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# Immunotherapy of the Pancreatic Cancer

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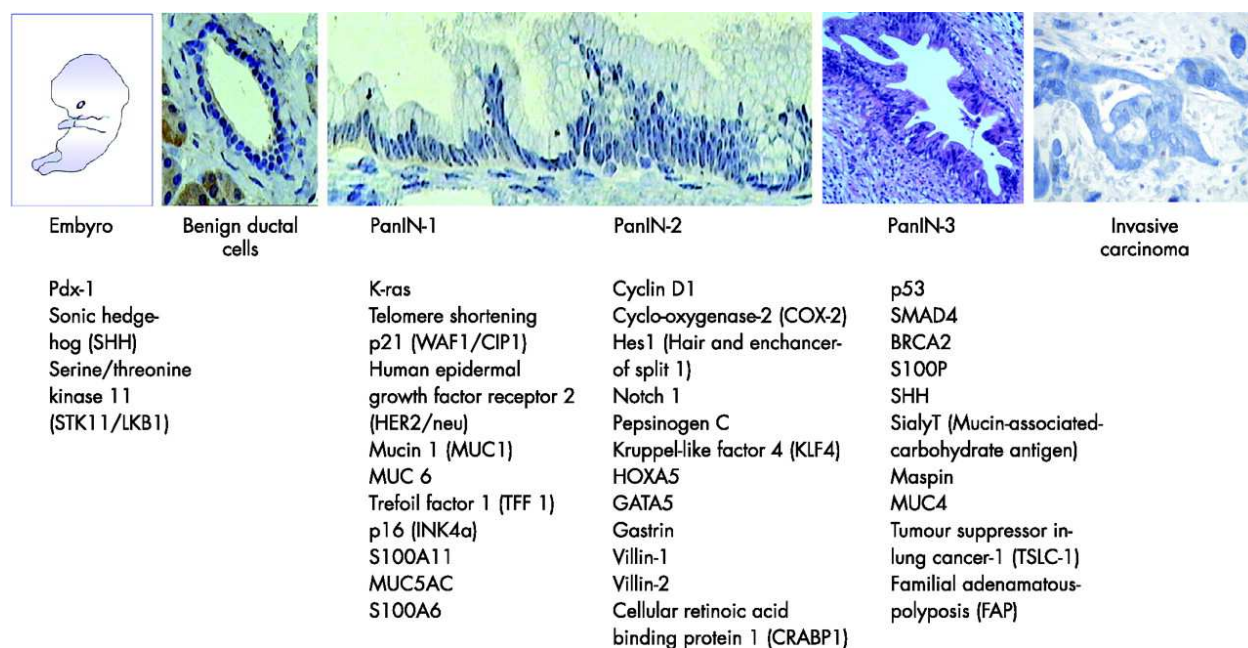
## 1. Introduction

Pancreatic cancer, which we refer to pancreatic ductal adenocarcinoma, is the fourth most common cause of cancer-related-death disease. In 2010, there were 43,140 new cases and 36,800 patients died of pancreatic cancer in USA<sup>(1)</sup>. Although surgical resection may be the only available treatment for this horrible disease, there are beyond 80% patients when diagnosed cannot be cured by surgical treatment<sup>(2)</sup>. In the past 50 years, despite of the progress in surgery skill, hospital morbidity and mortality rates were decreased from 59% and 24% to 36% and 2%, respectively<sup>(3)</sup>, in the patients received the most optimal surgical operation, the median survival ranged from 15 to 19 months, and 5-year survival rate was still approximately 20%<sup>(4)</sup>. Even if Gemcitabine became the new standard of chemotherapy in pancreatic cancer in 1997<sup>(5)</sup>. The outcome of the pancreatic cancer is still dismal, overall five-year survival rate is below 5%. With an increasing incidence of pancreatic cancer in the world and conventional treatments often have limited effects and substantial toxicity, a strong need exists for novel therapies. Biological approaches, including gene therapy and immunotherapy, which are targeting pancreatic cancer at a molecular or protein level, are rapidly evolving and seem to be promising strategies for this devastating and virtually unexceptionally lethal malignancy.

## 2. Immune target and Immune response in pancreatic cancer

Cancer is fundamentally a gene associated disease, it has become increasingly clear that some genomic instability and aberrant gene expression lead to biologic behaviour abnormality in tumor cells. In pancreatic cancer, several genes have high mutation rate in different phase, so the tumor cell may express abnormal antigens that make them immunologically distinct and potential targets for the host immune system.

**K-Ras:** The mutation of K-ras oncogene (homologous to the ras gene of Kirsten murine sarcoma virus) occurs in 75-100% of pancreatic cancer<sup>(6)</sup>. With the progression from minimally dysplasia epithelium (PanIN 1A, 1B) to more severe dysplasia (PanIN 2, 3) and invasive cancer<sup>(7)</sup>, the mutation rate of K-ras oncogene is increasing successively, denotes k-ras oncogene plays a very important role in tumor origination and progression<sup>(8)</sup>.



Picture 1. Associated genes in pancreatic cancer progression. from Paula Ghaneh, et al. Biology and management of pancreatic cancer. Gut 2007;56:1134-1152.

K-ras gene encodes a 21 kDa membrane-bound guanosine triphosphate(GTP) -binding protein. Before localization at cell membrane, K-ras protein must be farnesylated or geranylgeranylated on the same cysteine residue, it is involved in the transduction of signal from growth factor receptors and other signal inputs, as an upstream activator, it will activate several signaling pathways including Raf/MEK/ERK, P13K/Akt and RalEGF/Ral<sup>(9)</sup> to regulate gene expression and prevent apoptosis. The mutation of the K-ras oncogene, which occurs mostly at codon 12 but also occasionally at codon 13 and 61, will lead to impaired GTPase activity, resulting in lock the protein locked in GTP-bound state and thus activating downstream signalling cascades<sup>(10)</sup>. According to the META Analyse, point mutation occurred in codon 12 mainly divided into several types, the wild type GGT is replaced by GAT(47%), GTT(28%), CGT(15%), TGT(7%), AGT(2%) and GCT(1%). so in the protein, the 12th amino acid Guanine is replaced by Aspartic acid, Valine, Arginine, Cysteine, Alanine, Serine<sup>(11)</sup>. The K-RAS function changes due to the abnormality in protein structure. The mutation also provide the epitope which might be the target in immunotherapy.

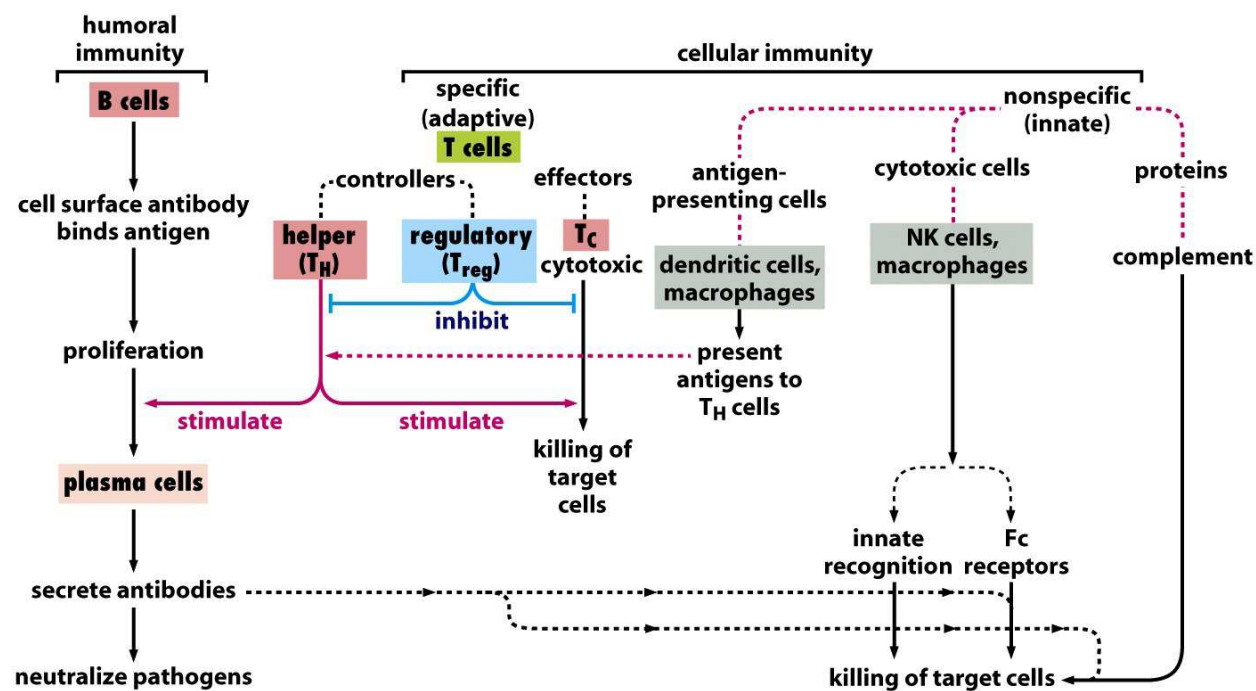
**MUC1:** Mucins are large glycoproteins with carbohydrate content and marked diversity both in the apoprotein and in the oligosaccharide moieties<sup>(12)</sup>. MUC1 is a heavily glycosylated type I membrane protein with several extracellular tandem repeat domains, which is expressed by nearly all human glandular epithelial and its expression is limited to the apical membrane of the cells. In pancreatic cancer, MUC1 expression is upregulated with an expression pattern over the entire cell surface<sup>(13)</sup>. The core peptide of MUC1 not only serves as a counter-receptor for myelin-associated glycoprotein in pancreatic cancer and is related to perineural invasion<sup>(14)</sup>, but also block death receptor-mediated apoptosis by binding to caspase 8 and FADD<sup>(15)</sup>. MUC1 molecular has sialic acid-containing oligosaccharides in a highly O-glycosylated tandem-repeat domain, the structure has wide range and a large molecular weight<sup>(16)</sup>. Although the core protein of MUC1 is similar in both

