Published in final edited form as:

Curr Pharm Des. 2011 ; 17(21): 2221–2238.

Molecular Targeted Approaches for Treatment of Pancreatic Cancer

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Abstract

Human pancreatic cancer remains a highly malignant disease with almost similar incidence and mortality despite extensive research. Many targeted therapies are under development. However, clinical investigation showed that single targeted therapies and most combined therapies were not able to improve the prognosis of this disease, even though some of these therapies had excellent anti-tumor effects in pre-clinical models. Cross-talk between cell proliferation signaling pathways may be an important phenomenon in pancreatic cancer, which may result in cancer cell survival even though some pathways are blocked by targeted therapy. Pancreatic cancer may possess different characteristics and targets in different stages of pathogenesis, maintenance and metastasis. Sensitivity to therapy may also vary for cancer cells at different stages. The unique pancreatic cancer structure with abundant stroma creates a tumor microenvironment with hypoxia and low blood perfusion rate, which prevents drug delivery to cancer cells. In this review, the most commonly investigated targeted therapies in pancreatic cancer treatment are discussed. However, how to combine these targeted therapies and/or combine them with chemotherapy to improve the survival rate of pancreatic cancer is still a challenge. Genomic and proteomic studies using pancreatic cancer samples obtained from either biopsy or surgery are recommended to individualize tumor characters and to perform drug sensitivity study in order to design a tailored therapy with minimal side effects. These studies may help to further investigate tumor pathogenesis, maintenance and metastasis to create cellular expression profiles at different stages. Integration of the information obtained needs to be performed from multiple levels and dimensions in order to develop a successful targeted therapy.

Keywords

Pancreatic ductal adenocarcinoma; molecular targeted therapy; pancreatic stroma; drug resistance; gene therapy; monoclonal antibody; kinase inhibitors

INTRODUCTION

For decades, scientists, both in clinical as well as basic research areas, have worked intensively to make a major breakthrough in human pancreatic cancer treatment and to improve its clinical prognosis. Unfortunately, little progress has been achieved. In a recent

CONFLICT OF INTEREST

Dr. Buchsbaum has intellectual property interests related to the TRA-8 anti-DR5 antibody.

report from Ellison *et al.*, the five-year relative survival for pancreatic cancer remains at 6% [1]. Human pancreatic cancer treatment remains a serious challenge in oncology.

Although complete surgical resection of the tumor mass is the most effective regimen in pancreatic cancer treatment, it can only be used in limited cases with localized tumors and there exists a significant rate of cancer relapse [2]. Chemotherapy, radiation therapy and adjuvant therapy together with palliative treatment are other approaches for pancreatic cancer treatment. Among these therapies, gemcitabine has been recognized by many oncologists as the first-line drug to treat pancreatic cancer for more than a decade [3]. With the progression of in-depth research on pancreatic cancer, several important pathologic processes related to pancreatic cancer pathobiology have been elucidated and many therapeutic strategies targeting key molecules in these processes have been developed. For example, cetuximab as well as erlotinib are used to target the EGFR pathway to inhibit tumor growth. Bevacizumab is used for targeting VEGF to inhibit angiogenesis. Imetelstat was produced to inhibit telomerase activity in both pancreatic cancer cells as well as cancer stem cells [4]. GDC-0449 is used to target hedgehog pathway inhibiting Smoothened activation [5]. In recent years, many of these targeted therapeutic approaches in pancreatic cancer treatment have been tested in clinical trials (<http://www.cancer.gov>). However, most of these trials, including combination treatment with gemcitabine, did not improve overall survival rate of pancreatic cancer. It is reasonable to conclude that single pathway targeted treatments and currently used combined therapies do not block the pathogenesis and metastasis of pancreatic cancer sufficiently. Multidimensional therapeutic strategies for pancreatic cancer should be considered and designed by using network and systems biology approaches [6, 7]. In this review, currently available targeted therapies are discussed, with an emphasis on those that have been developed pre-clinically and translated into clinical trials. However, much work needs to be done in terms of how to use these therapies effectively to achieve success in pancreatic cancer treatment.

The optimal treatment of pancreatic cancer may rely on an improved understanding of genetics and biology of pancreatic cancer [8], which may be closely associated with tumorigenesis and metastasis formation. Further research to identify critical genes and the role of their downstream signals in pancreatic cancer pathogenesis is still an urgent need. However, individual signal transduction pathways may have cross-talk with other signaling cascades. In normal cells, the specific biological effect of a pathway in response to a stimuli may be maintained by filtering out spurious cross-talk through mutual inhibition [9]. Specific targeted therapy can easily hit the target in these conditions. In cancer cells, abundant crosstalk between different pathways could make the target a “moving target” [10]. Multiple points of interaction of two pathways allow for the discrete pairing of cell cycle activation and regulation of protein translation [11]. Thus, the specific target inhibitor may be ineffective and downstream signaling to promote tumor growth may escape the treatment. Cross-talk between insulin/insulin-like growth factor 1 (IGF-1) and G protein-coupled receptor (GPCR) signaling pathways has been proposed as a mechanism of pancreatic cancer cell proliferation [12]. Metformin, a drug used to treat diabetes was reported to break this cross-talk [13]. Similarly, crosstalk may disturb normal feedback mechanisms. In cancer, taking the interaction between kinases and proteases as an example, enormous diversity and complexity of their bidirectional communication processes were found [14]. To break this interrelationship among pathways, combined molecular targeted therapies can be applied. However, a significant number of clinical trials with various combinations of chemotherapy and molecular targeted therapies have not led to a major breakthrough yet. Chemotherapeutic agents are still the main components in pancreatic cancer treatment. A recent phase III trial using FOLFIRINOX (5-FU, leucovorin, irinotecan and oxaliplatin) vs. gemcitabine showed a significant prolongation of overall survival in metastatic pancreatic cancer patients (11.1 vs. 6.8 months) [15]. Minimizing side effects

from the treatment is also an important issue. Integration of targeted therapies with chemotherapies may still be an applicable strategy to reach that goal. Additional investigation to further explore these promising regimens and to search for critical genes or proteins may supply valuable information leading to important new targeting agents. Identifying and combining molecular targeted therapies against different targets in pancreatic cancer treatment requires extensive analysis and integration of crucial interacting pathways [6]. Network and systems biology approaches are necessary in understanding and evaluation of multiple drug combinations in cancer treatment [6].

Another challenge in pancreatic cancer treatment is to identify underlying pathological defects and to integrate them with molecular biomarkers or gene mutations [10]. Analysis of histological classification and anatomical extent of tumor combined with their molecular features may help to create tailored, personalized multidimensional therapies [7]. Identification and analysis of effective, combined targeted therapies may help to generate better strategies in treating pancreatic cancer.

Targeted Molecules on the Cell Surface

In targeting cell surface molecules, monoclonal antibodies are the main tools that have been used. Monoclonal antibodies may serve as a receptor pathway inhibitor or as a ligand to activate certain pathways. Humanized monoclonal antibodies are used clinically to decrease the immune response to murine products.

