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Monoclonal antibodies in the treatment of pancreatic cancer

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Abstract

Human pancreatic cancer is a malignant disease with almost equal incidence and mortality. Effective diagnostic and therapeutic strategies are still urgently needed to improve its survival rate. With advances in structural and functional genomics, recent work has focused on targeted molecular therapy using monoclonal antibodies. This review summarizes the 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. Targeted molecules include cell-surface receptors, such as the EGF receptor, HER2, death receptor 5 and IGF-1 receptor. Effects of monoclonal antibodies against these target molecules alone or in combination with chemotherapy, small-molecule signal transduction inhibitors, or radiation therapy are also discussed. Also discussed are the use of toxin or radioisotope conjugates, and information relating to the use of these targeting agents in pancreatic cancer clinical trials. Although targeted molecular therapy with monoclonal antibodies has made some progress in pancreatic cancer treatment, especially in preclinical studies, its clinical application to improve the survival rate of pancreatic cancer patients requires further investigation.

Keywords

cancer therapy; immunotherapy; monoclonal antibody; pancreatic cancer

Pancreatic cancer remains a disease with high mortality despite numerous efforts that have been made to improve its survival rates. In a recent analysis using a database from 1973 to 2003 based on modeled period analysis, 5-year survival of pancreatic cancer patients was 7.1% and 10-year survival was below 5% [1]. The survival rate is apparently related to the disease stage with a low rate at 1.6–3.3% among patients with distant metastases [1,2]. Early diagnosis and effective treatment to control the advanced stages of disease may prolong the survival rate of pancreatic cancer [3]. The search for effective treatment of pancreatic cancer has involved in-depth translational research for decades. With the achievements made in molecular biology and cell biology, studies have focused on structural genomics, which have evolved to functional genomics (proteomics) in recent years [4]. Extensive studies to explore molecular mechanisms involved in pancreatic cancer oncogenesis, cancer stem cells, cell proliferation control and metastasis supplied valuable information and directed the treatment strategies towards targeted therapies. In targeted therapies, tumor-associated or tumor-specific proteins were targeted and their roles in corresponding signal transduction pathways were investigated [5]. Targeted therapies could be accomplished by using specific monoclonal antibodies (mAbs) against these proteins or by pathway-specific small-molecule inhibitors [5]. In this review, we focus on the application of mAbs in human pancreatic cancer treatment.

Ideally, the cellular targets in the targeted therapies should be expressed in high concentration on the surface of tumor cells. They might be required for tumor cell survival or play an

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important role in maintaining tumor cell proliferation. The targets should not be shed/secreted from the cell surface and their concentration or function should not be modulated after binding to the administered antibodies [4,6,7]. The targets are generally classified into three major categories: cell surface proteins; antigens associated with the tumor stroma; and antigens on tumor-associated vasculature and angiogenic ligands [8].

Monoclonal antibodies against human tumor targets were initially raised in rodents, most of them from mice. However, the immunogenicity of the mouse antibodies induced immunologic responses from patients, which produced human antimouse immunoglobulin antibodies. This immune response exacerbates after multiple administrations of mouse antibodies, which subsequently induces their rapid clearance from the bloodstream. Recombinant DNA technology was used to overcome such immune responses against mouse antibodies. Chimeric antibodies (consisting of variable regions from mouse antibodies and constant regions of human immunoglobulin), antibody fragments or intact fully human antibodies were produced and tested clinically [8]. Immunoconjugation with chemotherapy agents, radioisotopes or toxins directly to targeted antibodies enhanced their efficacy [9,10]. Recently, recombinant techniques were used to generate multivalent antibodies to optimize the targeting and to create bispecific antibody molecules [11].

Many antibodies binding different target molecules have been tested in clinical trials to treat pancreatic cancer (Table 1) [201]. In most of the clinical trials, antibodies were combined with other chemotherapy agents, small molecule signal transduction inhibitors, or radiation. In some studies, radioisotopes were conjugated with antibodies before administration (Table 2).

Target molecules on the cell surface

EGF receptor

The EGF receptor (EGFR) belongs to a large family of cell surface receptors, which comprise four isotypes EGFR/ErbB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. Ligands for these receptors include EGF, TGF- α , heparin-binding EGF-like growth factor and neuregulins. EGFRs are dimerized upon binding by ligands and induce activation of Ras-MAPK and PLC- γ pathways. The EGFR signal transduction pathway is essential for tissue and organ development, and regulation of cell migration, adhesion and proliferation. In many cancers, including pancreatic cancer, activated EGFR has been demonstrated to be related with many pathophysiology mechanisms, including oncogenesis, angiogenesis, apoptosis inhibition and tumor metastasis.

Cetuximab is a chimeric mouse-human antibody against an epitope located in the extra-cellular part of EGFR. Preclinical studies revealed cetuximab could decrease cell proliferation and phosphorylation of EGFR, and blocked the binding of the adaptor protein Grb2 to EGFR upon activation by EGF [12]. Clinically, cetuximab was administered to patients at an initial dose of 400mg/m² body area followed by weekly doses of 250 mg/m² [13]. Synergistic effects were observed using combination therapy of cetuximab and chemotherapy agents. In a multicenter Phase II trial, patients were treated with cetuximab and gemcitabine. The results showed the median survival duration was 7.1 months [14]. In a Phase II trial, patients with pancreatic adenocarcinoma were treated with irinotecan/docetaxel with or without cetuximab. The results showed median overall survival of 7.4 months in the group treated with irinotecan/docetaxel and cetuximab, compared with 6.5 months in the group treated with irinotecan or docetaxel only [15]. Recently, Kullmann *et al.* reported the results of combination treatment with cetuximab and gemcitabine/oxaliplatin from a multicenter Phase II study. The overall response rate from 34 evaluable patients was 38% (with one complete and 12 partial remissions). There were 24% of patients with stable disease and 38% with progressive disease (Box 1). Median time to progression was 155 days with a preliminary 6-month survival estimate of 54% (95%

