with increased micro-vessel density[66].

Monoclonal antibodies that target a range of these pathways have demonstrated efficacy in preclinical models[65,67,68]. In addition, monoclonal antibodies that target EGFR and VEGF receptor have been tested in patients with a range of cancers, including pancreatic cancer[69,70] (TABLE 2). Although these antibodies have demonstrated only modest results as single agents, the pathways they affect are also candidate targets for immune intervention.

Preclinical evidence has also shown that specific inhibitors of these signalling pathways can also increase immune activation. For example, VEGF is a key inhibitor of pro-inflammatory cytokines as well as dendritic-cell maturation, and it can also directly inhibit T-cell development[58]. So antibodies that block signalling by this growth factor can promote antitumour immune responses. Furthermore, downregulation of the ERBB-receptor-family members with drugs such as herceptin promotes tumour-antigen presentation by HLA class I molecules, improving the potential for T-cell recognition and lysis[71]. Monoclonal antibodies that target these signalling pathways are now being developed for clinical trials as agents that potentially synergize with other immune-based approaches, including vaccines.

Vaccines against pancreatic tumour antigens

To develop the ideal vaccine for pancreatic cancer, the following wish list would probably need to be fulfilled. First, specific cell-surface proteins must be identified that are that are crucial in the cancer growth or progression pathway and are unique to pancreatic cancer tumours. Second, these tumour-exclusive proteins

DESMOPLASTIC STROMAL REACTION
A pathological hallmark of pancreatic cancer characterized by an intense inflammatory reaction by host cells to the invasive tumour.

POST-VACCINATION DTH RESPONSE
A hallmark of the cell-mediated immune response against inactivated autologous tumour proteins, as measured by extent of skin induration.

should be shown to elicit a vigorous tumour-protein-specific immune response. Third, the best carrier to deliver the appropriate immunogenic tumour proteins should be identified. Fourth, molecules that are immune stimulatory as well as molecules that can abrogate the natural immune-inhibition signalling that is seen in pancreatic cancer should be identified to enhance the immune response. Fifth, additional synergistic immune help should be identified (for example, antibodies or *ex vivo* tumour-reactive T cells). Several proteins, such as carcinoembryonic antigen (CEA), mutated KRAS, mucin-1 (MUC1) and gastrin, have in fact been identified to be specifically overexpressed in most pancreatic cancers[72–78]. These antigens were identified over 10 years ago using various methods to analyse gene expression in cancer cells. Vaccines and antibodies designed to target these antigens have been tested in early-phase clinical trials[69,70,78–85] (TABLE 2). As these antigens are known to have weak inherent immune potential, various immune-modulating agents were co-administered, including granulocyte–macrophage colony-stimulating factor (GM-CSF), and interleukin-2 (IL-2). So far, a few studies have demonstrated post-vaccination immune responses to the relevant peptides or whole proteins. Significant clinical responses have not yet been observed. This might be due to the lack of pooling of the right antigens, to the existence of host mechanisms of immune tolerance, the inability of the relevant immune cells to effectively localize to the sites of disease, or a combination of these factors.

As discussed previously, monoclonal antibodies have so far been the most successful form of immunotherapy clinically. They are being used as diagnostic tools, prognostic indicators, and for the treatment of many cancers. Advantages include their specific targeting of tumour cells while sparing normal tissue, their relative ease of administration, and their low toxicity profile. The major disadvantages include the absence of T-cell activation, which therefore precludes T-cell-mediated cytotoxic killing and the generation of memory immune responses. In addition, a potential limiting factor in its use involves tumour heterogeneity. Specifically, all tumour cells within a proliferating mass might not express the antigen that is being targeted. Inhibitors to EGFR and to VEGF have been tested in combination with gemcitabine (TABLE 2) and are currently in Phase III trials either with chemotherapy or other vaccine strategies (TABLE 3).

Other approaches have used dendritic cells as the carrier of the antigen of interest. To date, CEA and MUC1 antigens have been among the initial antigens tested, with mixed results[80,81]. The use of adoptively transferred pancreatic-cancer-specific T cells has been proposed to be another opportunity to augment the immune response. Although this strategy has been promising preclinically, and has been used with some success in melanoma, there have not been any clinical trials in pancreatic cancer so far, as a major obstacle lies in the generation of pancreatic-cancer-specific T-cell lines/clones[86,87].