EGF Receptor

EGF receptors comprise four subtypes EGFR/ERBB1, HER2/ErbB2, HER3/ErbB3 and HER4/ErbB4. EGF, TGF- α , heparin-binding EGF-like growth factor and neuregulins serve as their ligands. The EGFR signal transduction pathway is essential for tissue and organ development and regulation of cell migration, adhesion and proliferation. Activation of EGFR in cancer is associated with oncogenesis, angiogenesis, apoptosis inhibition and tumor metastasis. Recently, Fujita *et al.* reported that pancreatic cancer patients with high EGFR mRNA expression in tumor tissue had shorter disease-free-survival and overall survival [16].

Cetuximab is a chimeric mouse-human antibody that binds to EGFR. Pre-clinical studies showed cetuximab decreased cell proliferation and phosphorylation of EGFR, and blocked the binding of the adaptor protein Grb2 to EGFR upon activation by EGF [17]. Morgan *et al.* reported cetuximab together with gemcitabine and radiation effectively prolonged pancreatic cancer xenograft volume doubling time, and produced a synergistic effect with gemcitabine and radiation [18]. However, an enhanced antitumor effect was not observed in pancreatic cancer clinical trials with cetuximab combined with gemcitabine, or gemcitabine/cisplatin [19–21]. In a phase III study of gemcitabine plus cetuximab vs. gemcitabine alone the median survival was 6.3 months with progression-free survival of 3.4 months in the combination treatment group, vs. 5.9 months and 3 months respectively in the gemcitabine group [20]. Recently, cetuximab was used in combination with capecitabine, radiation and ixabepilone in phase II clinical trials, although no obvious improvement was observed [22, 23]. There are several other ongoing clinical trials although using a combination of cetuximab, irinotecan and oxaliplatin, or cetuximab, everolimus and capecitabine [<http://www.cancer.gov>].

Other antibodies against EGFR include panitumumab, matuzumab (EMD 72000), and nimotuzumab. Several studies have been conducted in pre-clinical as well as clinical trials using these antibodies [24–26]. Another chimeric monoclonal antibody CH 806 is an

antibody against mutant EGFR. There is no information available yet for this antibody in pancreatic cancer treatment.

HER2/ErbB2

HER2/ErbB2, a member of the EGFR family, was found to be overexpressed in pancreatic cancer. Tsiambas *et al.* reported 42% positive staining of HER2 with 16% gene amplification in pancreatic cancer tissue [27]. A recent report showed overexpression of HER2 in 61.2% of tissue samples from pancreatic cancer patients without metastasis and overexpression of HER2 positively correlated with shorter survival time of these patients [28]. Expression of HER2 was found to be related with the expression of MUC4 protein. Down-regulation of MUC4 led to decreased expression and phosphorylation of HER2, with subsequent decreased phosphorylation of its downstream signal proteins FAK and p44/42 [29]. Trastuzumab (Herceptin) is a humanized antibody against HER2. Pre-clinical studies using this antibody showed significant growth inhibition of pancreatic cancer cell lines and xenografts. Additive effects were found when trastuzumab was combined with fluoropyrimidine S-1 [30]. In addition, trastuzumab induced antibody-dependent cellular cytotoxicity (ADCC) through antibody Fc receptors expressed on immune effector cells. ADCC contributed to the cytotoxicity of trastuzumab against pancreatic cancer cell lines [30, 31]. Clinical trials of trastuzumab have not yet achieved an improved prognosis for pancreatic cancer. Combination of trastuzumab with capecitabine treatment yielded a progression free survival at 12 weeks of 23.5% and median overall survival of 7.0 months [32]. Vidalpla *et al.* recently reported trastuzumab combined with cetuximab and gemcitabine induced a 2.1 fold increase in pancreatic cancer cell death and 2.3 fold reduction of vascularization in xenografts compared with gemcitabine plus either antibody as a double treatment [33]. Whether this pre-clinical study can be translated into a clinical trial with similar outcomes needs to be determined. ¹¹¹In-labeled trastuzumab was generated and is now in a phase I clinical trial [34].

Another recombinant humanized monoclonal antibody against HER2, pertuzumab, has been used to treat solid tumors, including pancreatic cancer. Two pancreatic cancer patients showed partial responses with stable disease for 15.3 months in one patient [35]. A phase II clinical trial using this antibody together with erlotinib is ongoing [<http://www.cancer.gov>].

Death Receptor 5

Death receptor (DR) 5 belongs to the tumor necrosis factor receptor (TNFR) superfamily. Its ligand is TNF-related apoptosis-inducing ligand (TRAIL). This transmembrane receptor forms a homotrimer after ligand binding and subsequently induces the formation of a death-inducing signaling complex (DISC) which activates caspase 8-triggered apoptosis. The intrinsic mitochondrial pathway is also activated and contributes to apoptosis [36–39]. Normal cells are in general resistant to TRAIL induced apoptosis despite the presence of DR5 on the cell surface. Tumor cell lines from diverse origins including pancreatic cancer are sensitive to this apoptotic process [40].

Tigatuzumab (CS-1008) is a humanized antibody against DR5 [41]. CS-1008 is derived from a mouse anti-human DR5 antibody TRA-8 [41]. TRA-8 activates the apoptosis pathways in a variety of tumor cell lines [42]. In an orthotopic pancreatic cancer mouse model, TRA-8 produced synergistic cytotoxicity with gemcitabine in a panel of pancreatic cancer cell lines [43]. TRA-8 combined with irinotecan prolonged mouse survival time longer than monotherapy with TRA-8 or irinotecan [44]. In another pancreatic cancer xenograft model, TRA-8 treatment combined with gemcitabine significantly prolonged tumor doubling time compared with gemcitabine monotherapy. Combination treatment

reduced the pancreatic cancer stem cell population in the tumor tissues as compared to monotherapy with gemcitabine [45].

Conatumumab (AMG655) is another humanized antibody against DR5 developed by Amgen Inc. In pre-clinical studies, AMG 655 produced synergistic cytotoxicity when combined with gemcitabine and irinotecan [46]. Ongoing phase I clinical trials are testing this antibody in solid tumor treatment [47, 48]. Another human single chain antibody against DR5, Apomab, was developed by Genentech. In pre-clinical models of pancreatic cancer, complete remission of tumors was observed in 100% of mice after a single injection of Apomab (10 mg/kg). Combination treatment with this antibody and gemcitabine induced partial remission in seven and a complete remission in one of ten mice [49]. It was reported recently that the sensitivity to Apomab was correlated with the expression of O-glycosyltransferase GALNT14 in pancreatic cancer cells [50]. More information needs to be obtained from further evaluation of TRAIL death receptor antibodies in clinical trials.