CI: 37–78%) [16]. In one preclinical study reported recently, the combination of cetuximab together with gemcitabine and radiation effectively prolonged the tumor xenograft volume doubling time (30.1 ± 3.3 days), compared with gemcitabine monotherapy (11.6 ± 3.1 days), radiation monotherapy (16.7 ± 3.1 days), cetuximab with gemcitabine (20.1 ± 3.1 days) or cetuximab with radiation (22.5 ± 3.3 days) [17]. However, cetuximab failed to show a synergistic effect in combination with gemcitabine/cisplatin treatment in a Phase II trial. No significant differences were found in objective response rate (17.5% in cetuximab group, 12.2% in noncetuximab group), median progression-free survival (3.4 months in cetuximab group, 4.2 months in noncetuximab group) and median overall survival (7.5 months in cetuximab group, 7.8 months in noncetuximab group) [13]. Similarly, a Phase III study of gemcitabine plus cetuximab versus gemcitabine alone showed median survival of 6.5 months and progression-free survival of 3.5 months in the combination treatment group, versus 6 months and 3 months in the gemcitabine and cetuximab monotherapy groups, respectively [18]. Whether the sensitivity to cetuximab is related to the level of EGFR expression is uncertain. Affinity of expressed EGFR to cetuximab may be an important parameter to consider. Other factors have been reported to be related to cetuximab sensitivity, such as mutation of KRAS, PTEN expression, or host complement level [19–21]. Mutation of EGFR was a topic many investigators focused on regarding the relationship to sensitivity to cetuximab. A recent review summarized that in pancreatic cancer, the EGFR tyrosine kinase domain is highly conserved and EGFR mutation may not be predictive of sensitivities to EGFR tyrosine kinase inhibitors [22].

Box 1

National Cancer Institute response evaluation criteria in solid tumors

- Complete response: disappearance of all target lesions
- Partial response: 30% decrease in the sum of the longest diameter of target lesions
- Progressive disease: 20% increase in the sum of the longest diameter of target lesions
- Stable disease: small changes that do not meet above criteria

Other monoclonal antibodies against EGFR

Panitumumab: Panitumumab is a fully human IgG₂ antibody against EGFR. It blocked the binding of EGF and TGF- α to EGFR and subsequent phosphorylation of EGFR tyrosine kinase. Clinical efficacy of panitumumab to treat pancreatic cancer is under investigation in various clinical trials [23].

Matuzumab (EMD 72000): This is a humanized IgG₁ mAb against EGFR. Phase I studies showed this antibody significantly inhibited EGFR downstream signal transduction and was well tolerated by patients. In a Phase I clinical trial, matuzumab was given to pancreatic cancer patients at a dose of 400–800 mg once weekly for 8 weeks, followed by gemcitabine 1000 mg/m² weekly for two cycles (3 weeks of treatment with 1 week rest in one cycle). The partial response or stable disease in 12 evaluated advanced pancreatic cancer patients was 66.7% [24]. The partial response or stable disease in 22 evaluated advanced solid tumors was 50% in another study [25]. In an early report by Burris *et al.* in 1997, gemcitabine was administered as a monotherapy at a similar dose for up to two cycles. Of 56 patients, 44% had partial response or stable disease [26].

Nimotuzumab: Another humanized antibody against EGFR, nimotuzumab, is currently in a clinical trial to treat pancreatic cancer [201]. In a preclinical study using non-small-cell lung cancer (NSCLC), this antibody enhanced antitumor efficacy of radiation [27].

CH 806: A chimeric mAb against mutant EGFR, CH 806, has been tested in patients with solid tumors [28]. The advantage of using this antibody is its very low normal tissue distribution. Currently, there is no information available for this antibody in pancreatic cancer treatment.

ErbB2/HER2

One isotype of the EGFR family, ErbB2/HER2, has been found to be overexpressed in many tumors, including pancreatic cancer. A recent report using improved techniques showed about 42% HER2-positive staining and 16% *HER2* gene amplification in pancreatic cancer tissue samples [29]. Preclinical studies using the humanized mAb trastuzumab (Herceptin®) showed significant growth inhibition of a pancreatic cancer cell line and xenografts established with that cell line. Additive effects were observed when trastuzumab was combined with fluoropyrimidine S-1 both *in vitro* and *in vivo* (fluoropyrimidine S-1 is an oral preparation of 5-fluorouracil [5-FU] that consists of tegafur [a prodrug of 5-FU], 5-chloro-2,4-dihydropyridine and potassium oxonate, where the latter two can inhibit degradation of 5-FU and reduce gastrointestinal toxicity). In addition to the inhibition of the HER2 signal transduction pathway, antibody-dependent cellular cytotoxicity (ADCC) induced by trastuzumab contributed to cell growth inhibition [30]. Many effector cells in the immune system express IgG Fc receptors, which may activate the effector cells upon binding with specific antibodies. Activated effector cells may possess the ability of cytolytic, endocytic or phagocytic functions. This function of Fc receptors plays a key role in immune defense and immune surveillance. Kimura *et al.* tested cytotoxicity of trastuzumab in four pancreatic cancer cell lines and reported no inhibitory effect and no synergistic or additive effect of this antibody when combined with gemcitabine [31]. However, ADCC was observed in three of four cell lines and was proportional to HER2 expression [31]. Trastuzumab administration significantly inhibited tumor growth in Capan-1 xenografted mice and prolonged survival. This inhibitory effect was enhanced by the addition of gemcitabine [31]. Dual inhibition of EGFR and HER2 has been reported. Larbouret *et al.* showed that combination of matuzumab (anti-EGFR) and trastuzumab enhanced the inhibitory effect on HER2 phosphorylation. Combination treatment with both antibodies significantly decreased xenograft tumor sizes or induced more complete remissions as compared with either antibody alone, and prolonged survival in mice inoculated with BxPC-3 or MIA PaCa-2 pancreatic cancer cells [32]. Combination treatment with trastuzumab and other tumor inhibitory agents, such as the histone deacetylase inhibitor vorinostat, the farnesyltransferase inhibitor tipifarnib or IL-12, are being tested in clinical trials [33–35].

Pertuzumab, another antibody against HER2, is a recombinant humanized mAb. The binding site of this antibody on HER2 is different from that of trastuzumab. The binding of this antibody to HER2 blocked the receptor dimerization and subsequent signaling [36]. In a group of 21 incurable, locally advanced, recurrent or metastatic solid tumors patients, including two pancreatic cancer patients, pertuzumab was administered (0.5–15 mg/kg) every 3 weeks. Two of the patients (one pancreatic cancer patient with stable disease 15.3 months) showed partial responses. Histochemistry did not show overexpression of HER2 and *HER2* gene amplification. Stable disease of greater than 2.5-month duration was observed in six patients (29%). Three patients with prostate cancer had stable disease for 2.6, 2.7 and 5.5 months. Three others who had lung cancer, colorectal cancer and ovarian cancer had stable disease for 4.1, 2.8 and 4.0 months, respectively [37].