normal and tumor cells, there is a remarkable diversity in oligosaccharide moieties between normal and cancer cells<sup>(17)</sup>.

**Mesothelin:** Mesothelin is a 40-kDa glycosyl phosphatidylinositol anchored cell surface protein and is a c-terminus membrane-bound form of a 69-kDa precursor protein encoded by the Mesothelin gene(MSLN). Normally, mesothelin is only expressed on mesothelial cells which lining peritoneal, pleural and pericardial cavities<sup>(18)</sup>. The biologic functions are not clearly understood. Some early studies have shown mesothelin playing role in tumor adhesion and dissemination<sup>(19)</sup>. In pancreaticobiliary adenocarcinomas, the expression rate of mesotheline is 100%, whereas none in normal pancreas and chronic pancreatitis<sup>(20)</sup>.

**2.1 Immune escape and immunosuppression**

In the past 50 years, with the advances in cellular, molecular biology of cancer and development of immunology, people comes to realizes the relationship between tumor and immune cells is just like a cat and mouse game. The human immune system assume the responsibility to get rid of the extrinsic and endogenic abnormal antigen, it can produce activated immunocyte or immune material such as antibody to react anomalous antigen and finally eliminate the target, but the fact is not under our desire. (Picture 2)



Picture 2. Immune system : From Robert A. Weinberg, *The Biology of Cancer*. 2007

At the genesis of the cancer, under ideal condition, the innate immune system responds to “danger signals”, macrophages and fibroblasts are enlisted to construct the microenvironment surrounding the cancer cell, just like inflammation, many cytokines and growth factors are produced to activate innate effector cells with antitumor activity, stimulate professional antigen-presenting cells (APCs, mainly dendritic cell) to capture tumor-derived antigens and migrate to draining lymph nodes to priming an adaptive response by activating T and B lymphocytes. Unfortunately, the growth factors can also

stimulate cancer cells proliferation and progression. The cancer cells are so clever that can learn to avoid detection or to escape or overwhelm the immune response. Immunosuppressive tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg) reside in tumors, and their products along with tumor derived products (such as VEGF, TGFbeta and IL-10), create a microenvironment that resists immune activation and attack.

Many strategies are found to escape from immune surveillance<sup>(21)</sup>. 1) Physical exclusion of immune cells from tumor site. It has been proved in epithelial cancer that basal-membrane-like structures around the tumor can prevent lymphocytes from infiltrating and tumor-specific T cells expanding<sup>(22)</sup>. 2) Poor immunogenicity by reducing expression of major histocompatibility complex(MHC) or co-stimulatory proteins<sup>(23)</sup> and disruption of natural killer(NK) and natural killer T (NKT) cell recognition<sup>(24)</sup>. The other ways are to change themselves by losing whole protein or TAA expression, which changes in immunodominant T-cell epitopes that alter T-cell recognition, antigen processing or binding to the MHC. 3) Secreting soluble immunosuppressive proteins such as interleukin (IL-10) to prevent inflammatory response from triggering, or vascular endothelial growth factor(VEGF) to interfere with dendritic cells(DC) activation and differentiation<sup>(25)</sup>. 4) Increasing expression of STAT3 protein to block the production of pro-inflammatory molecules<sup>(26)</sup>.

If the specific reaction had been established, being attacked by activated NK cells, antibodies or cytotoxic T lymphocytes, cancer cells can escape elimination according to down-regulating targeted antigens, rendering tumor-reactive cell anergic<sup>(27)</sup>, or inducing responding T cell apoptosis specifically. The pro-apoptotic function of FasL on carcinoma cells has been demonstrated in both in vitro and in vivo, FasL expressed cancer cells can induce apoptosis of lymphocytes in Fas-dependent manner<sup>(28)</sup>, and in patient's biopsies, the present of FasL on cancer cells is in parallel with reduced number<sup>(29)</sup> and apoptosis<sup>(30)</sup> of tumor-infiltrating immune cells (TICs). In pancreatic ductal carcinoma, the expression of FasL is 82% in primary versus 100% in hepatic metastases and is associate with shorter survival<sup>(31)</sup>. At last, the eventual developed tumor reflects immunoediting with selection of poorly immunogenic and/or immune-resistant malignant cells<sup>(32)</sup>.

**Treg cells:** CD4+25+Foxp3+ regulatory T cells (Tregs) have been discovered in the 1960s, which can suppress T-cell response and compromise the development of effective tumor immunity<sup>(33)</sup>. these cells are distinguished in high expressed CD4, CD25, CTLA-4, the glucocorticoid-induced TNF-related receptor (GITR) and the forkhead transcription Foxp3. they can arise in response to persistent antigen stimulation in the absence of inflammatory signals, especially in the presence of TGF- $\beta$ <sup>(34)</sup>. The tumor-induced expansion of regulatory T cells by conversion of CD4+CD25+ lymphocytes is thymus and proliferation independent<sup>(35)</sup>.

Tregs play a critical role in the induction of tolerance to tumor-associated antigens and suppression of antitumor immunity. Additional evidence showed that Tregs were increased locally within the tumor microenvironment by a mechanism that seems dependent on TGF-beta receptor expression and the presence of tumor derived TGF-beta. The murine pancreas cancer cell line Pan02 produces high levels of TGF-beta both in vitro and in vivo. In contrast, the esophageal murine cancer cell line, Eso2, does not. Immunohistochemical staining of Foxp3 in explanted tumors showed an identifiable population of Treg in the Pan02 (TGF-

beta positive) tumors but not Eso2 (TGF-beta negative). Naive CD4+25-Foxp3- T cells, when adoptively transferred into Rag-/- mice, were converted into Foxp3+ Treg in the presence of Pan02 but not Eso2 tumors. Induction of Treg in Pan02 mice was blocked by systemic injection of an anti-TGF-beta antibody. If Rag-/- mice were instead reconstituted with naive CD4+25- T cells expressing a mutated TGF-beta receptor, induction of Foxp3+ Treg in Pan02 bearing mice was blocked. Collectively, The observations supported the role of TGF-beta in the induction of Treg in pancreas adenocarcinoma<sup>(36)</sup>.

Recent studies have shown that increased numbers of tumor-infiltrating Tregs were associated with poorer prognosis in pancreatic cancer<sup>(37)(38)</sup>, so the presence of Tregs in pancreatic cancers highlights the importance of targeting the suppressive function of these cells in future immunotherapy research.

In Yamamoto's study, a cytotoxicity assay, enzyme-linked immunosorbent spot (ELISPOT) assay and measuring cytokine secretion, were used to study the efficacy of Treg depletion by anti-CD25 antibody added to a dendritic cell/tumor cell (DC/TC) fusion hybrid vaccine in a murine pancreatic cancer model. All the mice treated with the combined therapy of fusion hybrid vaccine and Treg depletion rejected tumor growth in a challenging test, although the rejection rate was 20% both for mice that received the fusion hybrids alone or Treg depletion alone. In addition, combined therapy showed a significantly improved survival in comparison to other treatment or control groups. The NK cell activity for DC/TC fusion + Treg depletion was significantly higher than that for the other treatment groups. Cytotoxic T lymphocyte (CTL) activity for DC/TC could potentially be enhanced by the addition of Treg depletion therapy. The treatments including DC/TC fusion induced IFN-gamma secreting effector cells in ELISPOT assays. Furthermore, a cytometric beads array assay used to measure cytokine secretion showed that DC/TC fusion + Treg depletion stimulated the highest levels of IFN-gamma Th1/Th2 ratios and Th17. The results demonstrate that Treg depletion combined with DC/TC fusion hybrid vaccine enhanced the efficacy of immunotherapy in pancreatic cancer by activating CTLs and NK cells<sup>(39)</sup>.