Insulin-like Growth Factor-1 Receptor (IGF-1R)

IGF-1R is a transmembrane receptor tyrosine kinase and has been found to be overexpressed in pancreatic cancer. Activation of IGF-1R has been correlated with decreased apoptosis, cancer cell proliferation and angiogenesis [51, 52]. IGF-1R induced pancreatic cancer proliferation and invasion were related to the down-regulation of the tumor suppressor chromosome 10 (PTEN) phosphorylation and activation of the PI3K/Akt signaling pathway [53]. Blockage of IGF-1R activation also decreased the expression of the matrix metalloproteinase, MMP7, which was shown to be related with tumor invasion [54]. Pancreatic cancer cell proliferation and motility were significantly suppressed by a specific IGF-1R inhibitor picropodophyllin *in vitro* [55]. Insulin receptor and IGF-1R share high homology in amino acid sequences. In human multiple myeloma cells, insulin was found to be a myeloma growth factor and to induce insulin receptor (IR) and IGF-1R hybridization and phosphorylation [56]. In a pancreatic neuroendocrine cancer mouse model, high expression level of IR (or high IR to IGF-1R expression ratio) was related to anti-IGF-1R treatment resistance [57]. Investigation of the receptor expression profile in tumor tissues may help to determine if dual targeting against IR and IGF-1R would be beneficial. As mentioned above, there exists cross-talk between IR/IGF-1R and GPCR signaling pathways. This cross-talk may occur at multiple levels, from receptors to downstream cascades, causing a series of long term biological consequences [12]. In a recent report by Kisfalvi *et al.*, the cross-talk between IR/IGF-1R and GPCR pathways was mediated by mTOR, which was inhibited by rapamycin. Metformin, a drug to treat type II diabetes, disrupted this cross-talk by inhibition of mTOR function and inhibited human pancreatic PANC1 and MIA PaCa-2 xenograft growth in nude mice [13].

Cixutumumab (IMC-A12) is a human IgG1 antibody that binds to IGF-1R developed by ImClone Systems Incorporated. In mice bearing BxPC-3 tumors, IMC-A12 used a single agent at 1 mg/kg every 3 days inhibited tumor growth by 80% [58]. Additive effects were observed when IMC-A12 was combined with cetuximab and gemcitabine in pancreatic cancer xenograft models [59]. Combination therapy using this antibody together with gemcitabine and erlotinib is in a phase II clinical trial of pancreatic cancer patients [21].

AMG479, another human anti-IGF-1R antibody has been tested *in vitro* and in pancreatic cancer mouse models. AMG 479 completely inhibited ligand (IGF-1, IGF-II and insulin) induced activation of IGF-1R homodimer and IGF-1R/IR heterodimer, and decreased cell viability. In a mouse model, it inhibited by 80% xenograft tumor growth and an additive effect with gemcitabine was observed [60]. This antibody is now in a phase I clinical trial in patients with solid tumors and non-Hodgkin's lymphoma [61]. RAV12 is a chimeric antibody against an N-linked carbohydrate antigen (RAAG12). RAAG12 was found to

coexist with many membrane proteins, including IGF-1R, ALCAM, IFN- γ R1, TfR, IR, and EGFR. RAV12 inhibited pancreatic cancer cell growth *in vitro*. Additive or synergistic effects were observed when combined with MAPK inhibitors. Tumor cells with less expression of RAAG12 had no response to RAV12 treatment [62]. In a pre-clinical study of a colon cancer xenograft mouse model, RAV12 showed a dose dependent inhibition of tumor growth [63]. However, this antibody has shown some intolerable side effects at effective doses in a recent phase I clinical trial to treat gastrointestinal tumors, including pancreatic cancer patients. Reengineering of this antibody and humanized product have been suggested to eliminate these side effects [64]. Another monoclonal anti-IGF-1R antibody, figitumumab (CP-751,871) has been tested in pre-clinical studies and showed the ability to suppress tumor growth in a dose-dependent manner [65, 66]. MK-0646, another humanized anti-IGF-1R antibody, is now in a phase I/II clinical trial [67, 68].

Carboni *et al.* found the IGF-1R/IR inhibitor BMS-754807 inhibited growth of a broad range of human tumor cells *in vitro*, including a series of pancreatic cancer cell lines. BMS-754807 synergized with other anti-tumor treatment. Combination of cetuximab and BMS-754807 showed improved outcome *vs.* single treatment in a pre-clinical study [69].

Mesothelin

Mesothelin is a 40 kDa cell surface protein anchored by glycosyl phosphatidylinositol. Mesothelin is a cleaved product from a precursor protein encoded by the Mesothelin gene (*MSLN*). Expression of mesothelin is normally seen in mesothelial cells lining peritoneal, pleural and pericardial cavities. Overexpression of the *MSLN* gene as well as mesothelin was found in about one third of cancers, including pancreatic cancer [70, 71]. The physiological roles of this protein are not fully understood. However, due to its unique tissue distribution profile in cancer and normal tissues, mesothelin has become an important target in cancer diagnosis and treatment. A truncated mutant of *Pseudomonas* exotoxin A was fused genetically with a single-chain Fv against mesothelin isolated from a phage display library (SS1P). In a pre-clinical study, SS1P plus radiation treatment significantly prolonged tumor doubling time in a mesothelin-expressing tumor xenograft model [72]. Synergy was observed when gemcitabine was used in combination with SS1P and this treatment induced complete xenograft regressions [73]. SS1P was found to be well tolerated in a phase I clinical trial. Of 34 patients treated, 12% showed minor responses (tumor size decreased between 20–50% and lasted for more than 4 weeks), 56% showed stable disease and 29% of the patients had progressive disease [74]. Kreitman *et al.* reported a recent phase I clinical trial and suggested bolus dosing in combination with other therapies [75]. Another chimeric antibody derived from a phage-display library, MORAB-009, was used in a pre-clinical study with a mesothelin expressing xenograft model. Monotherapy with MORAB-009 moderately inhibited tumor growth, while combination therapy with gemcitabine significantly improved the inhibitory effect [76]. M912, another humanized monoclonal antibody obtained from a phage-display library was generated and the cytotoxicity to mesothelin expressing cancer cells was mediated by ADCC [77].

MUC1

MUC1 is a heavily glycosylated type 1 membrane protein with several extracellular tandem repeat domains. These domains undergo cleavage between amino acid 1097 and 1098 after translation. MUC1 is expressed in nearly all human glandular epithelial tissues and throughout all regions of the gastrointestinal tract on the apical side of cells [78]. MUC1 expression is upregulated in almost all human adenocarcinomas with an expression pattern over the entire cell surface [78–80]. The expression of MUC1 in cancer cells is regulated by DNA methylation and histone H2 lysine 9 modification of the MUC1 promoter. Inhibition of DNA methylation enhanced MUC1 gene expression [81]. 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 [82]. The intact tandem repeats and cytoplasmic tail of MUC1 may play a role in preventing tumor invasion and metastasis [83]. Overexpressed MUC1 upregulated expression of transcription factors slug and snail with inhibition of E-cadherin, thus enhancing epithelial to mesenchymal transition, which was associated with cancer invasion and metastasis [84]. In the TNF-R1 signaling pathway, 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 [85]. MUC1 was also found to block death receptor-mediated apoptosis by binding to caspase 8 and FADD [86]. Down-regulation of MUC1 by RNA interference upregulated β -catenin and E-cadherin expression, promoting E-catenin/catenin complex formation which decreased *in vitro* cell invasion [87]. In PANC-1 cells, knockdown of MUC1 expression significantly decreased cell proliferation and slowed the growth rate of xenografts in SCID mice [88].