Death receptor 5

Death receptor (DR)5 is a transmembrane protein which belongs to the TNF receptor (TNFR) superfamily. It contains an extracellular domain and a cytosolic death domain. Upon binding by its ligand, TNF-related apoptosis-inducing ligand (TRAIL), the receptor forms a homotrimer which induces the formation of a death-inducing signaling complex (DISC). The trimerization of the receptor death domain recruits adaptor molecules, including Fas-associated death domain (FADD) protein in the DISC, which subsequently recruits and activates caspase 8-triggered apoptosis (Figure 1) [38–41]. Apoptosis is also induced by activation of the intrinsic mitochondrial pathway [38,42]. The intrinsic pathway is mediated by caspase 8 cleavage of the protein Bcl-2 homology domain 3 (BH3)-interfering domain death agonist (Bid). Cleaved Bid triggers mitochondrial cytochrome C release through the proapoptotic protein Bax and Bak [38,42]. Although DR5 is present in a variety of normal tissues as well as tumors, normal cells are in general resistant to TRAIL induced apoptosis. By contrast, tumor cell lines of diverse origins are sensitive to TRAIL [43].

CS-1008 is a humanized antihuman DR5 receptor antibody derived from the mouse antihuman DR5 antibody TRA-8 [44]. TRA-8 (IgG₁κ) binds to the extracellular domain of human DR5 and competes with TRAIL for binding to DR5. TRA-8 has been shown to inhibit the proliferation of a variety of tumor cell lines and induce apoptosis both *in vitro* and *in vivo* [45,46]. We tested five human pancreatic cancer cell lines and found they had different levels of DR5 expression and different sensitivities to TRA-8. The sensitivities to TRA-8 did not correlate with the expression levels of DR5 [47]. Preclinical studies using an orthotopic pancreatic cancer mouse model (MIA PaCa-2) showed synergistic effects when gemcitabine was used together with TRA-8 [47]. In a subcutaneous mouse model (MIA PaCa-2), gemcitabine plus TRA-8 prolonged tumor-size doubling time from untreated (38 days), monotherapy with TRA-8 (49 days), or with gemcitabine (32 days) to 64 days [48]. Preclinical studies in this orthotopic mouse tumor model (MIA PaCa-2) also showed 3 weeks of treatment with TRA-8 (200 μg twice a week) and irinotecan (25 mg/kg twice a week) prolonged survival time. Mean survival was 70 ± 6.2 days in untreated mice, 84.3 ± 3.8 days in mice treated with CPT-11 alone, 90.4 ± 8.2 days in mice treated with TRA-8 and 120.6 ± 6.6 days in those receiving combination therapy. A 6-week treatment prolonged survival time by 169 days [49].

Another human antibody against DR5 (AMG 655) was developed recently by Amgen, Inc. (CA, USA), and is being evaluated in clinical trials. AMG 655 induced activation of caspase 3/7 in MIA PaCa-2 cells 3 h after treatment and decreased cell viability at 18 h. In the MIA PaCa-2 xenograft mouse model, AMG 655 significantly inhibited tumor growth at doses of 3 μg or greater biweekly. This effect was enhanced by combination treatment with gemcitabine [50].

Adams *et al.* reported on a human single chain antibody against DR5 (Apomab®). This antibody bound specifically to DR5 and had an overlapping DR5 binding site with the ligand TRAIL. It caused DR5 internalization and association of FADD and caspase 8. In a mouse model injected with MIA PaCa-2 cells, tumor sizes were followed for 32 days after a single injection of Apomab (10 mg/kg). Complete remission was observed in ten of ten mice. On day 32, nine of the ten mice remained tumor free. In mice bearing BxPC-3 pancreatic cancer xenografts, a single treatment of Apomab induced a partial remission (reduction of tumor volume between 50–100% of its original volume) in five of ten mice. Combination of this antibody with gemcitabine (160 mg/kg, once every 3 days for four-times) caused partial remission in seven and a complete remission in one of ten mice [51]. Data from preclinical studies using TRA-8 and Apomab provide encouraging results in xenograft models of pancreatic cancer treatment. However, the pharmacologic information and toxicity of these products, especially liver

damage assessment, and whether these products produce similar effects in patients need to be evaluated by clinical trials.

Other anti-DR5 antibodies including lexatumumab, HGS-ETR2, HW1 and WD1 have been reported and tested in preclinical or clinical trials [52–55]. No information is available in regard to pancreatic cancer treatment using these antibodies.

Insulin-like growth factor-1 receptor

Insulin-like growth factor-1 receptor (IGF-1R) is a transmembrane receptor tyrosine kinase and has been reported to be overexpressed in many types of tumors, including human pancreatic cancer. Activation of IGF-1R has been shown to enhance cell growth and inhibit apoptosis. In a majority of pancreatic cancers (64%), IGF-1R expression was accompanied by expression of c-Src [56] and its antiapoptotic effect was mediated by activation of MAPK and phosphatidylinositol 3-kinase (PI3K). Neid *et al.* reported that IGF-1R signaling played an important role in pancreatic cancer angiogenesis [57]. IGF-1R physically interacts with focal adhesion kinase (FAK) leading to proliferation and cell survival. Blocking this interaction can inhibit cell viability and increase apoptosis [58].

IMC-A12, a human IgG₁ antibody (ImClone Systems Incorporated, Branchburg, NJ, USA) was tested in preclinical studies [59] and is now under clinical investigation. Blockage of activation of PI3K/AKT, p38 pathway via IGF-1R signaling may be the main mechanism for its antitumor activity. In mice bearing BxPC-3 tumors, IMC-A12 used as a single agent at 1 mg/kg every 3 days inhibited xenograft growth by 80%. A Phase I clinical trial showed two patients among 11 with refractory solid tumors had stable disease after treatment with 3 mg/kg of IMC-A12. One of these two patients had stable disease for 9 months and circulating cancer markers decreased in another patient [59].