Because few other pancreatic tumour antigens have been identified, the whole tumour cell has been postulated to serve as the best source of immunogen. As an example, a Phase I study of an allogeneic, GM-CSF-secreting whole-cell tumour vaccine approach was tested in sequence with adjuvant chemoradiation in patients who had resected pancreatic adenocarcinoma (TABLE 2). This approach is based on the concept that certain cytokines are required at the site of the tumour to effectively prime cancer-specific immunity. In the only study to directly compare a large number of immune-stimulating cytokines[79], GM-CSF stood out as the most potent cytokine capable of inducing systemic antitumour immunity when it was expressed by the tumour cells for the initial 24–72 hours of immune priming. GM-CSF is now recognized to be the crucial growth and differentiation factor for dendritic cells, which are the most potent professional antigen-presenting cells and are responsible for priming immune responses against infectious agents and tumour antigens. In the study, POST-VACCINATION DELAYED-TYPE HYPERSENSITIVITY (DTH) RESPONSES were observed in 3 of 8 patients who were vaccinated with either 10^8 or 5×10^8 cells following surgical resection of the tumour[79]. Post-vaccination DTH responses to autologous tumour cells have been

Table 1 | **Mechanisms of tumour immune evasion**

Alteration to immune response	Local factors*	Systemic factors‡	References
Molecules downregulated on immune cells	HLA class I, TAP, β2-microglobulin	N/A	4,7
Immune-cell inhibitors upregulated	IL-10, TGFβ, COX2,VEGF, B7-H1, B7-H4	IL-1, IL-6, IL-10, TGFβ	8–17
Immune checkpoints suppressed	B7-H1 signalling disrupted	B7.1–B7.2–CTLA4 signalling by dendritic cells; B7-DC–PD-1 signalling by dendritic cells; B7-H1–PD-1 signalling by dendritic cells	15–30
Defects in immune-cell localization	Accumulation of T_{Reg} cells in tumours; peripheral deletion of activated T cells through T_{Reg} cells	Peripheral deletion of activated T cells through T_{Reg} cells	31–51
Loss of co-stimulation	N/A	B7 family of molecules; OX-40; CD-40	52–57
Cellular effects	T-cell apoptosis increased	Inhibition of dendritic-cell maturation by production of VEGF and COX2	58–60

*Direct cell–cell interactions. ‡Cytokine-mediated interactions. COX2, cyclooxygenase-2; HLA, human leukocyte antigen; IL, interleukin; N/A, not applicable; TAP, transport-associated protein; TGFβ, transforming growth factor-β; T_{Reg}, $CD4^+CD25^+$ T regulatory cells; VEGF, vascular endothelial growth factor.

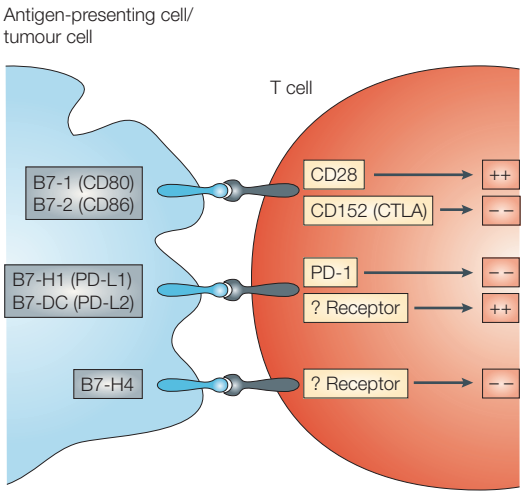

Figure 2 | **T-cell activation by B7-family proteins.** Efficient immune priming against tumour cells is dependent on T-cell-receptor recognition of specific peptide fragments derived from the tumour cell and processed by the antigen-presenting cell in the context of the appropriate human leukocyte antigen class I molecule and co-stimulatory molecule. The context in which the antigen is presented to the T cell determines whether or not the T cell subsequently becomes activated (indicated by plus (+) signs) or suppressed (indicated by minus (–) signs). In the absence of the appropriate co-stimulatory signals, engagement of the T-cell receptor can lead to ignorance (absence of immune responsiveness to antigens), anergy (functional silencing to antigen) or even apoptotic T-cell death. There are several families of regulatory molecules that have been identified and that have a role in T-cell activation/regulation. The B7 family is the most well characterized. What is now known is that these molecules have both stimulating and downregulating ligands. Some of these B7-family members preside predominately on professional antigen-presenting cells (for example, B7-1, B7-2, B7-DC and B7-H1), whereas others preside predominately on peripheral organs or on the tumour (for example, B7-H4).