PM4 is a murine antibody against MUC1. The binding of this antibody to MUC1 is conformation dependent and its epitope may contain carbohydrate. HuPAB4 is a humanized form. This antibody has been radiolabeled with ^{90}Y and is now under investigation in phase I/II clinical trials. In a phase I study, 15 patients with advanced pancreatic cancer were treated. Three patients showed 32–51% tumor shrinkage and other three patients had stable disease at 4 weeks after treatment [89]. TF-10, a humanized bispecific monoclonal antibody, which is divalent for PAM4 and monovalent for monoclonal antibody 679, was used in a CaPan1 human pancreatic cancer xenograft model. Antibody 679 is reactive against the histamine-succinyl-glycine hapten (HSG-hapten peptide). Pretargeting TF-10 followed by ^{111}In -labeled peptide IMP-288 containing HSG groups showed high ^{111}In -IMP-288 uptake in tumors [90]. Recently, the same group reported combination treatment of CaPan1 xenografts with gemcitabine and TF-10 followed by ^{90}Y -HSG peptide enhanced the tumor growth inhibition by gemcitabine monotherapy [91]. Glazer *et al.* recently reported administration of gold nanoparticles (AuNP) conjugated to PAM4 antibody induced intracellular hyperthermia of tumors. Combination treatment using PAM4 conjugated with AuNP followed by exposure to radiofrequency field 36 hours after the antibody limited the tissue destruction inside tumors despite whole body radiofrequency exposure [92].

CC49 is a mouse monoclonal antibody against a tumor-associated antigen TAG-72, which is a cell surface glycoprotein and has characteristics of a mucin. This antibody has been conjugated to radioisotopes for diagnosis and treatment purposes. Humanized CC49 was engineered. Branowska-Kortylewicz *et al.* reported that ^{131}I -CC49 significantly prolonged pancreatic cancer xenograft quadrupling time and this effect was enhanced by a tyrosine kinase inhibitor imatinib [93].

A humanized monoclonal antibody HuC242 against CanAg (a glycoform of MUC1) was conjugated with antimicrotubule agent DM1 ($\text{N}^{2'}$ -deacetyl- $\text{N}^{2'}$ -[3-mercapto-1-oxopropyl]-maytansine, cantuzumab mertansine) and tested in a phase I clinical trial of 37 patients with solid tumors. Two patients with chemotherapy resistance had minor regression and four patients had stable disease during treatment [94]. Conjugation of HuC242 to another maytasinoid derivative (HuC242-DN4) is now in a phase I clinical trial for pancreatic cancer treatment.

Carcinoembryonic Antigen (CEA)

CEA has been recognized as an overexpressed cell surface antigen in many cancers, mainly in gastrointestinal tract malignancies. A humanized anti-CEA antibody MN-14 (labetuzumab) is now under clinical trial for pancreatic cancer treatment together with filgrastim (G-CSF). Labetuzumab has recently been conjugated with SN-38, an active form

of CPT-11, and tested in pancreatic cancer and other cancer xenograft models. The results showed conjugated antibody prolonged the median survival time of mice and decreased the systemic toxicity due to a lower amount of drug in the conjugate [95].

Small Molecule Kinase Inhibitors

Many kinases including receptor tyrosine kinases or non-receptor kinases, have been found to be activated in pancreatic cancer tissues. Inhibition of these kinase activities using small molecules is now an important approach in pancreatic cancer treatment. Many kinase inhibitors have been tested in combination with other treatments in a variety of cancer therapy studies. Among these inhibitors, erlotinib (EGFR tyrosine kinase inhibitor) has been the one tested most in pancreatic cancer treatment with other drugs (Table (1)).

In a phase I study, 18 pancreatic cancer patients and 7 biliary cancer patients were administrated lapatinib at 1,500 mg/day daily with weekly gemcitabine or gemcitabine/oxaliplatin. Dose-limiting side effects of nausea or anorexia were found in 2 of 5 patients that received treatment combined with gemcitabine/oxaliplatin. Median survival in all patients was 11 months and 1-year survival was 48% [96]. Results of a phase III trial using erlotinib plus gemcitabine *vs.* gemcitabine alone in 569 patients with unresectable, locally advanced or metastatic pancreatic cancer showed significant improvement of clinical outcome. Median survival in the combination group was 6.24 months *vs.* 5.91 months in the gemcitabine group. One-year survival and progression-free survival were 23% and 3.75 months respectively in the combination group *vs.* 17% and 3.55 months in the monotherapy group [118]. Erlotinib combined with gemcitabine improved overall survival significantly for patients younger than 65 years old compared with a group treated with gemcitabine only as reported in a phase III trial recently [105]. Patients with skin rashes during treatment with erlotinib had better overall survival than the patients with fewer or no rashes [103, 104]. Combination of erlotinib (100 mg/day) with gemcitabine (1,000 mg/m²) did not show any benefit in overall survival rate compared with gemcitabine (1,000 mg/m²) combined with docetaxel (35 mg/m²) in a phase II study from 69 metastatic pancreatic cancer patients [106]. In a phase III study, erlotinib (150 mg/day) was given to 141 patients combined either with gemcitabine (1,000 mg/m²/week for 7 weeks), or capecitabine (2,000 mg/m²/day, day 1–14 every three weeks) until disease progression or toxicity appeared (TIF1). It was found that TIF1 was longer in the group receiving erlotinib with gemcitabine than that treated with erlotinib with capecitabine (3.4 months *vs.* 2.4 months, *p*=0.0036). Overall survival was longer in patients with wild type KRAS (8.0 months) than mutant type (6.6 months, *p*=0.011) in response to the treatment [100]. Watkins *et al.* reported a phase II study using the combination of gemcitabine (1,000 mg/m², on day 1, 8, and 15), capecitabine (1,400 mg/m² on day 1–21), bevacizumab (5 mg/m² on day 1 and day 15) and erlotinib (100 mg daily) for every 28 days. Median and one year overall survival was 11.1 months and 49% in patients with metastatic disease and 14.8 months and 89% in patients with local advanced disease [102]. Erlotinib (150 mg daily) plus bevacizumab (15 mg/kg every 21 days) did not show any treatment benefit in gemcitabine-resistant metastatic pancreatic cancer patients [101]. Erlotinib was proposed to down-regulate activated phosphorylation of Akt induced by the mTOR inhibitor rapamycin, and thus produce a synergistic anti-tumor effect. Whether it is effective clinically is still under investigation [108].

The VEGFR inhibitor pazopanib was tested in a phase I trial of solid tumors together with paclitaxel. Among twenty-six patients enrolled (17 patients received maximum tolerated regimen of pazopanib at 800 mg daily with weekly paclitaxel at 80 mg/m²), six patients (23%) had a partial response and 15 (58%) of patients had stable disease [110]. Another inhibitor axitinib was evaluated in a phase II trial from 103 unresectable, locally advanced or metastatic pancreatic cancer patients. The results showed a small, non-significant difference in median overall survival between the group treated with gemcitabine plus axitinib and

gemcitabine only (6.9 months vs. 5.6 months) and the drug needs to be further evaluated in a phase III study [111].