AMG 479, a human anti-IGF-1R antibody is now under investigation in a clinical trial to treat pancreatic cancer and has been tested against Ewing's sarcoma xenografts in mice [60]. Two other antibodies against human IGF-1R, CP-751,871 and h7C10 were tested in preclinical studies of other tumors [61,62].

A chimeric antibody against an *N*-linked carbohydrate antigen (RAAG12), RAV12, is now in a clinical trial in pancreatic cancer patients. RAAG12 was found to selectively coexist with many membrane proteins, including IGF-1R, ALCAM, IFN- γ R1, TfR, IR, EphA2 and EGFR [63]. RAV12 induced enhanced IGF-1 mediated IGF-1R phosphorylation followed by quick desensitization, as well as phosphorylation of downstream signaling components IRS1, Erk/MAPK1/2, Akt/PKB and p70s6K [63]. Cell growth was inhibited by RAV12 only and additive or synergistic effects from MAPK inhibitors were observed *in vitro*. In a colon cancer xenograft model, RAV12 showed a dose-dependent inhibition of tumor growth. Tumor sizes were inhibited by 60% at 1 mg/kg intraperitoneally, three-times per week for 2 weeks. The inhibition reached 92–100% at a dose 25 mg/kg or 50 mg/kg [64]. Tumors derived from cells expressing less RAAG12, such as A549 NSCLC cells, had no responses to RAV12 treatment. Modulation of IGF-1R-activity by RAV12 is considered to be the major mechanism of this antibody [63].

Mesothelin

Mesothelin is a 40-kDa glycosyl phosphatidylinositol anchored cell surface protein. It is a cleaved product from a precursor protein encoded by the Mesothelin gene (*MSLN*). Another part of the cleaved product is a soluble 31-kDa megakaryocyte potentiating factor [65]. The physiologic roles of these proteins have not been identified completely. Normally, expression of mesothelin is only seen in mesothelial cells lining peritoneal, pleural and pericardial cavities.

Overexpression of mesothelin protein as well as *MSLN* gene were found in about one third of cancers, including ovarian, pancreatic, biliary and gastroesophageal cancers [65]. One study showed that in pancreaticobiliary adenocarcinomas, mesothelin was expressed in 100% of pancreatic adenocarcinomas and bile duct adenocarcinomas, and in 89% of ampullar adenocarcinoma, but not in normal pancreas and chronic pancreatitis [66]. Overexpression of *MSLN* is regulated by transcription enhancer factor (TEF)-1 and its cofactor [65]. Due to its unique tissue distribution pattern in cancers and normal tissues, mesothelin has become an important target in cancer diagnosis and treatment.

Single-chain Fv against mesothelin and immunotoxin SS1(dsFv) PE38 (SS1P)

—Single-chain Fv (scFv) against mesothelin was isolated from a phage display library obtained from the spleen of mice immunized with mesothelin-expression plasmid. The scFv was then fused genetically with a truncated mutant of *Pseudomonas* exotoxin A to obtain recombinant immunotoxin. In one preclinical study using SS1P plus radiation in treating mesothelin-expressing tumor xenografts, combination treatment significantly prolonged the doubling time of tumors [67]. Similarly, synergy was observed when gemcitabine was used together with SS1P and this treatment induced complete regression of xenografts [68]. In a Phase I clinical study, SS1P was found to be well tolerated. SS1P was administered by intravenous infusion in 34 patients with mesothelin-expressing tumors, including two pancreatic cancer patients. The dose was 8–28 µg/kg in the groups receiving the drug every other day for six doses followed by 25–60 µg/kg every other day for three doses. Of the patients tested, 12% showed minor responses (tumor size decreased between 20–50% and lasted for more than 4 weeks). In addition, 56% of the patients showed stable disease and 29% of the patients had progressive disease [69].

MORAb-009—This antibody is a chimeric of a mouse and human mAb derived from a phage-display library as described above and re-engineered [70]. In a preclinical mesothelin expressing xenograft mouse model, monotherapy using antibody alone moderately inhibited tumor growth. Combination therapy with gemcitabine significantly enhanced the effects of chemotherapy. Blockage of binding between MUC16 and tumor cells by this antibody may be a possible mechanism for this synergistic effect [70].

Carcinoembryonic antigen

Carcinoembryonic antigen (CEA) has been recognized as an overexpressed cell surface antigen in many cancers, mainly in gastrointestinal tract malignancy. The pathophysiological role in cancer growth is not well defined. It was reported to possess antiapoptotic function and may be involved in cancer invasion, adhesion and metastasis [71]. A humanized anti-CEA antibody MN-14 (labetuzumab) is now under clinical trial to treat pancreatic cancer together with filgrastim (G-CSF). It has been demonstrated that MN-14 increased median survival of lung cancer xenograft mice by 10.7–42.7 weeks when combined with G-CSF, indicating an ADCC mechanism [72]. Enhanced antitumor effect was observed when MN-14 was combined with 5-FU or CPT-11 [72]. MN-14 was labeled with ¹³¹I for radioimmunotherapy (RIT) and was combined with gemcitabine in a preclinical colon cancer xenograft study [73]. BALB/c mice bearing peritoneal tumors from LS174T cells were treated with gemcitabine (0.11 or 0.33 mg/mouse × 4), RIT monotherapy (20 µg/mouse × 1) or RIT plus gemcitabine. Median survival was 39 days in untreated mice, 52 and 57 days in gemcitabine monotherapy groups, 66 days in RIT monotherapy group, 73 days (0.11 mg/mouse of gemcitabine) and 94 days (0.33 mg/mouse) for RIT combined with gemcitabine. Another humanized anti-CEA antibody, hPRIA3, has been shown to induce tumor cells lysis *in vitro*, mainly via ADCC [74].

Epithelial cell adhesion molecule

Epithelial cell adhesion molecule (EpCAM) is a 40-kDa transmembrane glycoprotein normally expressed on the basolateral surface of a majority of epithelial cells. The function of this protein is not fully understood and was considered as a Ca^{2+} -independent homophilic intercellular adhesion molecule. EpCAM was found to be overexpressed in many types of cancers. Human antibodies against EpCAM have been tested in preclinical as well as clinical trials and were shown to produce antitumor activity mediated via ADCC [75–77]. EpCAM overexpression was observed in 56% of pancreatic cancers [78]. No information is available regarding clinical trials using antibodies against EpCAM in pancreatic cancer patients.