used in previously reported vaccine studies as a surrogate to identify and characterize specific immune responses that are associated with vaccination. Toxicities were limited to minor local reactions at the vaccine site, and self-limited systemic rashes. A confirmatory Phase II study has recently completed patient enrollment (TABLE 3).

A current limitation to the development of vaccines for pancreatic cancer has been the inability to correlate *in vitro* measures of antitumour immunity with *in vivo* responses. Post-vaccination DTH responses to autologous tumour are a potential useful surrogate, but this approach is not ideal. At present, it is technically challenging to produce sufficient quantity and purity of autologous tumour material for testing, as tumours vary in their composition of tumour cells versus other cell types between patients. Although other biological end points, such as an antibody response or *in vitro* CYTOLYTIC T LYMPHOCYTE (CTL) ASSAY against a vaccine-delivered tumour antigen (or antigens), have been measured and provide important 'proof of concept' data, these end points have also not been demonstrated to be predictors of traditional clinical end points, including tumour response and survival benefit.

CYTOLYTIC T LYMPHOCYTE ASSAYS
A method to directly quantify the antigen-specific T-cell response.

It is difficult to assess whether the lack of improved survival after immunotherapy is due to inefficient antigen delivery, which could result in ineffective immunization, inappropriate selection of antigen targets, or both. As discussed above, there are formidable barriers to inducing an antitumour immune response, even when the vaccine itself is potent enough to reduce significant cancer burdens in more immunogenic tumour systems. Effective immunization will therefore require the targeting of relevant pancreatic tumour antigens using optimized antigen-delivery systems with immune-stimulating cytokines, in sequence with other therapeutic interventions that alter immune checkpoints in the tumour microenvironment, such as inhibitors to regulatory molecules on T cells (for example, antibody to CD152/CTLA4).

New immunotherapy targets

The inability of previously tested antigens (including CEA, KRAS, MUC1 and gastrin) to induce immune-specific responses underscores the challenge to identify more relevant immunogenic targets. Indeed, these antigens were chosen only because they were overexpressed or had altered expression in pancreatic tumours, and not because they had been shown to be immunogenic. Therefore, there might be additional as-yet-unidentified antigens that might be more immunogenic for inducing effective immunity against pancreatic cancers. How will such new candidate pancreatic cancer antigens be discovered? Two methods are routinely used in an attempt to identify new targets. The first method, serological analysis of recombinant tumour cDNA expression libraries (SEREX), uses serum to screen phage-display libraries prepared from tumour cells to identify candidate antigen targets that have elicited both humoral and cell-mediated immune responses in cancer patients. This method has identified coactosin-like protein (an actin-filament-binding protein that interacts directly with 5-liopoxygenase and has an important role in cellular leukotriene synthesis) as a potential pancreatic cancer target antigen. This protein seems to be recognized by antibody and T-cell responses in patients with pancreatic cancer[88].

The second method uses tumour-specific T cells that have been isolated from patients with pancreatic cancer to screen cDNA libraries prepared from autologous tumour cells. This method requires the isolation and culture of tumour-specific T cells, along with tumour cells, from patients with pancreatic cancer and is a technically challenging approach. This approach has been most successful in identifying melanoma-associated antigens[89,90].

A relatively newer, more promising method of tumour-antigen identification is the use of the patient's lymphocytes to evaluate proteins that are found to be differentially expressed by pancreatic cancers[91,92] (FIG. 1). This approach has several advantages. First, it allows for a rapid screen of a large number of candidate antigens but requires the isolation from patients of only a few lymphocytes, which are limited in availability. Second, this approach is not dependent