Imatinib (400 mg daily), a PDGFR inhibitor, was combined with different doses of gemcitabine and oxaliplatin and tested in a phase I trial with gemcitabine resistant locally advanced or metastatic pancreatic cancer patients. Twenty-six patients were enrolled. Two out of 26 patients had a partial response and 11/26 had stable disease. Median progression free survival and overall survival were 4.6 and 5.7 months respectively [112].

mTOR is an important kinase in the signaling pathway of PI3k/Akt, which is a downstream component of many growth factor receptors. Deregulation of mTOR is associated with tumor pathogenesis. mTOR inhibitors have been used as anti-cancer agents and have been summarized in a recent review [119]. Everolimus (RAD001) was evaluated in 58 specialized models of human cancers, including three cell lines of pancreatic cancer. The drug was given at 10 mg/kg/day, five days a week for three weeks. The mean change of relative tumor volume in treatment group/control group in pancreatic cancer models was $21 \pm 5\%$ [120]. Although mTOR inhibitors inhibit tumor growth and down-regulate protein synthesis, longer exposure to these inhibitors induced phosphorylation of Akt, which favors tumor growth as mentioned previously. This negative feedback loop might be related to rapid disease progression observed by Javle *et al.* in a phase II trial of the mTOR inhibitor temsirolimus (CCL-779) [108]. Schmid *et al.* reported in 2009 in a pancreatic cancer xenograft model (CA20948) that combination of the mTOR inhibitor everolimus (RAD001) with a multi-receptor ligand somatostatin analogue pasireotide (SOM230) produced additional tumor growth inhibition compared with monotherapy. The mechanism is still unknown [121]. Combination treatment with a MEK inhibitor RDEA119 and rapamycin in three pancreatic cancer xenograft models (OCIP 19, 21 AND 23) produced somewhat better tumor growth inhibition than rapamycin only, although the difference was not statistically significant [122].

Another target of kinase inhibitors is the Raf/MEK/ERK cascade. The anti-tumor activity of the MEK inhibitor AS703026 was tested in mouse xenografts of the pancreatic cancer cell line MIA PaCa-2. Tumor regression was observed at a low dose of 10 mg/kg twice a day [123]. Tumors bearing Ras and Raf mutations were more sensitive to this inhibitor [124].

Sorafenib, GSK1363089 (foretinib) and sunitinib are a group of agents which inhibit multi-kinases. *In vitro* tests showed that sorafenib induced apoptosis in pancreatic cancer cell lines by different mechanisms in different cell lines, which included inhibition of MEK/MAPK, dephosphorylation of BAD ser 122, up-regulation of PUMA, caspase 3 and 9 cleavage, down-regulation of the anti-apoptotic protein Mcl-1 and decreased Akt expression and phosphorylation, accompanied by different degrees of apoptosis [125]. In another *in vitro* study, sorafenib was found to inhibit constitutive signal transducer and activator of transcription 3 (STAT3) phosphorylation and suppress expression of Mcl-1 and Bcl_{xL}. Sorafenib was also found to enhance the effect of TRAIL to induce apoptosis in pancreatic cancer cells [126]. GSK1363089, when combined with the HER1/2 inhibitor lapatinib, was found to decrease phosphorylation of Akt and ERK significantly in tumors overexpressing HER1/2 [127]. Hong *et al.* tested a combination of sorafenib with a farnesyltransferase inhibitor tipifarnib in a phase I clinical trial with 50 cancer patients, including 4 pancreatic cancer patients. One pancreatic cancer patient showed prolonged stable disease for 6 months [115]. In another phase I study of 40 solid tumor patients (including one pancreatic cancer patient), three patients had a partial response and another 22 patients had stable disease with a duration from 1 to 10 months [116]. Negative data was reported by Kindler *et al.* from a phase II study in pancreatic cancer patients. Patients received sorafenib 400 mg twice daily and gemcitabine 1,000 mg/m² on days 1, 8 and 15 of a 28 day cycle. Thirteen patients were

evaluated for response. Eighteen percent of the patients had stable disease. Median overall survival was 4.0 months (95% CI: 3.4, 5.9), median progression-free survival was 3.2 months [128].

Src kinase has been recognized as a proto-oncogene and is regulated by a variety of signals including activation by receptor tyrosine kinases and cytoplasmic phosphatases [129]. Many downstream substrates of Src have been identified, which makes Src a critical kinase in cellular signal transduction [129]. Inhibition of Src activity has been a focused target in cancer treatment. Cellular location of Src expression was correlated with patient survival in pancreatic cancer. Increased membranous Src expression was associated with a significantly lower overall survival, while a higher expression in the cytoplasmic compartment was associated with improved survival [130]. An *in vitro* study showed that the Src inhibitor dasatinib inhibited pancreatic cancer cell proliferation, migration and invasion accompanied by stimulated apoptosis, decreased phosphorylation of Src, focal adhesion kinase, paxillin, Akt, STAT3, ERK and MAPK. In an *in vivo* study, tumor volume in untreated mice bearing BxPC3 xenografts increased 62% compared with 17% in sorafenib treated (10 days treatment) mice. Tumor volume in untreated mice bearing PANC 1 xenografts increased 174% compared with 50% in treated mice [130]. Recently, a triple combination of dasatinib, erlotinib and gemcitabine were used in a pre-clinical study. The treatment was able to inhibit multiple signaling pathways including FAK, Akt, ERK, JNK, MAPK and STAT3, while single agent treatment or double agent treatment was ineffective. Similar results were reported in another *in vivo* xenograft study. Tumor volume decreased 93.6% with triple treatment compared with dasatinib plus erlotinib 79.8%, dasatinib plus gemcitabine 77.3%, and gemcitabine plus erlotinib 81.1% [131]. In another pre-clinical study, Evans found administration of dasatinib decreased the metastasis rate of pancreatic cancer in mice [132]. It was proposed that dasatinib not only inhibited Src activity but also inhibited Eph receptor tyrosine kinase and its downstream effectors [133]. Another Src inhibitor saracatinib (AZD5030) was evaluated in a phase I/II clinical trial in combination with gemcitabine. The results did not show any advantage when compared with gemcitabine alone [134]. In another phase II trial using saracatinib single agent therapy in a group of unselected, previously treated advanced pancreatic cancer patients, this drug failed to improve 6-month survival [117].

Stroma-Targeted Therapy

The abundance of stroma in pancreatic cancer tissue has drawn investigator's attention regarding its role in aggressive tumor growth and metastasis. In pancreatic cancer, the extensive desmoplastic reaction may expand the stromal volume up to 90% of the whole tumor [135]. Pancreatic cancer stroma contains extracellular matrix, fibroblasts, myofibroblasts, and blood vessels. One important type of cell, pancreatic stellate cells (PSC), was found to play an important role in mediating epithelial-stromal interaction. This interaction and stromal biology were discussed in a recent review [135]. In animal models, PSC were found to migrate with cancer cells, and PSC were able to migrate through transendothelial monolayers, which was stimulated by cancer cells [136]. Ikenaga *et al.* recently reported that PSC in tumor tissue showed higher frequency expression of CD10, which significantly increased tumor growth and invasiveness than CD10⁻ PSC cells in co-culture with pancreatic cancer cells. The stimulation of tumor growth and metastasis may be mediated by matrix metalloproteinase 3 secreted by CD10⁺ PSC [137]. The growth, angiogenesis and invasiveness of pancreatic cancer were also found related to the level of hepatocyte growth factor (HGF) from stromal cells. Also, the HGF level was stimulated by cancer cell derived IL-1 α [138]. The desmoplastic reaction is accompanied by a relatively avascular tumor microenvironment, subsequent hypoperfusion and hypoxia of cancer tissue, which dramatically influences drug delivery to the tumor tissue. This partially explains why

excellent pre-clinical therapy in xenograft models has not been translated into clinical trials successfully. Xenografts tend to be better vascularized. It was proposed that using transgenic mouse models of pancreatic cancer in pre-clinical studies would yield more accurate information about the potential for cancer treatment [11], since abundant pancreatic cancer stroma forms in these models. To improve drug delivery into tumor parenchyma, targeting cancer stroma seems a step which cannot be neglected. A hypothesis for “stroma depletion” to enhance drug delivery to tumor tissue was proposed [139].