MUC1

MUC1 is a heavily glycosylated type I membrane protein with several extracellular tandem repeat domains. These domains undergo a proteolytic cleavage from the full length of MUC1 between amino acid 1097 and 1098 after translation. However, they remain associated with the transmembrane region and cytoplasmic tail and are transported to the cell surface. The dissociation of these domains from MUC1 may be achieved by mechanical stress and may be related to the protection of the apical cell membrane of the epithelial cells from rupture [79]. MUC1 is expressed by nearly all human glandular epithelial tissues and throughout all regions of the gastrointestinal tract and its expression is largely limited to the apical membrane of the cells [80]. MUC1 expression is upregulated in almost all human adenocarcinomas with an expression pattern over the entire cell surface [80–82]. The expression of MUC1 in cancer cells was regulated by DNA methylation and histone H3 lysine 9 modification of the MUC1 promoter. Inhibition of DNA methylation enhanced *MUC1* gene expression [83]. MUC1 serves as a counter-receptor for myelin-associated glycoprotein in pancreatic cancer and their interaction is considered to be related to pancreatic cancer perineural invasion [84]. The intact tandem repeats and cytoplasmic tail of MUC1 may play roles in preventing tumor invasion and metastasis [85]. Recently, Ahmad *et al.* reported that MUC1 was recruited to the TNF-R1 complex upon stimulation by TNF- α and interacted with the I κ B kinase complex resulting in phosphorylation and degradation of I κ B α , which favored cell survival [86]. MUC1 was found to block death receptor-mediated apoptosis by binding to caspase 8 and FADD [87]. Downregulation of MUC1 by RNA interference upregulated β -catenin and E-cadherin expression, promoting E-cadherin/catenin complex formation which decreased *in vitro* cell invasion [88].

PAM4 is a murine antibody obtained from mice immunized with purified mucin from a human pancreatic cancer xenograft sample. The binding of this antibody to MUC1 is conformation dependent and its epitope may contain carbohydrate. The humanized form, HuPAM4, has been radiolabeled with ^{90}Y and is now under investigation in a clinical trial of pancreatic cancer patients. In a Phase I study, 15 patients with advanced pancreatic cancer received ^{90}Y -HuPAM4 at 15–25 mCi/m ^{2} . At 4 weeks after treatment, three patients showed 32–51% tumor shrinkage, and three patients had stable disease. The major toxicity was hematologic at 25 mCi/m ^{2} [89]. Recently, a humanized bispecific mAb – TF10 – was reported that is bivalent for mAb PAM4 and monovalent for mAb 679. mAb 679 is reactive against the histamine-succinyl-glycine hapten (HSG-hapten peptide). Pretargeting TF10 in the CaPan1 human pancreatic cancer xenograft model followed by ^{111}In -labeled peptide IMP-288 (containing HSG groups) showed high ^{111}In -IMP-288 uptake in tumors [90].

CC49 is a mouse mAb against a tumor-associated antigen TAG-72. TAG-72 is a cell surface glycoprotein and has characteristics of a mucin. It is expressed in many types of malignancies, including pancreas, colon, ovary, prostate, lung and esophagus, while absent in normal tissues. The antibody has been conjugated to radioisotopes for diagnosis and treatment purposes. A sFv of CC49 was developed and was shown to localize in tumors. Humanized CC49 was

engineered to decrease its immunogenicity and increase the retention time in circulation after it was labeled with radioisotope. In a recent report by Baranowska-Kortylewicz *et al.*, ¹³¹I-CC49 significantly prolonged pancreatic cancer xenograft quadrupling time and this effect was enhanced by administration of a tyrosine kinase inhibitor, imatinib [91]. The therapeutic effect of imatinib was considered to be related to inhibition of phosphorylation of platelet-derived growth factor receptor (PDGFR), a reduction in the tumor interstitial fluid pressure, and an increase in the absorbed radiation dose in tumor by 60%.

A humanized mAb HuC242 against CanAg (a glycoform of MUC1) was conjugated with an antimicrotubule agent DM1 (N²-deacetyl-N²-[3-mercapto-1-oxopropyl]-maytansine; cantuzumab mertansine) and tested in a Phase I clinical trial of 37 patients with solid tumors [92]. The results showed two patients with chemotherapy resistance had minor regression and four patients had stable disease during treatment. Conjugation of huC242 to another maytansinoid derivative (huC242-DM4) is now in a clinical trial of pancreatic cancer treatment.

Target molecules in tumor stroma & vasculature

VEGF

The VEGF family consists of VEGF-A, -B, -C, -D and placenta growth factor (PLGF). The receptors for the VEGF family are VEGFR-1 (Flt-1), VEGFR-2 (KDR) and VEGFR-3. The VEGF (usually referred to as VEGF-A) family as well as its receptors have been studied intensively and have been found to be very important molecules in tumor angiogenesis [93]. VEGF was found to be expressed in 93% of ductal pancreatic adenocarcinoma samples and was correlated with liver metastasis and prognosis. VEGF was not only found to be expressed in tumor cells but also in endothelial cells. It was also detected in bone marrow derived cells or mesenchymal stem cells [94,95]. Expression of VEGF-C and -D in pancreatic adenocarcinoma was found to be correlated with lymph node metastasis, lymphatic invasion and venous invasion [96]. Dineen *et al.* reported recently that tumor-associated macrophage infiltration was mediated through VEGF-VEGFR-2 [97]. Blocking the VEGF signals is an important antiangiogenesis approach.