In both human pancreatic adenocarcinoma and a murine pancreatic tumor model (Pan02), tumor cells produce increased levels of ligands for the CCR5 chemokine receptor and, reciprocally, CD4(+) Foxp3(+) Tregs, compared with CD4(+) Foxp3(-) effector T cells, preferentially express CCR5. When CCR5/CCL5 signaling is disrupted, either by reducing CCL5 production by tumor cells or by systemic administration of a CCR5 inhibitor, Treg migration to tumors is reduced and tumors are smaller than in control mice. Thus, the study demonstrates the importance of Tregs in immune evasion by tumors, how blockade of Treg migration might inhibit tumor growth, and, specifically in pancreatic adenocarcinoma, the role of CCR5 in the homing of tumor-associated Tregs. Selective targeting of CCR5/CCL5 signaling may represent a novel immunomodulatory strategy for the treatment of cancer<sup>(40)</sup>.

In murine mesothelin-expressing pancreatic tumor model (Panc02), vaccine with the immune-relevant mesothelin-derived peptides and in sequence with low-dose cyclophosphamide (CY) and an anti-CD25 IL-2R $\alpha$  monoclonal antibody (PC61), which are known to deplete subpopulations of T regulatory cells (Tregs), showed that combined Treg-depleting therapies synergize to enhance vaccine efficacy<sup>(41)</sup>.

**Myeloid-derived suppressor cells:** Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expand during cancer, inflammation and infection,

and have a remarkable ability to suppress T-cell responses<sup>(42)</sup>. They contribute to negative regulation of immune response and can be activated by factors produced by activated T cells and tumor stromal cells<sup>(43)</sup>.

Myeloid derived suppressor cells (MDSC), which are observed with increased prevalence in the peripheral blood and tumor microenvironment of cancer patients, including pancreatic cancer. Accumulation of MDSC in the peripheral circulation has been related to extent of disease, and correlates with stage. MDSC have primarily been implicated in promoting tumor growth by suppressing antitumor immunity. There is also compelling evidence MDSC are also involved in angiogenesis and metastatic spread. Two main subsets of MDSC have been identified in cancer patients: a monocytic subset, characterized by expression of CD14, and a granulocytic subset characterized by expression of CD15. Both subsets of MDSC actively suppress host immunity through a variety of mechanisms including production of reactive oxygen species and arginase. Just as in humans, accumulation of monocytic and granulocytic MDSC has been noted in the bone marrow, spleen, peripheral circulation, and tumors of tumor bearing mice. Successful targeting of MDSC in mice is associated with improved immune responses, delayed tumor growth, improved survival, and increased efficacy of vaccine therapy. By further elucidating mechanisms of MDSC recruitment and maintenance in the tumor environment, strategies could be developed to reverse immune tolerance to tumor<sup>(44)</sup>.

In a comprehensive analysis of circulating myeloid-derived suppressor cells (MDSCs) and T regulatory cells (Tregs) in pancreatic, esophageal and gastric cancer Patients Peripheral blood was collected from 131 cancer patients (46 pancreatic, 60 esophageal and 25 gastric) and 54 healthy controls. PBMC were harvested with subsequent flow cytometric analysis of MDSC (HLADR(-) Lin1(low/-) CD33(+) CD11b(+)) and Treg (CD4(+) CD25(+) CD127 (low/-) FoxP3(+)) percentages. MDSCs and Tregs were statistically significantly elevated in pancreatic, esophageal and gastric cancer compared with controls, and MDSC numbers correlated with Treg levels. Increasing MDSC percentage was associated with increased risk of death, and in a multivariate analysis, MDSC level was an independent prognostic factor for survival. A unit increase in MDSC percentage was associated with a 22% increased risk of death (hazard ratio 1.22, 95% confidence interval 1.06-1.41). The result showed MDSCs are an independent prognostic factor for survival<sup>(45)</sup>.

In mice with spontaneous pancreatic tumours, mice with premalignant lesions as well as wild-type mice, Myeloid-derived suppressor cells (MDSC) were analysed. An increase in the frequency of MDSC early in tumour development was detected in lymph nodes, blood and pancreas of mice with premalignant lesions and increased further upon tumour progression. The MDSC from mice with pancreatic tumours have arginase activity and suppress T-cell responses, which represent the hallmark functions of these cells. The study suggests that immune suppressor mechanisms generated by tumours exist as early as premalignant lesions and increase with tumour progression and highlight the importance of blocking these suppressor mechanisms early in the disease in developing immunotherapy protocols<sup>(46)</sup>.

Nagaraj reported use of the synthetic triterpenoid (CDDO-Me) can completely abrogate immune suppressive activity of MDSC in vitro, CDDO-Me reduced reactive oxygen species in MDSCs but did not affect their viability or the levels of nitric oxide and arginase.

Treatment of tumor-bearing mice with CDDO-Me did not affect the proportion of MDSCs in the spleens but eliminated their suppressive activity. This effect was independent of antitumor activity. CDDO-Me treatment decreased tumor growth in mice. Experiments with severe combined immunodeficient-beige mice indicated that this effect was largely mediated by the immune system. CDDO-Me substantially enhanced the antitumor effect of a cancer vaccines. treatment of pancreatic cancer patients with the synthetic triterpenoid (CDDO-Me) didn't affect the number of MDSCs in peripheral blood but significantly improved the immune response. The research demonstrated MDSCs is the key of the immunotherapy<sup>(47)</sup>.

### 3. Nonspecific immunotherapy - Innate Immune system and cytokine

Nature kill cells are the central component of the innate immunity and play an important role in cancer immunosurveillance. It has been reported that NK cells can recognize and control tumor growth by direct cellular cytotoxicity and secrete immunostimulatory cytokines such as IFN- $\gamma$ . The further researches have demonstrated NK cells can eliminate tumor cell by inhibiting cellular proliferation, angiogenesis, promoting apoptosis and stimulate the adaptive immune system. In mouse experimental models, NK cell-mediated elimination of tumor cells induced the subsequent development of tumor-specific T cell responses to the parental tumor cells as a bridge between innate and adaptive immune responses<sup>(48)</sup>.

In 1984, K. Funa has found patients with pancreatic adenocarcinomas expressing deficiencies in the NK-IFN system at least three levels:(1)diminished basal NK activities, (2)decreased sensitivity of NK to IFN in vitro, (3)decreased atypical IFN production by *staphylococcus aureus* cowan I (SACoI)<sup>(49)</sup>.

In a recent clinical trial, a patient exhibited regression of several pancreatic cancer metastases following the administration of the immune modulator Ipilimumab (anti-CTLA-4 antibody). Tumor infiltrating lymphocytes (TIL-2742) and an autologous tumor line (TC-2742) were expanded from a regressing metastatic lesion excised from this patient. Natural killer (NK) cells predominated in the TIL (92% CD56(+)) with few T cells (12% CD3(+)). A majority (88%) of the NK cells were CD56(bright)CD16(-). TIL-2742 secreted IFN- $\gamma$  and GM-CSF following co-culture with TC-2742 and major histocompatibility complex mismatched pancreatic tumor lines. After sorting TIL-2742, the purified CD56(+)-CD16(-)-CD3(-) subset showed reactivity similar to TIL-2742 while the CD56(-)-CD16(-)-CD3(+) cells exhibited no tumor recognition. In co-culture assays, TIL-2742 and the NK subset expressed high reactivity to several pancreatic cancer cell lines and could lyse the autologous tumor as well as pancreas cancer lines. Reactivity was partially abrogated by blockade of TRAIL. This represents the first report of CD56(+)-CD16(-) NK cells with apparent specificity for pancreatic cancer cell lines and associated with tumor regression following the treatment with an immune modulating agent<sup>(50)</sup>.

Clinical and experimental evidence demonstrate the extent of NK cell activity in peripheral blood is associated with cancer risk in adults<sup>(51)</sup>. In recent years, novel studies have discovered the phenotypic status and functionality of NK cells in tumor site and also in peripheral blood of cancer patients. Research has shown that only a few infiltrating NK cells which are unlikely to greatly contribute to eliminate the tumoe cells<sup>(52)</sup>. Due to NK's



inefficient homing into malignant tissues, the situation may be overcome by cytokine-mediated activation in immunotherapeutic regimen<sup>(53)</sup>. However, novel studies of tumor-associated NK cells demonstrated a striking phenotype, supporting the notion that tumor-induced alterations of activating NK cell receptor expression may hamper immune surveillance and promote tumor progression.