One of the stroma targeting strategies is to use an antagonist of the hedgehog (Hh) signaling pathway. Hedgehog ligand receptor, Patched (Ptc) is a trans-membrane protein with 12 membrane spanning regions. Normally, Ptc represses its downstream protein, Smoothened (another trans-membrane protein with 7 membrane spanning regions, Smo) in the absence of Hh ligand. When Hh ligand binds Ptc, it is inactivated, thus activating Smo. Activation of Smo subsequently activates Gli transcription factors and activates Hh targeted genes (Fig. (1)) [140]. Activated Hh pathway was found in pancreatic cancer and its precursor lesions. Blocking this pathway with cyclopamine induced apoptosis and inhibited cancer cell proliferation [141, 142]. Over-expressed Hh ligand was found in pancreatic cancer cells [142], while Smo was found overexpressed in pancreatic cancer stromal cells [143]. Activation of the Hh signaling pathway through paracrine Hh ligand from cancer cells induced Hh signal activation in pancreatic cancer stroma [144], which made the Hh signal blocking strategy more specific towards stroma. The Smo inhibitor GDC-0449 (vismodegib) has been tested in some tumors. It is now in ongoing clinical trials to treat pancreatic cancer (NCT01096732, NCT01088814 and NCT00878163).

Another strategy of stromal targeting is administration of nab-paclitaxel. Nab-paclitaxel is an albumin-bound nanoparticle formulation of paclitaxel. The attached albumin molecules bind albumin receptor, glycoprotein 60, on endothelial cells and nab-paclitaxel molecules are transported into endothelial cells. Nab-paclitaxel is then transported across cells into the extracellular space through formation of caveolae, where it selectively binds to secreted protein, acidic and rich in cysteine (SPARC). Paclitaxel is then released from the complex (Fig. (2)) [145, 146]. SPARC is a glycoprotein with high affinity to albumin. The biological characterization of SPARC, its expression profile as well as its role in tumorigenesis was described in a review [147]. SPARC expression was found to be low in several pancreatic cancer cell lines while high in pancreatic stellate cells. The expression was mainly in peritumoral and distal stroma [147, 148]. Pancreatic cancer patients who expressed SPARC in stromal cells, but not pancreatic cancer cells, had worse prognosis than those did not express SPARC [149]. SPARC expression in distal stroma correlated inversely with overall survival of the pancreatic cancer patients [148]. High SPARC mRNA expression was also found associated with a poor prognosis and was proposed as a prognostic marker for pancreatic cancer patients [150]. Recently, analysis of SPARC gene transcriptional regulation region showed two hypermethylation wave peak regions, and one of them was highly associated with the tumor size. Methylation of the SPARC gene may serve as a tumorigenesis marker for early detection of pancreatic cancer [151]. In a phase II clinical trial using nab-paclitaxel to treat 19 advanced pancreatic cancer patients who were previously treated with gemcitabine, nab-paclitaxel was given at 100 mg/m² on days 1, 8, and 15 of a 28-day cycle. Six-month overall survival was 58%. Median overall survival was 7.3 months. One patient showed a partial response. Six patients showed stable disease. CA19-9 levels decreased by 52% in the patients who had stable disease or partial response compared with an 18% decrease in the patient who had progressive disease [152]. In a recent report by Thapaliya *et al.*, nab-paclitaxel was combined with gemcitabine in 13 patients. Nine of 13 patients showed a partial response and three of 13 patients showed stable disease. One patient had progressive disease [153]. Nab-paclitaxel was combined with low dose 5-FU, leucovorin, oxaliplatin and bevacizumab to treat 28 pancreatic cancer

patients. One patient showed a complete response and 13 patients showed a partial response. Overall survival was greater than 8 months [154]. Further studies using nab-paclitaxel in pancreatic cancer treatment is ongoing and is expanding to a larger cohort and is being tested in multiple institutions.

Anti-VEGF targeting is another important approach in controlling tumor growth and metastasis. 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. VEGF (usually referring to VEGF-A) was found to be expressed in 93% of pancreatic cancer samples and expression of VEGF was correlated with liver metastasis and prognosis. VEGF was also detected in bone marrow derived cells or mesenchymal stem cells [155, 156]. Expression of VEGF-C and -D in pancreatic cancer was found to be correlated with lymph node metastasis, lymphatic invasion and venous invasion [157]. Bevacizumab is a humanized murine anti-human VEGF-A monoclonal antibody and has been used in the treatment of pancreatic cancer together with other chemotherapy agents.

The preliminary clinical trial results of GDC-0449, nab-paclitaxel and bevacizumab, either monotherapy or combined with other drugs are listed in Table (2). It is still too early to identify the best combinations of these agents. According to most of the results, side effects or toxicities from these drugs were tolerable and manageable. However, a recent report showed that bevacizumab increased the treatment-related mortality in cancer patients. The most common side effects seen were hemorrhages, neutropenia and gastrointestinal tract perforation. These risks were seen more often when bevacizumab was combined with taxanes or platinum agents [158].

Heat Shock Proteins

Heat shock proteins (HSPs) are a set of highly conserved proteins whose expression is induced in response to a variety of physiological and environmental insults [162]. Once up-regulated, HSPs have been shown to protect against programmed cell death or apoptosis. By virtue of this pro-survival role, HSPs are believed to contribute to the pathogenesis of cancer. Heat shock protein 70 (HSP70) is one of the most extensively studied HSPs and multiple lines of evidence have established its role in the extreme resistance to cell death demonstrated by pancreatic cancer cells. Aghdassi *et al.* [163] showed that the HSP70 levels are markedly high in various pancreatic cancer cell lines at both protein and mRNA levels, thus suggesting increased transcription, as compared to normal pancreatic ductal cells, the cells of origin of pancreatic cancer. This finding is clinically relevant since HSP70 overexpression was also observed in human pancreatic cancer specimens when the levels were compared with normal pancreas margins [163]. This increased expression of HSP70 has been linked to increased level and activity of Heat Shock Factor-1 (HSF-1), the transcription factor that regulates the heat shock response [164]. Similar overexpression of HSP70 has been observed in other cancers like hepatocellular cancer [165] and colorectal cancer [166] and this increased expression has been correlated with advanced stage, poor differentiation and poor prognosis.