Table 2 | **Recently completed immunotherapy clinical trials**

Study	Number of patients/ stage of disease	Antigen	Delivery	Median survival	Comments	References
Jaffee (2001)	14 patients (resected pancreatic cancer)/post-treatment (adjuvant)	Whole cell	GM-CSF genetically engineered allogeneic vaccine (known as GM) administered intradermally	NR	Safe treatement — 3 long-term survivors (all now 7or more years) with positive DTH to autologous tumour responses and mesothelin-specific T cells	79
Achtar (2003)	18 patients (2 pancreatic, 15 colon and 1 lung cancer) metastatic	Mutated KRAS with GM-CSF/IL-2	Peptides administered subcutaneously	NR	Immune responses for mutant but not wild-type RAS	78
Morse (2004)	14 patients (11 colon and 3 lung cancers)/ metastatic	rFCEA–B7-1/ ICAM1/LFA3 (TRICOM)*	Antigen pulsed onto DCs	NR	CEA-specific immune responses	80
Finn (2004)	8 patients (pancreatic cancer)/post-treatment	MUC1	MUC1 pulsed onto DCs every 3 weeks for 3 vaccinations	NR	Isotype switch from IgM to IgG by ELISA in 2 patients	81
Marshall (2005)	58 CEA-expressing tumours (25 colon cancers, 7 other GI cancers)	CEA + B7-1/ ICAM1/LFA3	S1: dose escalate rFCEA–B7-1/ICAM1/LFA3 S2: rVCEA + MTD rFCEA S3: rVCEA/GM + rFCEA/GM S4: rVCEA/GM + split rFCEA/GM	NR	Safe treatment — 23 patients (40%) with stable disease at 4 months; trend towards enhanced CEA-specific immune responses for patients treated at S4	82
Gilliam (2004)	154 patients (pancreatic cancer)/ metastatic	G17DT gastrin peptide	G17DT versus placebo (treatment week 0,1,3,24,52)	151 versus 82 days (P=0.03)	Safe treatment — no immune end points reported	83
Laheru (2004)	50 patients (pancreatic cancer)/metastatic	Whole cell	Cohort A: 30 patients, GM administered intradermally Cohort B: 20 patients, cyclophosphamide (Cytoxan) and GM	Cohort A: 2.3 months Cohort B: 4.7 months	30/50 patients received over 2 previous chemotherapy schedules. Safe treatement — 3 long-term survivors (> 1 year). Mesothelin epitopes identified exclusively in patients with prolonged time to tumour progression and overall survival	85
Xiong (2004)	41 patients (pancreatic cancer)/EGFR-positive	EGFR	Gemcitabine (Gemzar) + cetuximab (Erbitux)	7.6 months	1 year suvival 32%	69
Kindler (2004)	33 patients (pancreatic cancer)/metastatic	Soluble VEGF	Gemcitabine + bevacizumab (Avastin)	12.4 months	1 year survival 54%	70

*TRICOM consists of three costimulatory molecules (lymphocyte function-associated antigen 3 (LFA3), intercellular-adhesion molecule 1 (ICAM1) and B7-1). CEA, carcinoembryonic antigen; DC, dendritic cell; DTH, delayed-type hypersensitivity; EGFR, epidermal growth factor receptor; ELISA, enzyme-linked immunosorbent assay; G17DT, gastrin 17 peptide linked to diphtheria toxoid; GI, gastrointestinal; GM-CSF, granulocyte–macrophage colony-stimulating factor; Ig, immunoglobulin; IL-2, interleukin-2; MUC1, mucin-1; MTD, maximum tolerated dose; NR, not reported; rV, recombinant vaccinia; rF, recombinant fowlpox; S1/2/3/4, stage 1/2/3/4; VEGF, vascular endothelial growth factor.

on the availability of autologous tumour cells, which are difficult to isolate in large enough numbers for generating cDNA libraries. Third, this approach can be used to identify tumour antigens that are expressed by any HLA type, allowing for the generalization of this approach to most patients. Finally, this approach has the potential to rapidly identify 'immune relevant' antigens, as it uses immunized lymphocytes from patients vaccinated with a whole-tumour-cell vaccine approach who ideally have demonstrated clinical evidence of immune activation following vaccination. So this method provides the best insurance that the antigens identified are ones that the patient's immune system is reacting to after immunization.