Bhat R reported the finding: besides its intrinsic oncolytic activity, parvovirus H-1PV is able to enhance NK cell-mediated killing of pancreatic adenocarcinoma cells. The experiment shows that H-1PV infection of Panc-1 cells increases NK cell capacity to release IFN- $\gamma$ , TNF- $\alpha$  and MIP-1 $\alpha/\beta$ . Multiple activating receptors are involved in the NK cell-mediated killing of Panc-1 cells. Indeed, blocking of the natural cytotoxicity receptors-NKp30, 44 and 46 in combination, and NKG2D and DNAM1 alone inhibit the killing of Panc-1 cells. Interestingly, H-1PV infection of Panc-1 cells overcomes the part of inhibitory effects suggesting that parvovirus may induce additional NK cell ligands on Panc-1 cells. The enhanced sensitivity of H-1PV-infected pancreatic adenocarcinoma cells to NK cell-dependent killing could be traced back to the upregulation of the DNAM-1 ligand, CD155 and to the downregulation of MHC class I expression. The data suggests that NK cells display antitumor potential against PDAC and that H-1PV-based oncolytic immunotherapy could further boost NK cell-mediated immune responses and help to develop a combinatorial therapeutic approach against pancreatic cancer<sup>(54)</sup>.

NK cells can eliminate tumor cells through their ability to mediate antibody-dependent cellular cytotoxicity (ADCC). NK cell recognition of an antibody-coated target cell results in rapid NK cell activation and degranulation<sup>(55)</sup>. NK-cell mediated ADCC plays a part in mechanisms of tumor-targeted mAbs which target CD20, Her2/neu, epidermal growth factor receptor (EGFR)<sup>(56)(57)</sup>. Because HLA class I is a ligand for inhibitory receptor family, killer cell immunoglobulin-like receptor of NK cells<sup>(58)</sup>, loss of HLA class I expression can lead to escape of antigen-dependent cytotoxicity of CD8+ CTL and increase the possibility as a target of NK cell cytotoxicity. In pancreatic cancer, total HLA class I loss is 6% in primary versus 43% in metastatic tumors; 0 in G1, 33% in G2 and 67% in G3<sup>(59)</sup>.

In research of nonspecific immunotherapy, many cytokines were used to elevate the ability of the immune system. It is possible to activate tumor-specific antitumor immune responses by systemic injection of cytokine or introduction of cytokine gene into tumors through activating natural killer (NK) cells and tumor-specific CD4+ T cells and cytotoxic T lymphocytes (CTL). Different cytokines may stimulate antitumor immune responses by different mechanisms.

Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF) and IL-2 are the most popular cytokines used in cancer immunotherapy. GM-CSF, which can stimulate bone marrow differentiating and maturing to neutrophils, monocytes and dendritic cells, is used to generate cancer immunotherapy called GAVX<sup>(60)</sup>. In clinical trials using the GAVX, induction of systemic antitumor immune response and clinical activity was observed in pancreatic cancer, melanoma, and renal cell carcinoma. In a study of combination of chemotherapy and immunotherapy, two GM-CSF secreting pancreas cancer cell lines (CG8020/CG2505) as immunotherapy were administered alone or in sequence with Cy in patients with advanced pancreatic cancer. Results showed GM-CSF secreting pancreas cancer cell lines demonstrated minimal treatment-related toxicity in patients with advanced

pancreatic cancer. Also, mesothelin specific T cell responses are detected/enhanced in some patients treated with CG8020/CG2505 immunotherapy. In addition, Cy modulated immunotherapy resulted in median survival in a Gemzar resistant population similar to chemotherapy alone<sup>(61)</sup>.

Interlukin-2 (IL-2) is a growth factor that stimulates innate immunity cells. Different dose of IL-2 has been proved either enhance or decrease cellular and humoral immune functions. Rosenberg used it developing lymphokine-activated killer(LAK) therapy for cancer<sup>(62)</sup>. In a randomized study, preoperative subcutaneously IL-2 immunotherapy at 12 million IU for 3 consecutive days before surgery is able to abrogate the effects of the surgical trauma and recover a normal immunofunction in pancreatic cancer patients<sup>(63)</sup>. Recombinant interleukin-2 (rIL-2) was used in a study which aimed to evaluate the toxicity of pre- and postoperative rIL-2 treatment and the effects on innate immunity both in peripheral blood and in cancer tissue of patients with resectable pancreatic adenocarcinoma. Seventeen patients received high dose rIL-2 preoperative subcutaneous administration and two low dose postoperative cycles. NK cell and eosinophil count were evaluated in blood and in pancreatic surgical specimens. The result showed toxicity was moderate. In the early postoperative period, blood NK cells and eosinophils significantly increased compared to basal values ( $p < 0.02$ ). Preoperative high dose rIL-2 administration is able to counteract surgery-induced deficiency of NK cells and eosinophils in peripheral blood in the early postoperative period, although it cannot overcome local mechanisms of immune tumor escape in cancer tissue. The amplification of innate immunity, induced by immunotherapy, may improve the control of metastatic cells spreading in the perioperative period<sup>(64)</sup>.

As a bridge between innate and adaptive immune response<sup>(65)</sup>, IL-12 is independently identified as natural killer-stimulating factor (NKSF) and cytotoxic lymphocyte maturation factor<sup>(66)</sup>, which induces proliferation of NK and T1 cells and production of cytokines, especially IFN- $\gamma$ , and also enhances the generation and activity of CTLs, through activation of STAT4<sup>(67)</sup>.

The combination of IL-12 and IL-27 can modify the polarization of Th2 effectors by both reduction of IL-5, GM-CSF and IL-13 and induction of IFN-gamma production, which lasted after cytokine removal. Besides, the combined treatment functionally modulated the Th2 polarization of CEA-specific CD4(+) T cells and enhanced pre-existing Th1 type immunity<sup>(68)</sup>.

In recent study, IL-12 was coformulated with the biodegradable polysaccharide chitosan which could enhance the antitumor activity of IL-12 while limiting its systemic toxicity. Antitumor efficacy of IL-12 alone and IL-12 coformulated with chitosan (chitosan/IL-12) was assessed in mice bearing established pancreatic (Panc02) tumors. Additional studies involving depletion of immune cell subsets, tumor rechallenge, and CTL activity were designed to elucidate mechanisms of regression and tumor-specific immunity. Coformulation with chitosan increased local IL-12 retention from 1 to 2 days to 5 to 6 days. Weekly i. t. injections of IL-12 alone eradicated  $\leq 10\%$  of established Panc02 tumors, while i. t. chitosan/IL-12 immunotherapy caused complete tumor regression in 80% to 100% of mice. Depletion of CD4(+) or Gr-1(+) cells had no impact on chitosan/IL-12-mediated tumor regression. However, CD8(+) or NK cell depletion completely abrogated antitumor activity. I. t. chitosan/IL-12 immunotherapy generated systemic tumor-specific immunity, as  $>80\%$

of mice cured with i. t. chitosan/IL-12 immunotherapy were at least partially protected from tumor rechallenge. Furthermore, CTLs from spleens of cured mice lysed MC32a and gp70 peptide-loaded targets. The research has demonstrated Chitosan/IL-12 immunotherapy increased local retention of IL-12 in the tumor microenvironment, eradicated established, aggressive murine tumors, and generated systemic tumor-specific protective immunity<sup>(69)</sup>.

#### 4. Specific immunotherapy

Specific immunotherapy, which seems to be more important in cancer treatment research, could be divided into 3 parts: monoclonal antibody, adoptive cellular therapy, and vaccine. Infusion of antibody or activated cells is called Passive Immunotherapy, on the other, vaccine can induce active immunotherapy. The simplest model of immune cell-mediated antigen-specific tumor rejection consists of three elements: appropriate antigen specific for the tumor, efficient antigen presentation and the generation of potent effector cells.

##### 4.1 Active immunotherapy

**Vaccine:** The development of human therapeutic cancer vaccines has come a long way since the discovery of major histocompatibility complex (MHC) restricted tumor antigens. As a new method to reconstituting immunity, cancer vaccination can actively harness the intrinsic power of the immune system to recognize and destroy tumors. The ideal designed vaccine should actively generate antigen-specific immune response to abnormal protein expressed in tumor cells, including activating distinct components of the immune system: antigen presenting cells, B cells and T cells, producing the advantages of high specificity, minimal toxicity and permanently effective immunologic memory. Antigen could be delivered in the form of DNA or peptide, as well as tumor cells or antigen-pulsed DCs.

**GM-CSF** is an important growth factor for granulocytes and monocytes, and has a crucial role in the growth and differentiation of DCs. Kimura M has found in vivo growth of AsPC-1 cells, which retrovirally transduced with the GM-CSF gene, was inhibited and associated with increased survival of the nude mice<sup>(70)</sup>.