Further support for the role of HSP70 in the pathogenesis of pancreatic cancer comes from the studies which have shown that decreasing HSP70 leads to cell death in pancreatic cancer cells. As a proof of principle, silencing HSP70 expression by specific siRNA, leads to caspase dependent apoptotic cell death suggesting that HSP70 down-regulation could emerge as novel therapeutic strategy for pancreatic cancer [163]. This has led to a search for pharmacologic inhibitors of HSP70 which could be used clinically. Two such inhibitors include quercetin [163] and triptolide [167]. Though quercetin is effective in inhibiting HSP70 expression [163] as well as inducing cell death in pancreatic cancer cells [163], its toxicity profile, low potency and insolubility in water have been barriers to its application in

clinical practice. Triptolide, a diterpene triepoxide from the Chinese plant *Tripterygium wilfordii*, was identified as an inhibitor of human heat shock gene transcription through a small molecule screen [168]. Inhibition of HSP70 expression in pancreatic cancer cells by triptolide [167] has been shown to induce apoptotic cell death in pancreatic cancer cells. Since cancer in itself is composed of a heterogeneous group of cells with variable aggressiveness, the effect of triptolide has been evaluated in both less aggressive pancreatic cancer cell lines like MIA PaCa-2 and Panc-1 developed from primary tumors, as well as in highly aggressive cell lines like S2013 and S2VP10 (developed from metastases) and ASPC-1 (developed from malignant ascites) and has been shown to be uniformly effective [167, 169]. Furthermore, in line with the hypothesis that HSP70 induces resistance to apoptosis, triptolide sensitizes pancreatic cancer cells to TRAIL induced apoptosis. Pancreatic cancer cells are known to be highly resistant to TRAIL induced apoptosis, and the fact that inhibition of HSP70 by triptolide could sensitize to TRAIL induce apoptosis opens up novel avenues for combination therapy for pancreatic cancer for which therapies have largely been ineffective.

Triptolide has been evaluated in multiple animal models of pancreatic cancer and has been shown to be very effective in reducing the growth (Fig. (3A) and (3B)) as well as loco-regional spread of pancreatic tumors (Fig. (3C)) [163, 167]. Remarkably, inhibition of HSP70 expression by quercetin or triptolide does not induce apoptosis in normal pancreatic ductal cells [163, 167]. Though the reason for this specificity of anti-HSP70 therapy to the cancer cells is unclear, the data points toward certain mechanisms. The level of HSP70 in the normal ductal cells is very low as compared to pancreatic cancer cells. Thus, it seems that HSP70 expression is important for the survival of pancreatic cancer cells but may not be required for the survival of normal non-neoplastic cells under unstressed conditions [163]. Similar observations have been reported by others [170] where in contrast to cancer cells, non-transformed cells do not undergo cell death on depletion of HSP70 suggesting that tumor cells but not non-transformed cells cannot survive endogenously activated cell death if they lack HSP70 [171]. This along with the fact that triptolide is non-toxic to experimental animals clearly suggests that inhibition of HSP70 expression can emerge as a tumor selective therapy.

Similar efficacy of anti-HSP70 therapy has been demonstrated in other gastrointestinal and non-gastrointestinal tumors. Phillips *et al.* reported that besides inducing apoptosis in pancreatic cancer cells, triptolide induces cell death in colon cancer cell lines as well suggesting that anti-HSP70 therapy will be of broader value rather than for pancreatic cancer only [167]. Triptolide also induces apoptotic cell death in neuroblastoma cells and markedly reduces tumor growth in animal models [172]. Similar to its effects in pancreatic cancer cells, triptolide induces apoptotic cell death in cholangiocarcinoma cells and also sensitizes them to TRAIL induced cell death [173]. Thus, development of effective and bioavailable inhibitors of HSP70 expression will be useful in a wide variety of cancers as monotherapy or in combination with other chemotherapeutic agents. In this regard, triptolide [167] appears to be a very promising molecule which at very low doses (0.2 mg/kg) is able to inhibit HSP70 expression in mouse models and also produce anti-cancer effects with inhibition of both local tumor growth and loco-regional spread. One of the limitations in taking triptolide to clinical trial is that it is not soluble in water. This has been circumvented at the University of Minnesota by synthesis of its water-soluble derivative named minnelide. In pre-clinical trials (unpublished work), minnelide has been shown to be as effective as triptolide and clinical trials are in design phase. Once in clinical trials, minnelide will be one of the first HSP inhibitors to undergo basic and translational research against pancreatic cancer.

Targeting Drug Resistance

One of the reasons for poor prognosis of pancreatic cancer is the resistance to chemotherapy. Diagnosis of drug resistance in individual patients may improve treatment efficacy by avoiding inefficient treatment [174]. Targeting drug resistance of pancreatic cancer has drawn investigators' attention. Drug resistance in cancers may be inherent (intrinsic) or acquired during treatment. Primary cancer cell culture or use of established cell lines to test sensitivities to chemotherapeutic drugs as well as targeted therapies may help to detect intrinsic resistance to drugs. Clinically, this may generate "assay-assisted" therapeutic regimens which may achieve more successful outcomes, decrease side effects from possible resistance to treatment and reduce the cost [174]. Recently, Villarroel *et al.* reported establishment of mouse xenografts using fresh human pancreatic cancer samples obtained at surgery and establishment of a PancXenobank of tumor samples maintained in mice. Sensitivities to various drugs were tested using these samples as well as genomic and proteomic analysis. In one patient with advanced, gemcitabine-resistant pancreatic cancer, a personalized regimen with mitomycin C and cisplatin was designed based on these studies. The patient was treated successfully with minimal side effects and this patient has remained symptom free three years after surgery [175]. Many factors have been found to be related to drug resistance in pancreatic cancer, mainly to gemcitabine and 5-FU. Table (3) lists recently reported factors associated with chemotherapy resistance. Most of the investigations were performed in *in vitro* studies. Although antagonists of these factors achieved some effect in reversing drug resistance, additional efforts are required through pre-clinical and clinical studies. TMS1 demethylation agent, azacitidine, is in phase I clinical trials to treat pancreatic cancer. Notch signal pathway inhibitors MK0752 and RO4929097 in combination with gemcitabine are in current clinical trials to treat pancreatic cancer. Notch signaling is a mechanism that controls cell fate in a broad spectrum of cells [176]. Notch receptors are trans-membrane proteins expressed on the cell surface with four isotypes (Notch 1–4). Binding of Notch receptors to their ligands induces proteolytic cleavage of the receptor by γ -secretase and releases its intracellular domain to the nucleus, which subsequently transactivates target genes. In normal pancreas, Notch signaling is relatively inactive. The signal may be activated if there exists acinar dedifferentiation and/or epithelial cell proliferation. Moderate to high level expression of Notch receptors, their ligands as well as their target genes has been found in pancreatic intraepithelial neoplasms and pancreatic cancer [177, 178]. Notch signaling plays a substantial role in pancreatic cancer initiation, progression, and maintenance [177]. In gemcitabine-resistant pancreatic cancer cells, acquired epithelial-mesenchymal transition (EMT) was found to be associated with highly upregulated Notch signaling. Down-regulation of Notch signaling with siRNA partially reversed EMT through down-regulation of NF- κ B, thus resulting in up regulation of E-cadherin [179]. Among the isotypes of Notch receptors, Notch 2 and 3 are related to gemcitabine resistance [178, 180]. *In vitro* studies showed down-regulation of Notch 3 with siRNA increased the gemcitabine-induced apoptosis in BxPC-3 and PANC-1 pancreatic cancer cell lines with concomitant down-regulation of PI3K/Akt activity [180]. There are no pancreatic cancer clinical trial results available regarding the application of γ -secretase inhibitors MK-0752 and RO4929097.