Mesothelin is a candidate pancreatic tumour antigen that was recently identified using this approach. Mesothelin is a transmembrane glycoprotein and derives from a larger protein, mesothelin/megakaryocyte potentiating factor[93]. Mesothelin is overexpressed by most pancreatic tumours[94,95]. This antigen was recently identified as a T-cell target using lymphocytes that were isolated from three pancreatic cancer patients who had been immunized with an allogeneic, GM-CSF-secreting pancreatic tumour vaccine and who demonstrated other evidence of immune and clinical responses. Antibodies against mesothelin are currently being tested as therapeutic agents for patients with advanced pancreatic cancer[96] (TABLE 3).

As additional 'immune relevant' pancreatic tumour antigens are identified, the next significant challenge lies in developing strategies to improve the *in vivo* delivery of these antigens to APCs and thereby allow effective antigen processing and presentation, and subsequent activation of a potent antitumour immune response. DCs are now accepted as the most efficient APCs in B- and T-cell activation. Several clinical trials have tested *ex vivo* expanded

Table 3 | **Immunotherapy clinical trials in progress**

Trial: investigator/location	Antigen selection	Approach	Stage of disease	Phase
Daniel Laheru/Johns Hopkins (accrual reached 1/05)	Whole-cell vaccine	GM-CSF allogeneic vaccine integrated with chemoradiation therapy	Adjuvant	II
Ghassan Abou-Alfa/MSKCC	RAS peptide	Peptide delivery following chemoXRT	Adjuvant	II
James Abbruzzese/Southwest Oncology Group	Antibody to EGFR (cetuximab (Erbitux))	Gemcitabine (Gemzar) +/– cetuximab	Metastatic 1st line	III
Hedy Kindler/Cancer and Leukemia Group B	Antibody to soluble VEGF (bevacizumab (Avastin))	Gemcitabine +/– bevacizumab	Metastatic 1st line	III
Hedy Kindler/University of Chicago	Bevacizumab with oral TKI (Erlotinib)	Gemcitabine + bevacizumab with either cetuximab or oral TKI	Metastatic 1st line	II
Margaret Tempero/UCSF	Bevacizumab	Gemcitabine + cisplatin + bevacizumab	Metastatic 1st line	II
Steven Cohen/Fox Chase Cancer Centre	Bevacizumab	Bevacizumab +/– docetaxel (Taxotere)	Metastatic 2nd line	II
John Marshall Therion Biologics/(Cambridge, Massachusetts)	rFCEA/MUC1–B7-1/ICAM1/ LFA3 (TRICOM) (PANVAC-VF*)	rFCEA/MUC1–B7-1/ICAM1/LFA3 versus best supportive care	Metastatic 1st line	III
Michael Morse/Duke University	rFCEA–B7-1/ICAM1/LFA3	rFCEA–B7-1/ICAM1/LFA3 pulsed onto dendritic cells	Metastatic 2nd line	II
Raffit Hassan/National Cancer Institute	Mesothelin	Immune toxin conjugated to mesothelin monoclonal antibody	Metastatic 2nd line	I
Daniel Laheru/Johns Hopkins	Whole-cell vaccine	Cyclophosphamide (Cytoxan) + GM-CSF allogeneic vaccine integrated with cetuximab	Metastatic 1st line	II

*The PANVAC-VF vaccine comprises two separate vaccine vectors, each of which contains genes encoding carcinoembryonic antigen (CEA) and mucin-1 (MUC1) plus TRICOM, which consists of three costimulatory molecules (lymphocyte function-associated antigen 3 (LFA3), intercellular-adhesion molecule 1 (ICAM1) and B7-1). EGFR, epidermal growth factor receptor; GM-CSF, granulocyte–macrophage colony-stimulating factor; MSKCC, Memorial Sloan-Kettering Cancer Center; rF, recombinant fowlpox; TKI, tyrosine-kinase inhibitor; UCSF, University of California at San Fransisco; VEGF, vascular endothelial growth factor.

and primed DCs as a vaccine approach. However, these studies have revealed the difficulty in reliably producing phenotypically mature DCs for clinical testing, as only mature DCs are capable of efficiently presenting antigens to T cells. If an antigen is not presented in the proper context by mature DCs, immune downregulation or tolerance can occur. It has been shown in animal models that immature DCs induce T-cell tolerance. As an alternative to DC-based delivery, recombinant viral- and bacterial-vector delivery systems are currently under development or are already undergoing clinical testing. The use of modified viral particles or targeted bacteria to deliver tumour antigens to the immune system is based on the innate ability of the agent to efficiently infect APCs *in vivo*. Early approaches have included viruses such as vaccinia[97–99]. However, the use of immunogenic vectors in cancer patients who have been previously exposed to a similar vector often induces vigorous immune responses against the vector before effective priming against the tumour antigen can occur. As such, other viral particles and bacterial delivery systems are currently nearing or are already undergoing clinical development for the treatment of pancreatic cancer, including fowlpox viruses and *Listeria monocytogenes*[100,101].