A series of clinical trials have been reported by researchers in John Hopkins in recent 10 years. Jaffee et al conducted a phase I study using allogeneic GM-CSF-secreting whole-cell tumor vaccine for pancreatic cancer. As vaccines, Two pancreatic cancer lines (PANC 10.05 and 6.03), which had been genetically modified to express GM-CSF, were given to patients who had undergone pancreaticoduodenectomy eight weeks prior. Three of the eight patients who received  $\geq 10 \times 10^7$  vaccine cells developed post-vaccination delayed-type hypersensitivity (DTH) responses associated with increased disease free survival time, and remained disease-free for longer than 25 months after diagnosis. Side effects were mainly limited to local skin reactions at the site of vaccination<sup>(71)</sup>. Further phase II study of 60 patients with resected pancreatic adenocarcinoma, patients received five treatments of  $2.5 \times 10^8$  vaccine cells, together with 5-Fu and radiotherapy. The reported median survival was 26 months, with a 1- and 2-year survival of 88% and 76% respectively<sup>(72)</sup>. In latest report, a single institution phase II study of 60 patients with resected pancreatic adenocarcinoma was performed, each treatment consisted of a total of  $5 \times 10^8$  GM-CSF-secreting cells distributed equally among 3 lymph node regions. Subsequently, had received

5-FU based chemoradiation, patient received 5 immunotherapy. The median disease-free survival was 17.3 months with median survival of 24.8 months. The administration of immunotherapy was well tolerated. Besides, the postimmunotherapy induction of mesothelin-specific CD8+ T cells in HLA-A1+ and HLA-A2+ patients correlates with disease-free survival. The research concluded that an immunotherapy approach intergated with chemoradiation is safe and helpful for resected pancreas cancer<sup>(73)</sup>.

**VEGF** Vascular endothelial growth factor receptor 2 (VEGFR2) is an essential factor in tumor angiogenesis and in the growth of pancreatic cancer. Immunotherapy using epitope peptide for VEGFR2 (VEGFR2-169) is expected to improve the clinical outcome. A phase I clinical trial combining of VEGFR2-169 with gemcitabine was conducted for patients with metastatic and unresectable pancreatic cancer. Gemcitabine was administered at a dose of 1000 mg/m<sup>2</sup> on days 1, 8, and 15 in a 28-day cycle. The VEGFR2-169 peptide was subcutaneously injected weekly in a dose-escalation manner (doses of 0.5, 1, and 2 mg/body, six patients/one cohort). No severe adverse effect of grade 4 or higher was observed. Of the 18 patients who completed at least one course of the treatment, 15 (83%) developed immunological reactions at the injection sites. Specific cytotoxic T lymphocytes (CTL) reacting to the VEGFR2-169 peptide were induced in 11 (61%) of the 18 patients. The disease control rate was 67%, and the median overall survival time was 8.7 months. This combination therapy for pancreatic cancer patients was tolerable at all doses. Peptide-specific CTL could be induced by the VEGFR2-169 peptide vaccine at a high rate, even in combination with gemcitabine. From an immunological point of view, the optimal dose for further clinical trials might be 2 mg/body or higher<sup>(74)</sup>.

**Ras** peptide is the first agent tested in immunotherapy in pancreatic cancer. Gjertsen used an intradermal vaccine of APCs loaded ex vivo with synthetic ras peptide corresponding to the mutation found in patients. In this phase I/II trial, two of five patients with advanced pancreatic cancer showed induced immune response<sup>(75)</sup>. In further phase I/II trial in 48 pancreatic cancer patients with different clinic stages, ras peptide in combination with GM-CSF could induce peptide-specific immunity in 58% patients. Compared to non-responders, survival time were prolonged in patients with advanced disease, the association between prolonged survival and an immune response against the vaccine suggests that a clinical benefit of ras peptide vaccination may be obtained for this group of patients<sup>(76)</sup>.

In 24 Patients with resected pancreatic cancer, with K-ras mutations at codon 12, were vaccinated once monthly for 3 months with a 21-mer peptide vaccine containing the corresponding K-ras mutation of the patient's tumor. Immune responses were evaluated by delayed-type hypersensitivity (DTH) tests and the enzyme-linked immunosorbent spot assays. Results showed there were no grade 3-5 vaccine-specific toxicities. The only National Cancer Institute grade 1 and 2 toxicity was erythema at the injection site (94%). Nine patients (25%) were evaluable for immunologic responses. One patient (11%) had a detectable immune response specific to the patient's K-ras mutation, as assessed by DTH. Three patients (13%) displayed a DTH response that was not specific. Median recurrence free survival time was 8.6 months (95% confidence interval, 2.96-19.2) and median overall survival time was 20.3 months (95% confidence interval, 11.6-45.3). It suggested K-ras vaccination for patients with resectable pancreatic adenocarcinoma proved to be safe and tolerable with however no elicitable immunogenicity and unproven efficacy<sup>(77)</sup>.

In another phase II study, a specific mutant ras peptide vaccine was tested as an adjuvant immunotherapy in pancreatic and colorectal cancer patients. Five pancreatic and seven colorectal cancer patients were vaccinated subcutaneously with 13-mer mutant ras peptide, corresponding to their tumor's ras mutation. Vaccinations were given every 4 weeks, up to a total of six vaccines. The result showed no serious acute or delayed systemic side effects were seen. Five out of eleven patients showed a positive immune response. Furthermore, the five pancreatic cancer patients have shown a mean disease-free survival (DFS) of 35.2+ months and a mean overall survival (OS) of 44.4+ months. The study suggested it is feasible to use mutant ras vaccine in the adjuvant setting. This vaccine is safe, can induce specific immune responses, and it appears to have a positive outcome in overall survival<sup>(78)</sup>. In a follow-up study, Twenty-three patients who were vaccinated after surgical resection for pancreatic adenocarcinoma (22 pancreaticoduodenectomies, one distal resection). The vaccine was composed of long synthetic mutant ras peptides designed mainly to elicit T-helper responses. Seventeen of 20 evaluable patients (85%) responded immunologically to the vaccine. Median survival for all patients was 27.5 months and 28 months for immune responders. The 5-year survival was 22% and 29%, respectively. Strikingly, 10-year survival was 20% (four patients out of 20 evaluable) versus zero (0/87) in a cohort of nonvaccinated patient treated in the same period. Three patients mounted a memory response up to 9 years after vaccination. The observation indicates that K-ras vaccination may consolidate the effect of surgery and represent an adjuvant treatment option for the future<sup>(79)</sup>.

**MUC1** In order to create MUC1-specific immune response, a vaccine composed of MUC1 peptide and SBAS2 adjuvant was tested in a phase I study, There was an increase in the percentage of CD8+ T cells and MUC1-specific antibody<sup>(80)</sup>.

The other approach to induce MUC1-specific immune response is antigen-pulsed DCs. A Phase I/II clinical trial of a MUC1 peptide-loaded DC vaccine was carried out in 12 pancreatic and biliary cancer patients following resection of their primary tumors. The vaccine was well tolerated and no toxicity was observed. Prior to vaccination, patients entered onto this trial had a significantly higher percentage of FoxP3+CD4+T cells compared to age matched healthy controls. The percentage of these cells also increased transiently following each injection, returning to baseline or below before the next injection. Vaccinated patients have been followed for over four years and four of the twelve patients are alive, all without evidence of recurrence<sup>(81)</sup>.

Another phase I/II trial used human autologous DCs transfected with MUC1 cDNA as vaccine, 4 of 10 patients showed a two- to ten-fold increase in the frequency of mucin-specific IFN- $\gamma$ -secreting CD8+ T cells, suggesting an immune response<sup>(82)</sup>. But in a phase III trial of 255 patients using vaccine consisted of recombinant vaccinia and fowlpox viruses coexpressing CEA/MUC1/TRICOM, researchers failed to improve overall survival compared to palliative chemotherapy or best supportive care<sup>(83)</sup>.

In Kondo H's clinical trial, Peripheral blood mononuclear cells (PBMCs) of twenty patients with unresectable or recurrent pancreatic cancer were separated into adherent cells for induction of MUC1-DCs and floating cells for MUC1-CTLs. MUC1-DCs were generated by culture with granulocyte monocyte colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) and then exposed to MUC1 peptide and TNF-alpha. MUC1-CTLs were induced by co-culture with YPK-1 and then with interleukin-2 (IL-2). MUC1-DCs were injected

intradermally and MUC1-CTLs were given intravenously. The result showed one patient with multiple lung metastases experienced a complete response. Five patients had stable disease. The mean survival time was 9.8 months. No grade II-IV toxicity was observed. The research suggested adoptive immunotherapy with MUC1-DC and MUC1-CTL may be feasible and effective for pancreatic cancer<sup>(84)</sup>.