Bevacizumab is a humanized murine anti-human VEGF-A mAb and has been used in the treatment of colon and breast cancer, among others, together with different chemotherapy agents [95]. A Phase I study of bevacizumab plus radiation and capecitabine in pancreatic cancer patients showed some side effect of gastrointestinal bleeding. Approximately 20% of the assessable patients had partial responses. Overall median survival was 11.6 months [98]. A Phase II trial with bevacizumab plus gemcitabine in 52 patients showed 21% of patients had partial responses and 46% had stable disease. Median survival was 8.8 months [99]. Ko *et al.* reported the results of a Phase II study with bevacizumab together with fixed-dose rate gemcitabine and low-dose cisplatin. Approximately 19.2% of patients had unconfirmed response, and 57.7% had stable disease with a median survival of 8.2 months [100]. In the 2007 American Society of Clinical Oncology's Gastrointestinal Cancer Symposium, Kindler *et al.* reported the CALGB study results using bevacizumab and gemcitabine from 602 patients with advanced pancreatic cancer. Combination therapy with bevacizumab plus gemcitabine did not show any beneficial effect compared with gemcitabine alone [101]. It seems that bevacizumab did not improve the response to chemotherapeutic agents in pancreatic cancer treatment [101]. Only a small portion of patients had experienced benefit from anti-VEGF therapy. Attempts to identify markers that may predict the response to bevacizumab, including plasma angiogenic factors, baseline plasma VEGF levels, as well as circulating tumor cells before and after bevacizumab treatment, were unsuccessful [100].

Integrin $\alpha_5\beta_1$

Integrins are heterodimeric receptors. Binding of integrins to their ligands can induce morphological, motility and growth rate changes in endothelial cells. Ligation of integrin $\alpha_5\beta_1$ promotes endothelial cell survival, migration and proliferation rates, thus inducing angiogenesis [102]. A chimeric mAb – volociximab – has been demonstrated to inhibit tumor growth in a preclinical study [103]. A Phase II clinical trial using this antibody together with gemcitabine in 20 pancreatic cancer patients (volociximab 10 mg/kg every 2 weeks plus gemcitabine 1000 mg/m² on days 1, 8 and 15 of a 28-day cycle) showed one patient (5%) with partial response, and ten patients (50%) with stable disease, with an overall survival of 34% at 1 year. In another group of 20 patients who received volociximab 15 mg/kg/week on days 1, 8, 15 and 22 with gemcitabine on days 1, 8 and 15, preliminary results showed two patients with partial response (10%), seven patients with stable disease (35%), and overall survival was 4.8 months. It seems too early to evaluate the objective response of this mAb. Overall survival did not benefit from this regimen. Most frequent toxicities observed were gastrointestinal with nausea (75%), constipation (60%), diarrhea (55%) and abdominal pain, (50%). Four patients were found to have pulmonary embolism [104].

Other target molecules studied in pancreatic cancer treatment are connective tissue growth factor (CTGF), extradomain (ED)-B of fibronectin and prostate-specific membrane antigen. A human IgG₁ mAb against CTGF, FG-3019, inhibited orthotopic pancreatic tumor growth in nude mice, enhanced apoptosis and decreased lymph node metastasis of tumor cells [105, 106]. Combination of FG-3019 with gemcitabine had an additional effect in decreasing tumor growth [105]. Wagner *et al.* recently reported that IL-2-conjugated human scFv (L19) antibody against ED-B in an orthotopic pancreatic cancer mouse model inhibited tumor growth in a dose-dependent manner [107]. Tumor volumes were reduced up to 2.7% of their original size and lymph node metastasis was diminished [107]. An antibody J591 against the extracellular domain of prostate-specific membrane antigen (BZL Biologics Inc., Framingham, MA, USA) was conjugated to ¹¹¹In and tested in a clinical trial of 27 patients with advanced solid tumors, including three pancreatic cancer patients [108]. The results showed the conjugated antibody successfully targeted the vascular endothelium of metastatic sites. No objective regression was observed. Three of 27 patients had stable disease [108].

Other target molecules

TNF- α

TNF- α is a cytokine that induces an inflammatory process, and has been shown to be elevated in pancreatic cancer samples. Overexpressed TNF- α either from endogenous cancer cells or exogenous sources (from immune or stromal cells) is recognized to play a role in pancreatic cancer development and progression [109]. It is also considered to be related with surgery-associated tumor recurrence and metastasis [109]. The chimeric monoclonal IgG₁ κ antibody against human TNF- α , infliximab, has been tested in a preclinical orthotopic xenograft model of human pancreatic cancer and is now in a clinical trial in pancreatic cancer patients [110, 111]. In mice bearing xenografts, tumor volumes were inhibited with infliximab treatment by 20–30% [109]. In a Phase II clinical trial to evaluate the safety and efficacy of infliximab given together with gemcitabine to treat cancer cachexia, lean body mass was measured in the groups treated with gemcitabine plus placebo, gemcitabine plus 3mg/kg of infliximab or gemcitabine plus 5 mg/kg of the antibody. The differences among these three groups were not statistically significant. Even the infliximab (5 mg/kg, together with gemcitabine) treated group showed a higher lean body mass at 1.7 kg compared with 0.4 kg in a placebo group and 0.3 kg in a group that received 3 mg/kg of antibody [111].

Cytotoxic T-lymphocyte antigen-4

Tumor antigens can induce host immunosurveillance and initiate activation of cytotoxic T lymphocytes through the reaction between tumor antigens presented by antigen-presenting cells (APCs) with the T-cell receptor. The further activation of T cells requires binding of CD28 of T cells with CD80 (B7-1) or CD86 (B7-2) from APCs. Cytotoxic T-lymphocyte antigen (CTLA)-4, a homolog of CD28, can bind to CD80 or CD86 with a much higher affinity than CD28. This binding prevents further activation of T cells and reduces the T-cell population to a small pool of memory cells [112]. The ability of host immunosurveillance is thus compromised (Figures 2A & B). Besides CTLA-4, a similar molecule programmed death (PD)-1 was discovered, which binds to B7-H1 to prevent activation of T cells [113]. *CTLA-4* polymorphism has been detected in many diseases. Expression of CTLA-4 and PD-1 was found in tumor-infiltrating immune cells in human pancreatic cancers [113]. Thus, CTLA-4 or PD-1 blockade with antibodies may serve as an important regimen for cancer treatment.

Ipilimumab and tremelimumab are two human anti-CTLA-4 mAbs that have been tested in clinical trials in patients with melanoma [114–116]. The treatment included monotherapy of antibody or combination therapy with chemotherapy agents, vaccines or cytokines. Immune-related adverse events (IRAEs) such as enterocolitis, dermatitis or hepatitis were reported. Ipilimumab is currently in a clinical trial of pancreatic cancer.