Targeting immune checkpoints

There are extensive murine and human data demonstrating that tumours grow despite the simultaneous existence of tumour-specific immune responses. To explain this observation, it has long been thought that patients with cancer develop peripheral tolerance to their tumour. Insights into the mechanisms that underlie immune tolerance have provided opportunities for designing combinatorial immune-based interventions that enhance the antitumour immune response. For example, preclinical studies and early clinical trials in patients with prostate cancer and melanoma have demonstrated that downregulation of signalling through CTLA4, using an antagonist monoclonal antibody, increases antitumour immunity in some patients, even when administered as a single agent. Phase I clinical trials that analyse the effects of combining antibodies that block CTLA4 signalling with antigen-targeted vaccination in patients with pancreatic cancer are about to begin.

T_{Reg} cells are now accepted as another immune checkpoint for the systemic regulation of the antigen-specific T-cell responses at the tumour site. Several preclinical studies have demonstrated that the administration of T_{Reg}-inhibiting agents — either immune-modulating doses of chemotherapy or an IL-2-receptor-targeted antibody that depletes T_{Reg} cells — to naive hosts increases the antitumour effects of immune-based therapies[102,103]. A Phase II study compared a whole-cell pancreatic cancer vaccine given either alone or in combination with immune-modulating doses of the T_{Reg}-inhibiting chemotherapeutic agent cyclophosphamide (Cytoxan) in patients with metastatic pancreatic cancer who were previously treated with two or more chemotherapies. The study reported an increased number of patients

experiencing progression-free survival in the cohort that received cyclophosphamide plus the vaccine (40% of patients at 16 weeks), compared with the cohort that received the vaccine alone (16% of patients at 16 weeks)[85] (TABLE 2). More importantly, mesothelin epitopes are identified exclusively in patients with prolonged survival. The side effects associated with this vaccine approach are limited to local, transient, vaccine skin-site reactions. These side effects are usually tolerable and self-limiting, lasting no more than 2 weeks and requiring minimal, if any, intervention. The fact that the side effects are minimal and tolerable allows such a vaccine approach to be easily integrated with other treatment modalities. The results of these studies will provide direction for the future development of vaccines in pancreatic cancer. For example, immune-based therapies are currently being combined with targeted therapies that are believed to have multiple mechanisms (immune and non-immune mediated) of antitumour activity such as inhibitors to EGFR and VEGF receptor.

Future directions

The limitations of currently available therapy for pancreatic cancer are more clearly exposed as we begin to appreciate the molecular changes behind the complex transformation of normal pancreatic ductal cells into frank pancreatic cancers, and the mechanisms of pancreatic cancer resistance to traditional anticancer modalities. It is clear that the most effective therapy will require a combined approach incorporating the best targeted interventions taken from each respective modality. Preclinical models have already revealed the synergy between immunotherapy and other targeted therapeutics, such as inhibitors of VEGF and EGF signalling. These combinations are about to be tested in patients with pancreatic cancer.

Pancreatic cancer remains one of the most resistant cancers to traditional forms of therapy. Until techniques for early detection can be developed, most patients will continue to present with incurable disease. The pancreatic cancer research community is committed to developing new therapies for this disease. Pancreatic cancer patients and their families, through a number of national pancreatic cancer non-profit organizations such as Pancreas Cancer Action Network have organized to support this effort. It is crucial that we move forward with scientifically driven innovative therapies, as the empirical approaches have failed. Recent developments in the design of mouse models that recapitulate early pre-invasive genetic changes in *KRAS* activation, inactivation of *CDKN2A*, *TP53* and *SMAD4* tumour-suppressor genes should provide the opportunity to test such approaches in a timely manner[104–106].

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Competing interests statement

The authors declare competing financial interests: see web version for details.

Online links

DATABASES

The following terms in this article are linked online to:

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