**Mesothelin** It is first reported by Thomas that specific CD8+ T-cell response which targeting mesothelin epitopes in pancreatic cancer can be induced via cross-presentation by an approach that recruits APCs to the vaccination site<sup>(85)</sup>. Combined with anti-glucocorticoid-induced TNF receptor antibody (anti-GITR), the mesotheline DNA vaccine can induce immune protection in mice with syngeneic mesothelin-expressing pancreatic cancer. 50% of animals treated with mesothelin were tumor-free 25 days after tumor injection compared to 0 in untreated mice<sup>(86)</sup>.

DNA vaccines employing single-chain trimers (SCT) have been shown to bypass antigen processing and presentation and result in significant enhancement of DNA vaccine potency. In a study, a DNA vaccine employing an SCT targeting human mesothelin and characterized the ensuing antigen-specific CD8+ T cell-mediated immune responses and anti-tumor effects against human mesothelin-expressing tumors in HLA-A2 transgenic mice. The results showed that vaccination with DNA employing an SCT of HLA-A2 linked to human mesothelin epitope aa540-549 (pcDNA3-Hmeso540-beta2m-A2) generated strong human mesothelin peptide (aa540-549)-specific CD8+ T cell immune responses in HLA-A2 transgenic mice. Vaccination with pcDNA3-Hmeso540-beta2m-A2 prevented the growth of HLA-A2 positive human mesothelin-expressing tumor cell lines in HLA-A2 transgenic mice in contrast to vaccination with DNA encoding SCT linked to OVA CTL epitope. Thus, the employment of SCT of HLA-A2 linked to the human mesothelin epitope aa540-549 represents a potential opportunity for the clinical translation of DNA vaccines against human mesothelin-expressing tumors, including pancreatic cancer<sup>(87)</sup>.

## 4.2 Passive immunotherapy

Passive immunotherapy could be accomplished by infusion monoantibody and tumor specific T-cell which was activated in vitro. With advances in structural and functional genomics, recent work has focused on targeted molecular therapy using monoclonal antibodies. Many monoantibodies were used to target molecules on the tumor cell surface and normal tissue stroma, which are related to pancreatic cancer oncogenesis, tumor growth or resistance to chemotherapy, as well as molecules involved in regulating inflammation and host immunoresponses. Although progress made by monoantibody in pancreatic cancer treatment, especially in preclinical studies, its clinical application requires further investigation. Besides the function bind to target antigen to block the corresponding signal transduction pathways, antibody-dependent cellular cytotoxicity (ADCC) can also be observed in some pancreatic cancer cell lines.

### 4.2.1 Antibody

Monoclonal antibodies against human tumor targets were initially in rodents, which will induce immunologic responses from patient against mouse antibodies. With the

development of recombinant DNA technology, this problem was solved and chimeric antibodies, antibody fragments or intact fully human antibodies were produced and tested clinically. Based on moral principles, antibodies were used as adjunctive treatment with chemotherapy agents, small molecule signal transduction inhibitors, or radiation in clinical trials, to study if they can help patients lengthen the survival. The targets are generally classified into three major categories: cell surface proteins; antigen associated with the tumor stroma; antigen on tumor-associated vasculature and angiogenic ligands<sup>(88)</sup>.

#### 4.2.1.1 Anti-EGFR antibodies

Cetuximab is a chimeric mouse-human antibody against an epitope located in the extra-cellular part of EGFR. In preclinical studies, cetuximab could decrease cell proliferation and phosphorylation of EGFR, and blocked the binding of the adaptor protein Grb2 to EGFR upon activation by EGF<sup>(89)</sup>. Another preclinical study, the combination of cetuximab together with gemcitabine and radiation effectively prolonged the tumor xenograft volume doubling time ( $30.1 \pm 3.3$  days), compared with gemcitabine monotherapy ( $11.6 \pm 3.1$  days), radiation monotherapy ( $16.7 \pm 3.1$  days), cetuximab with gemcitabine ( $20.1 \pm 3.1$  days) and cetuximab with radiation ( $22.5 \pm 3.3$  days)<sup>(90)</sup>.

In many clinical trials, synergistic effects were observed using combination of cetuximab therapy and chemotherapy agents. In a multicenter phase II trial, patients with measurable locally advanced or metastatic pancreatic cancer who had never received chemotherapy for their advanced disease and had immunohistochemical evidence of EGFR expression were treated with cetuximab at an initial dose of 400 mg/m<sup>2</sup>, followed by 250 mg/m<sup>2</sup> weekly for 7 weeks. Gemcitabine was administered at 1,000 mg/m<sup>2</sup> for 7 weeks, followed by 1 week of rest. In subsequent cycles, cetuximab was administered weekly, and gemcitabine was administered weekly for 3 weeks every 4 weeks. In sixty-one patients who were screened for EGFR expression, 58 patients (95%) had at least 1+ staining, and 41 were enrolled onto the trial, results showed five patients (12.2%) achieved a partial response, and 26 (63.4%) had stable disease. The median time to disease progression was 3.8 months, and the median overall survival duration was 7.1 months. One-year progression-free survival and overall survival rates were 12% and 31.7%, respectively. The most frequently reported grade 3 or 4 adverse events were neutropenia (39.0%), asthenia (22.0%), abdominal pain (22.0%), and thrombocytopenia (17.1%). Cetuximab in combination with gemcitabine showed promising activity against advanced pancreatic cancer<sup>(91)</sup>.

Another multicenter phase II study which is combination treatment with cetuximab and gemcitabine/oxaliplatin. Patients which had histological or cytological diagnosis of metastatic pancreatic adenocarcinoma received cetuximab 400 mg m<sup>-2</sup> at first infusion followed by weekly 250 mg m<sup>-2</sup> combined with gemcitabine 1000 mg m<sup>-2</sup> as a 100 min infusion on day 1 and oxaliplatin 100 mg m<sup>-2</sup> as a 2-h infusion on day 2 every 2 weeks. The intention-to-treat analysis of 61 evaluable patients showed an overall response rate of 33%, including 1 (2%) complete and 19 (31%) partial remissions. There were 31% patients with stable and 36% with progressive disease or discontinuation of the therapy before re-staging. The presence of a grade 2 or higher skin rash was associated with a higher likelihood of achieving objective response. Median time to progression was 118 days, with a median overall survival of 213 days. A clinical benefit response was noted in 24 of the evaluable 61

patients (39%). Although the addition of cetuximab to the combination of gemcitabine and oxaliplatin is well tolerated, the research failed to increase response or survival in patients with metastatic pancreatic cancer<sup>(92)</sup>.

But the effect of the cetuximab is limited by the affinity of expressed EGFR in pancreatic cancer, other factors including mutation of K-ras, PTEN expression or host complement level. these may be the reasons of failure in some trails. In a phase II trail, within the cetuximab group and noncetuximab group, no significant differences were found in objective response rate (17.5% Vs12.2%), median progression-free survival (3.4 months Vs 4.2 months), median overall survival (7.5 months Vs 7.8 months)<sup>(93)</sup>, The result can't prove a synergistic effect in combination of cetuximab and gemcitabine/cisplatin treatment in pancreatic cancer.

Another phase III trail of Patients with unresectable locally advanced or metastatic pancreatic adenocarcinoma were randomly assigned to receive gemcitabine alone or gemcitabine plus cetuximab. A total of 745 eligible patients were accrued. No significant difference was seen between the two arms of the study with respect to the median survival time (6.3 months for the gemcitabine plus cetuximab arm v5.9 months for the gemcitabine alone arm; hazard ratio = 1.06; 95% CI, 0.91 to 1.23; P = .23, one-sided). Objective responses and progression-free survival were similar in both arms of the study. Although time to treatment failure was longer in patients on gemcitabine plus cetuximab (P=.006), the difference in length of treatment was only 2 weeks longer in the combination arm. Among patients who were studied for tumoral EGFR expression, 90% were positive, with no treatment benefit detected in this patient subset. The author think in patients with advanced pancreas cancer, the anti-EGFR monoclonal antibody cetuximab did not improve the outcome compared with patients treated with gemcitabine alone. Alternate targets other than EGFR should be evaluated for new drug development<sup>(94)</sup>.

Matuzumab(EMD 72000) is a humanized IgG1 mAb against EGFR. Laboratory studies have shown promising inhibitory effects on tumor growth and angiogenesis, include L3. 6pl in an orthotopic rat model<sup>(95)</sup>. In an phase I clinical trail, matuzumab was given at a dose of 400-800 mg once weekly for 8 weeks, followed by gemcitabine 1000mg/m<sup>2</sup> weekly for two cycles. The partial response or stable disease in 12 evaluated advanced pancreatic cancer patients was 66.7%<sup>(96)</sup>.