In addition to blockade of CTLA-4 to optimize the activation of T cells, efforts have been made to directly activate T cells by stimulating a member of the TNF superfamily, TNFRSF9 (4-1BB). Ligation of this receptor by either its ligand (TNFSF9 or 4-1BBL) or agonistic antibodies has been shown to provide a potent signal in activation of T cells [117]. It is thus proposed that combination treatment with antibodies against CTLA-4 and 4-1BB may result in an enhanced immunomodulation effect in cancer treatment [117].

Monoclonal antibodies conjugated with protein toxins in pancreatic cancer therapy

Monoclonal antibodies have been conjugated to radioisotopes, chemotherapy drugs or ribonucleases and used in cancer treatment. mAbs conjugated to modified protein toxins, such as pseudomonas exotoxin and diphtheria toxin, have been used in solid tumor treatment after successful application in hematologic malignancy therapy. These products possess specific tumor tissue binding from the specific target antibodies, as well as cytotoxic activities. A mAb against the carbohydrate moiety Lewis Y was conjugated with *Pseudomonas aeruginosa* exotoxin A (LMB-9) and is in Phase I clinical trials for pancreatic, esophageal, stomach, colon and rectal cancer. The patients received LMB-9 4–12 µg/kg/day for 10 days. Allergic skin reaction, reversible renal function damage and proteinuria were reported. Most patients developed antitoxin antibodies [118]. No objective responses were reported. Antibody or antibody fragment against EpCAM has been conjugated with a molecularly engineered, truncated *Pseudomonas exotoxin A* (ETA 252–608). The internal furin site of the toxin was replaced by a cleavage site that can be cleaved by cancer-associated proteases, gelatinase A and B (MMP-2 and MMP-9) to increase the cancer tissue specificity [119]. Modified *P. aeruginosa* exotoxin A was also conjugated to a single chain variable fragment against human EGFR. Preclinical studies in disseminated human pancreatic cancer mouse models showed that treatment with this immunotoxin reduced the average number of lung metastases from 56.25 per mouse to 0.875 per mouse in single injection group and 0.286 per mouse in a multiple injection group [120]. Although the preclinical studies have showed encouraging results, this regimen still faces several obstacles to be used effectively. Immunogenicity from the toxin, unwanted toxicity, limited half-life and subsequently developed resistance need to be solved for the future application of immunotoxins clinically [121].

Conclusion

Immunotherapy using mAbs to treat human pancreatic adenocarcinoma has shown promising results in preclinical studies as well as in some clinical trials. A variety of antibodies have been developed to block the cancer cell growth or promote apoptosis. Targeted therapy with mAbs has established a new strategy in cancer treatment and has the potential to increase the tumor tissue specificity of chemotherapy as well as radiation therapy. Although monotherapy with mAbs alone could achieve significant effects, combination therapy together with classical chemotherapy agents or radiation has been proven more potent and is highly recommended. However, the clinical efficacy of mAb products available so far in human pancreatic cancer therapy trials is not satisfactory. Despite in-depth mechanistic studies *in vitro* and excellent efficacy of treatment in xenograft models, mixed results were obtained in clinical trials. Preclinical efficacy in pancreatic cancer therapy studies apparently were not translated perfectly to clinical application as expected. The mechanisms responsible for the discrepancy between clinical trials and preclinical studies are unknown. Sufficient drug delivery to tumor sites may be a critical factor that needs to be considered. Fast growth of tumor cells causes reduced vascular density and increases the space between vessels and cells. This may be more critical for protein products with large molecules, such as mAbs. A Phase III trial with the EGFR tyrosine kinase inhibitor erlotinib plus gemcitabine achieved a better overall survival rate than cetuximab plus gemcitabine [122]. Whether the small molecular inhibitor of EGFR signal transduction could be more efficiently delivered to tumor sites than cetuximab needs to be further studied. Some ligands of receptors, such as TNF- α or TRAIL, can induce dual effects in apoptosis after binding to receptors. An antiapoptotic process can be activated through the NF- κ B pathway at the same time as activation of apoptosis. The fate of the cells is dependent on the net effects of pro- and anti-apoptotic balance. The net effects of mAbs on tumor cells *in vivo* needs more investigation. Orthotopic xenograft models or molecular analysis from fine needle aspiration samples of patients may supply valuable information in future clinical trials. It will be too naive to consider that targeted therapy would eliminate all cancer cells from the body. Cancer is not a mass of autonomous malignant cells but is more like an 'organ' with its repertoire of cellular matrix, vasculature and immune cells, growth factors and other secreted molecules [123]. Even if an effective therapy induced massive destruction of cancer tissue, there may still remain a possible drug-resistant subpopulation, or possibly cancer stem cells that could escape the killing. Targeted therapy with mAbs must be applied in combination with not only traditional chemotherapy or radiation therapy but other regimens, including adjustment of immune surveillance and inflammation control, among others.

Future perspective

With the identification of the molecular mechanisms associated with pancreatic cancer, more target molecules which play critical roles in tumor pathophysiology will be identified. This may enable the molecular targeting techniques using mAbs to become more specific and potent. A combination therapy protocol using mAbs and conventional chemotherapy or radiotherapy will be established clinically for pancreatic cancer. The combination may also comprise more than one mAb. Further efforts to investigate target molecule expression, mutations and dynamic changes in cancer development may help to identify reliable biomarkers in order to guide the application of mAbs with other cytotoxic agents. DNA recombinant techniques to generate multivalent antibodies as well as bispecific antibodies may help to generate new targeting molecules. Advanced nanotechnology may develop more mAb preparations with different conjugates and different functions. This may simplify the treatment, decrease the systemic side effects, and enhance the efficacy. Although mAbs have great potential in cancer treatment, molecular targeted therapy with mAbs must be combined with other regimens aimed at multiple targets to improve the treatment efficacy.

Executive summary

Molecular targeting with monoclonal antibodies

- Monoclonal antibodies (mAbs) against EGF receptor (EGFR), HER2, VEGF and death receptor (DR)5 have been investigated and have produced promising results in preclinical studies. These antibodies are under extensive clinical investigation in a variety of human cancers, including pancreatic adenocarcinoma.

Combination therapy with mAbs & chemotherapy or radiotherapy

- There are reports that combination therapy with mAbs against EGFR, HER2, VEGF, DR5 and chemotherapy produces increased inhibition of tumor growth in preclinical and some clinical studies. Investigation of mAbs with different chemotherapy agents is highly recommended.