#### 4.2.1.2 Anti-ErbB2/HER2 antibodies

Trastuzumab(Herceptin) is a humanized mAb, which has shown significant growth inhibition of a pancreatic cancer cell line and xenografts established with the same line. In a study focusing on HER2 overexpressing pancreatic cancer, trastuzumab was combined with fluoropyrimidine S-1 to treat cancer cells in vivo and in vitro, pancreatic cell growth inhibition is observed not only by inhibition of the HER2 signal transduction pathway, but also by antibody-dependent cellular cytotoxicity(ADCC) induced by trastuzumab<sup>(97)</sup>. In another research, although in four pancreatic cell lines, trastuzumab didn't express inhibitor effect and synergistic effect with gemcitabine, ADCC were observed in three cells which expressed HER2 in mice. In Capan-1 xenografted mice, trastuzumab inhibited tumor growth significantly and prolonged survival<sup>(98)</sup>.



Larbouret reported combination treatment of matuzumab and trastuzumab could enhance the inhibitory effect on HER2 phosphorylation, lead to significantly decrease xenograft tumor sizes or induce more complete remissions when compared to antibody alone, then prolonged survival in BxPC-3 and MIA PaCa-2 pancreatic cancer cells xenograft mice<sup>(99)</sup>. The further study which took place in nude mice, bearing human pancreatic carcinoma xenografts, combined anti-EGFR (cetuximab) and anti-HER2 (trastuzumab) or gemcitabine were given as treatment and tumor growth was observed. Result showed in first-line therapy, mice survival was significantly longer in the 2mAbs group compared with gemcitabine ( $P < 0.0001$  for BxPC-3,  $P = 0.0679$  for MiaPaCa-2 and  $P = 0.0019$  for Capan-1) and with controls ( $P < 0.0001$ ). In second-line therapy, tumor regressions were observed after replacing gemcitabine by 2mAbs treatment, resulting in significantly longer animal survival compared with mice receiving continuous gemcitabine injections ( $P = 0.008$  for BxPC-3,  $P = 0.05$  for MiaPaCa-2 and  $P < 0.001$  for Capan-1). Therapeutic benefit of 2mAbs was observed despite K-Ras mutation. Interestingly, concerning the mechanism of action, coinjection of F(ab')<sub>2</sub> fragments from 2mAbs induced significant tumor growth inhibition, compared with controls ( $P = 0.001$ ), indicating that the 2mAbs had an Fc fragment-independent direct action on tumor cells. This preclinical study demonstrated a significant improvement of survival and tumor regression in mice treated with anti-EGFR/anti-HER2 2mAbs in first- and second-line treatments, compared with gemcitabine, independently of the K-Ras status<sup>(100)</sup>.

#### 4.2.1.3 Anti-MUC1 antibodies

PAM4 is a murine antibody to MUC1 obtained from mice immunized with purified mucin from a human pancreatic cancer xenograft sample. In a preclinical study, <sup>90</sup>Y-labelled PAM4 monoclonal antibody was combined with gemcitabine in mice bearing Capan-1, the result showed increased inhibition of tumor growth and prolonged survival of the mice<sup>(101)</sup>. The recent clinical trial took place in 21 patients with advanced pancreatic cancer. <sup>111</sup>In-hPAM4 showed normal biodistribution with radiation dose estimates to red marrow and solid organs acceptable for radioimmunotherapy and with tumor targeting in 12 patients. One patient withdrew before <sup>(90)</sup>Y-hPAM4; otherwise, 20 patients received <sup>(90)</sup>Y doses of 15 (n=7), 20 (n=9), and 25 mCi/m<sup>2</sup> (n=4). Treatment was well tolerated; the only significant drug-related toxicities were (NCI CTC v.3) grade 3 to 4 neutropenia and thrombocytopenia increasing with <sup>(90)</sup>Y dose. There were no bleeding events or serious infections, and most cytopenias recovered to grade 1 within 12 weeks. Three patients at 25 mCi/m<sup>2</sup> encountered dose-limiting toxicity with grade 4 cytopenias more than 7 days, establishing 20 mCi/m<sup>2</sup> as the maximal tolerated <sup>(90)</sup>Y dose. Two patients developed HAHA of uncertain clinical significance. Most patients progressed rapidly and with CA19-9 levels increasing within 1 month of therapy, but 7 remained progression-free by CT for 1.5 to 5.6 months, including 3 achieving transient partial responses (32%-52% tumor diameter shrinkage). The study concluded <sup>(90)</sup>Y-Clivatuzumab tetraxetan was well tolerated with manageable hematologic toxicity at the maximal tolerated <sup>(90)</sup>Y dose, and is a potential new therapeutic for advanced pancreatic cancer<sup>(102)</sup>.

In a Phase I trial for patients with stage III and IV pancreatic cancer, another antibody C595, which is targeting the protein core of MUC1, was conjugated with the  $\alpha$ -particle-emitting

213bismuch. In vitro study showed specific cytotoxic to MUC1-expressing pancreatic cancer cells in a concentration-dependent manner compared to controls<sup>(103)</sup>.

#### 4.2.1.4 Anti-mesothelin antibodies

SS1P is a recombinant immunotoxin that consists of an anti-mesothelin scFv(ss1) fused to PE38, a 38Kda portion of *Pseudomonas* exotoxin. Sing-chain Fv(scFv)v was isolated from a phage display library obtained from the spleen of mice immunized with mesothelin-expression plasmid. After binding to mesothelin and subsequent internalisation into cells, it inhibits protein synthesis and results in apoptosis.

In preclinical study, SS1P plus radiation in treating mesothelin-expressing tumor xenografts, combination treatment significantly prolonged the doubling time of tumors<sup>(104)</sup>; meanwhile, synergic result was observed when treat with gemcitabin, the tumors were induced regression completely<sup>(105)</sup>.

In the further phase I clinical study, SS1P was administered by intravenous infusion in 34 patients with mesothelin-expressing tumor, including 2 pancreatic cancer patients, the results showed that it was well-tolerated with self-limiting pleuritis as dose-limiting toxicity, 12% tumor size decreased from 20-50% and lasted for more than 4 weeks, 56% patients showed stable disease and 29% of the patients had progressive disease<sup>(106)</sup>.

Another monoclonal antibody against mesothelin, MORAb-009, is a chimeric of a mouse and human mAb derived from a phage-display library and re-engineered<sup>(107)</sup>. In a phase I clinical trial, treatment of MORAb-009 in patients with advanced mesothelin-expressing cancers has been determined if safety, dose-limiting toxicity (DLT), and maximum tolerated dose (MTD). A total of 24 subjects were treated including 13 mesothelioma, 7 pancreatic cancer, and 4 ovarian cancer patients. The median number of MORAb-009 infusions was 4 (range 1-24 infusions). At the 400 mg/m<sup>2</sup> dose level, 2 subjects experienced DLT (grade 4 transaminitis and a grade 3 serum sickness). Thus, although there were other contributing causes of these adverse events, 200 mg/m<sup>2</sup> was considered the MTD. Other adverse events at least possibly related to MORAb-009 included 7 drug hypersensitivity events (all grade 1 or 2) and a thromboembolic event (grade 4). Eleven subjects had stable disease. There was a dose-dependent increase in serum MORAb-009 concentration. The result suggested that MORAb-009 is well tolerated and the MTD when administered weekly is conservatively set at 200 mg/m<sup>2</sup>. Phase II studies of MORAb-009 in different mesothelin-expressing cancers are ongoing<sup>(108)</sup>.

#### 4.2.2 Adoptive cell transfer

In cellular antitumor immunity, T-cells must first be activated by bone marrow – derived APCs that present tumor antigens and provide essential co-stimulatory signals, migrate and gain access to the tumor microenvironment, and overcome obstacles to effective triggering posed by the tumor. Dendritic cells, which are the strongest antigen presenting cells in the body. Their generation for anti-tumor immunity has been the focus of a vast array of scientific and clinical studies. DC's specialized capacity to cross-present exogenous Ags onto major histocompatibility (MHC) class I molecules for the generation of T-Ag-specific cytotoxic T lymphocytes (CTLs) has made it possible to produce activated T cell in vitro and

in vivo. Adoptive immunotherapy involves harvesting the patient's peripheral blood T-lymphocytes, stimulating and expanding the autologous tumor-reactive T-cells, finally transferring them back into the patient.