Combination therapy with mAbs & chemotherapy may not have synergistic or additive effects

- No synergistic or additive effect was found in a Phase II study with cetuximab and gemcitabine/cisplatin.
- Combination therapy of bevacizumab and gemcitabine did not show any benefit compared with monotherapy in one clinical trial.

Lack of treatment efficacy using mAbs may be related to the special clinical & molecular characteristics of pancreatic cancer patients

- Despite the promising results obtained *in vitro* or in preclinical animal model studies, clinical efficacy using mAbs in pancreatic cancer is still a challenge.
- Further research on the clinical and molecular characteristics of pancreatic cancer may help to identify biomarkers and to establish their relationship to mAbs and combination treatment. This may promote therapy with mAbs and result in a more potent and specific pancreatic cancer treatment.

DNA recombinant technology & nanotechnology are two important tools in developing more potent mAb preparations

- DNA recombinant technology to generate multivalent or bispecific antibodies and nanotechnology to conjugate mAb and cytotoxic agents with multiple functions are expected to produce more potent anticancer effects with less-systemic side effects in the future.

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Figure 1. Death receptor 5 activation pathway

Death receptor 5 is a member of the TNF-receptor superfamily. When the TRAIL (or TRA-8) binds to receptor, the receptor forms a homotrimer, which subsequently induces the death-inducing signaling complex formation. DISC includes FADD, caspase 8 and cIAP or FLIP. Apoptosis is induced by the activation of caspase 8/caspase 3 as well as activation of the intrinsic mitochondrial pathway.

FADD: Fas-associated death domain protein; TRAIL: TNF-related apoptosis-inducing ligand.

Figure 2. T-cell activation in immunosurveillance

Tumor antigens may be recognized and expressed by antigen-presenting cells (APCs). **(A)** T cells are activated by the binding of expressed tumor antigen to T-cell receptor. This activation also requires the binding of CD80 or CD86 from APCs to CD28 on T cells. **(B)** CTLA-4, a homolog of CD28 expressed on T cells, can bind to CD80 or CD86 with a higher affinity. This binding prevents the activation of T cells despite the binding of tumor antigen to T-cell receptor. Blocking the binding between CTLA-4 with CD80 or CD86 with antibodies against CTLA-4 may promote T-cell activation and enhance immunosurveillance.

CTLA: Cytotoxic T-lymphocyte antigen.

Table 1

Monoclonal antibodies investigated in Phase I clinical trials of pancreatic cancer.

Target molecule	Antibody	Drugs in combined therapy	Ref.
EGFR	Cetuximab	Bortezomib	[124]
		Erlotinib + bevacizumab	[125]
		Gemcitabine + radiation	[17,126]
ABX-EGFR	Panitumumab		[23]
HER2	Trastuzumab	Tipifarnib	[33]
		IL-12	[35]
VEGF	Bevacizumab	Sorafenib	[127,128]
RAAG12	RAV 12		[129]
MUC1	HuPAM 4	Y-90 radiolabeled	[89]
Glucoprotein with mucin properties	CC49	Radioisotope conjugated to mAb	[130]
CanAg	HuC242-DM4	Maytansinoid DM4 conjugated mAb	[131]
Mesothelin	MORAb-009		[70]
	SS1(dsFv) PE38	Immunotoxin conjugated	[69]
Lewis Y	LMB-9	Immunotoxin conjugated	[118]

EGFR: EGF receptor; mAb: Monoclonal antibody; MUC: Mucin.

Table 2

Monoclonal antibodies investigated in Phase II and III clinical trials of pancreatic cancer.

Target molecule	Antibody	Drugs in combined therapy	Ref.
EGFR	Cetuximab	Gemcitabine	[14,18]
		Irinotecan	[15]
		Docetaxel	[15]
		Cyclophosphamide/GM-CSF tumor cell vaccine	[132]
		Bevacizumab/gemcitabine	[133]
		Gemcitabine/oxaliplatin	[134]
		Radiation	[126]
		Gemcitabine/radiation	[126]
		Bevacizumab/gemcitabine/capecitabine/radiation	[201]
		Bortezomib	[124,135]
		Bevacizumab/erlotinib	[201]
		Erlotinib	[136]
EGFR	Nimotuzumab		[27]
ABX-EGFR	Panitumumab	Radiation/fluorouracil/capecitabine	[137,138]
		Gemcitabine/erlotinib	[139]
HER2	Trastuzumab	Gemcitabine	[31]
		Vorinostat	[34]
		Tipifarnib	[33]
		IL-12	[35]
VEGF	Bevacizumab	Gemcitabine	[99,140]
		Gemcitabine/cisplatin	[100]
		Gemcitabine/oxaliplatin	[141]
		Gemcitabine/capecitabine	[142]
		Gemcitabine/radiation	[143–145]
		Docetaxel	[146]
		Fluorouracil/leucovorin/oxaliplatin	[147]
		Capecitabine/radiation	[98]
		Gemcitabine/oxaliplatin/fluorouracil	[201]
		Erlotinib/cisplatin	[201]
DR5	CS-1008	Gemcitabine	[44]
	AMG 655	Gemcitabine	[50,148]
IGF-1R	IMC-A12	Erlotinib	[59]
		Gemcitabine	[59]
	AMG 479	Gemcitabine	[60,149]

Target molecule	Antibody	Drugs in combined therapy	Ref.
MUC1	HuPAM 4	Y-90 radiolabeled	[89,150]
Glycoprotein with property of mucin	CC49	Radioisotope conjugated to mAb	[130]
CEA	MN-14	Filgrastim	[72]
RAAG12	RAV12	Gemcitabine	[63,64]
Mesothelin	SS1(dsFv) PE38	Immunotoxin conjugate	[68,69]
$\alpha 5$ - $\beta 1$ integrin	Volociximab		[104]
CTLA-4	Ipilimumab		[112]
TNF	Infliximab	Gemcitabine	[109]
CanAg	HuC242-DM4		[92,151]

CEA: Carcinoembryonic antigen; CTLA-4: Cytotoxic T-lymphocyte antigen-4; DR: Death receptor; EGFR: EGF receptor; IGF-1R: IGF-1 receptor; mAb: Monoclonal antibody; MUC: Mucin; RAAG12: N-linked carbohydrate antigen.