#### 4.2.2.1 K-ras-specific CTLs

In vitro, Immature DCs had pulsed with synthesized mutant K-ras peptide (YKLVVVGAV). When the DCs were matured, Kras antigen epitope can express on the DC's surface effectively and Cytotoxic T lymphocytes (CTLs) can be induced when autogeneic and homologous T cells co-cultured with the mutant K-ras peptide-pulsed DCs. The research demonstrated the induced CTLs can kill the pancreatic cancer cell line Patu8988 which express the same K-ras mutation type effectively in vitro and in vivo. Without damage the normal tissue cells, the killing rate of activated K-ras specific CTLs to the tumor cell when the ratios of CTL: Patu8988 cells were 10:1, 20:1, and 50:1 were (21.2+/-1.9)%, (32.4 +/- 2.1)%, and (45.7+/-5.3)% respectively, all while the killing efficiency significantly superior to those of the non-specific activated T lymphocyte (all  $P < 0.05$ ). Eight days after CTL injection into the nude mice the tumor size of the intratumor injection group was (68 +/- 13) mm<sup>3</sup>, significantly smaller than those of the control group and IL-2 activated non-specific CTL intra-tumor injection group [(87+/-14) mm<sup>3</sup> and (79 +/- 19) mm<sup>3</sup>, both  $P < 0.05$ ]. The survival rates of the nude mice of the K-ras specific CTL intra-tumor injection group, CTL caudal vein injection group, and IL-2 activated non-specific CTL intra-tumor injection group were all significantly higher than that of the control group (all  $P < 0.05$ ), and the survival rate of the K-ras specific CTL intra-tumor injection group was significantly higher than that of the IL-2 activated non-specific CTL intra-tumor injection group ( $P < 0.05$ ). Immunohistochemical staining confirmed that K-ras specific CTL had the ability to move toward tumor. The result showed antigen-specific-CTLs induced in vitro and transferred into the patient can used be a effective treatment for pancreatic cancer<sup>(109)</sup>.

#### 4.2.2.2 MUC1-specific CTLs

In MUC1 expressing Tumor-bearing mice , there were low affinity MUC1-specific CTLs that have no effect on the spontaneously occurring pancreatic tumors in vivo. However, adoptive transfer of these CTLs was able to completely eradicate MUC1-expressing injectable tumors in MUC1 transgenic mice, and these mice developed long-term immunity. These CTLs were MHC class I restricted and recognized peptide epitopes in the immunodominant tandem repeat region of MUC1. The MET mice appropriately mimic the human condition and are an excellent model with which to elucidate the native immune responses that develop during tumor progression and to develop effective antitumor vaccine strategies<sup>(110)</sup>.

In a study of 11 patients with lung metastases from different cancer, CTLs were generated in vitro using cultured DCs, synthetic peptide, peripheral blood lymphocytes, IL-2 and anti-CD3 antibody. The patients received either Muc-1, CEA, gp100, Her-2 or SART-3-PDAK cells generated in vitro, All transfers of peptide-pulsed dendritic cell-activated killer(PDAK) cells, which showed peptide/HLA-specific lysis, were well-tolerated in all patients, and adverse effects (elevation of transaminase, fever, and headache) were observed primarily at grade 1, but in no case greater than grade 2. One partial response (PR) of lung metastasis occurred in a pancreatic cancer patient who received  $3 \times 10^7$  Muc-1-PDAK cells/kg. The cytolytic units

of PDAK cells in this patient appeared to be substantially higher compared to those in PD patients. The results suggest that adoptive immunotherapy using PDAK cells for cancer patients with antigen-positive lung metastasis is safe and feasible<sup>(111)</sup>.

However, in another clinical study, data demonstrate that MUC1 peptide-based immunization elicits mature MUC1-specific CTLs in the peripheral lymphoid organs. The mature CTLs secrete IFN-gamma and are cytolytic against MUC1-expressing tumor cells in vitro. Unfortunately, active CTLs that infiltrate the pancreas tumor microenvironment become cytolytically anergic and are tolerized to MUC1 antigen, allowing the tumor to grow. The CTL tolerance could be reversed at least in vitro with the use of anti-CD40 co-stimulation. The pancreas tumor cells secrete immunosuppressive cytokines, including IL-10 and TGF-beta that are partly responsible for the down-regulation of CTL activity. In addition, they down-regulate their MHC class I molecules to avoid immune recognition. CD4+CD25+T regulatory cells, which secrete IL-10, were also found in the tumor environment. Together these data indicate the use of several immune evasion mechanisms by tumor cells to evade CTL killing. Thus altering the tumor microenvironment to make it more conducive to CTL killing may be key in developing a successful anti-cancer immunotherapy<sup>(112)</sup>.

#### 4.2.2.3 Telomerase-specific CTLs

In a syngeneic pancreatic tumor mouse model, T-cells were produced in vitro by coculturing human lymphocytes with telomerase peptide-pulsed dendritic cells(DCs) or in vivo by injection of peptide, animals treated with telomerase-specific T cells showed significantly delayed disease progression<sup>(113)</sup>.

#### 4.2.2.4 Mesothelin-specific CTLs

With the identification of novel mesothelin CTL epitopes, T-cell lines generated from one of these epitopes were shown to lyse pancreatic tumor cells. Several agonist epitopes were defined and were shown to (a) have higher affinity and avidity for HLA-A2, (b) activate mesothelin-specific T cells from normal individuals or cancer patients to a greater degree than the native epitope in terms of induction of higher levels of IFN-gamma and the chemokine lymphotactin, and (c) lyse several mesothelin-expressing tumor types in a MHC-restricted manner more effectively than T cells generated using the native peptide. External beam radiation of tumor cells at nontoxic levels was shown to enhance the expression of mesothelin and other accessory molecules, resulting in a modest but statistically significant increase in tumor cell lysis by mesothelin-specific T cells. The result supports and extends observations that mesothelin is a potential target for immunotherapy of pancreatic cancers, as well as mesotheliomas. Combination of immunotherapy and chemoradiotherapy may be a better choice for the patients<sup>(114)</sup>.

### 4.3 Future perspective

Although it is used as adjuvant treatment in preclinical or clinical trial, immunotherapy may be the next great hope for pancreatic cancer treatment. While monoclonal antibodies, cytokines, vaccines and CTL have individually shown some promise, it's hard to say which is better in nonspecific and specific immunotherapy. It seems to be the best strategy to

obtained more efficient results in combination with a variety of antigens, or vaccine and antibody combinations. A nonspecific and specific immunotherapy combination offers another potent strategy. With the combination, the ultimate achievable goal may be a durable anti-tumor immune response that can destroy and prevent it from recurrence over the course of a patient's life.

According to the existed profiles, The key of the immunotherapy on pancreatic cancer is to break through cancer microenvironment's defence. Suppressing the function of immunosuppression cells, such as immunosuppressive tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) reside in tumors is as important as inducing the specific immune agent, such as antibody or CTL.

Combating with each other, tumor and immune system are like two warriors on the other side of balance. What we can do is to break the balance and help the immune system win the war. Traditional methods, surgical operation, chemoradiation, can decrease the number of the tumor cells to the minimum while do harmful to immune system in the same time. So as the followed treatment, the passive immunotherapy may be the best choice to supply enough activated immune agents in a short period to kill the metastatic cancer cells. When patient recovered, Cytokines and vaccine will help to establish long-term specific immune response to keep watch on and get rid of residuary cancer cells. Owing to pancreatic cancer cells expressing different abnormal antigens, the combination of 2 or more epitopes vaccines will obtain better effect to prevent from recurrence. and metastasis.

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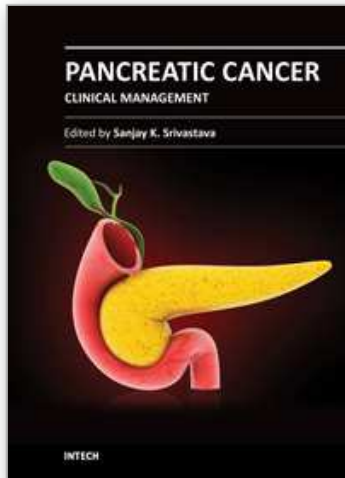
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## **Pancreatic Cancer - Clinical Management**

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This book covers pancreatic cancer risk factors, treatment and clinical procedures. It provides an outline of pancreatic cancer genetic risk factors, biomarkers and systems biology for the better understanding of disease. As pancreatic cancer suffers from lack of early diagnosis or prognosis markers, this book encompasses stem cell and genetic markers to identify the disease in early stages. The book uncovers the rationale and effectiveness of monotherapy and combination therapy in combating the devastating disease. As immunotherapy is emerging as an attractive approach to cease pancreatic cancer progression, the present book covers various aspects of immunotherapy including innate, adaptive, active, passive and bacterial approaches. Management of anesthesia during surgery and pain after surgery has been discussed. Book also takes the reader through the role of endoscopy and fine needle guided biopsies in diagnosing and observing the disease progression.

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