OTHER TARGETED THERAPIES

Immunotherapy

1. **Algenpantucel-L Immunotherapy:** Algenpantucel-L is a preparation of irradiated, live, allogeneic human pancreatic cancer cells expressing the enzyme α -1,3 galactosyl transferase (α -GT). An antibody against the α -GT epitope of algenpantucel-L was induced which might possess an anti-tumor effect. In a recent clinical trial reported by Hardacre *et al.*, algenpantucel-L was used in combination

with standard adjuvant therapy in patients with resected pancreatic cancer. Kaplan-Meier estimated survival rates at 12 and 24 months were 91% and 54%, respectively, comparing favorably to 63% and 32% expected [192]. Median progression free survival was 16 months which compared favorably to the 11 months observed in RTOG 9704 [192]. Algenpantucel-L is now being tested in an ongoing phase III clinical trial.

2. **Cytotoxic T-lymphocyte Antigen-4 (CTLA-4):** Tumor antigens 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. 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, thus compromising host immunosurveillance (Fig. (4)) [193]. CTLA-4 was found in tumor-infiltrating immune cells in human pancreatic cancer [194]. Blocking CTLA-4 may play an important part in immunotherapy of pancreatic cancer. Ipilimumab and tremelimumab are two human anti-CTLA-4 antibodies that have been tested in clinical trials in pancreatic cancer patients [195]. In a phase II trial reported by Royal *et al.* recently, one patient showed regression of the primary lesion and 20 hepatic metastases after single agent treatment using ipilimumab, although most of the patients enrolled in this phase II trial did not show responses. The response was evidenced by tumor marker normalization and clinically improved performance status [196].
3. **Pomalidomide (CL-4047):** Pomalidomide is an immunomodulatory derivative of thalidomide which has been used to treat multiple myeloma. Its immunomodulatory effects have been discussed in a recently published review [197]. It is now in an ongoing clinical trial with other chemotherapy drugs to treat pancreatic cancer. In twenty evaluable patients with metastatic pancreatic cancer, three patients (15%) showed partial responses, and 10 (50%) showed more than 50% decreased CA19-19 levels [198].

Gene Therapy

BC-819—BC-819 (DTA-H19) is a double stranded DNA plasmid carrying the gene for the A subunit of diphtheria toxin under the regulation of the H19 gene promoter. H19 gene RNA was found in a variety of cancers including pancreatic cancer. Its anti-tumor effects have been shown in pre-clinical xenograft models [199]. This drug is now under investigation in a clinical trial to treat pancreatic cancer [200]. No clinical results are yet available from these trials.

Rexin-G—Rexin-G is a nonreplicative retroviral vector, which encodes a mutant human cyclin G1 gene. Blockade of cyclin G1 activity with Rexin-G induced inhibition of pancreatic cancer cell proliferation. A phase I study with Rexin-G in gemcitabine refractory and metastatic pancreatic cancer patients was carried out [201]. Gordon *et al.* reported three cases of metastatic pancreatic cancer patients with responses from stable disease to complete remission of primary lesion and decreased size of metastatic lesions in the liver. One patient used Rexin-G as a neo-adjuvant treatment and underwent surgery to remove the primary tumor [202].

Reolysin—Reolysin is a reovirus strain, which inhibits activated Ras kinase activity. In a phase I trial in solid tumors, overall clinical response was seen in 45% of patients enrolled

with one partial response and seven stable disease [203]. This drug is now in a phase II clinical trial for pancreatic cancer.

Conditionally replicative adenovirus (CRAd)—A CRAd under control of cyclooxygenase (COX) 2 was produced, which possesses the ability to replicate and spread within the tumor. In a pre-clinical study, administration of CRAd in combination with gemcitabine or 5-FU showed synergistic effects in some pancreatic cancer cell lines. Synergistic effects were also observed in a MIA PaCa-2 pancreatic cancer xenograft model [204].

hTNF- α vector—The vector AdEgr.TNF.11D produces TNF- α which induces apoptosis through the TNF receptor. Intra-tumoral injection of AdEgr.TNF.11D increased TNF- α expression and produced additional effects when combined with gemcitabine in a human pancreatic cancer xenograft model [205]. It is now in an ongoing clinical trial of pancreatic cancer.

X-linked inhibitor of apoptosis protein (XIAP) targeting—Small hairpin RNA (shRNA) targeting XIAP was cloned into a modified pU6.ENTR plasmid to generate pBlockIt.shXIAP plasmid for XIAP knockdown. In a human pancreatic cancer xenograft mouse model, XIAP silencing enhanced the sensitivity of pancreatic cancer cells to apoptosis induced by tumor necrosis factor-related apoptosis (TRAIL), and blocked lymphatic tumor cell dissemination [206].

Targeting telomerase—Imetelstat (GRN163L) is an oligonucleotide which carries a sequence complementary to the template region of human telomerase. Telomerase was reported as a critical enzyme in maintaining infinite replicative potential of cancer cells, especially cancer stem cells. *In vitro* treatment with imetelstat on pancreatic cancer cell line PANC1 showed reduced tumor engraftment and reduction of cancer stem cell levels [4].

CONCLUSIONS

The long list of investigative treatment methods for pancreatic cancer with low success rates indicates human pancreatic cancer is not a disease controlled by a simple pathological process. Discoveries in pathogenesis of pancreatic cancer, including abnormalities at the gene level and signal transduction pathways, revealed very valuable information. However, targeted therapies based on these discoveries have not changed the overall survival of this malignant disease clinically, despite very promising results in some pre-clinical studies. Pancreatic cancer apparently possesses treatment resistance via multiple pathways. Overcoming this resistance requires further investigation of these pathways. In pancreatic cancer, homeostasis of tissue has been severely altered. Observed pathological phenomenon in pancreatic cancer may relate to oncogenesis or metastasis, or may be the biological responses associated with cancer. The role of molecular targets in pancreatic cancer development need to be evaluated not only with *in vitro* studies or preclinical studies, but also at different stages of this disease using clinical samples. The importance of molecular targets at different stages during cancer development needs to be analyzed with integration of information collected from multiple pathways. New therapeutic approaches for pancreatic cancer need to be considered based on combined analysis of pathophysiological information obtained from multiple levels and dimensions. Successful molecular targeted and chemotherapy based regimens may be established based on this information. Gene mutation determination, proteomic analysis and integration efforts using advanced informatics may help to uncover the complicated pathological processes and expose the resistance mechanisms in pancreatic cancer patients. Critical pathways may also be defined through

examination of tumor samples from patients who responded to targeted therapies, such as the patients treated with the FOLFIRINOX regimen. In this review, we also identified several patients who responded well to ipilimumab and Rexin-G treatment. A thorough analysis of the samples from these patients may help investigators to gain insight into an effective treatment for pancreatic cancer.

FUTURE PERSPECTIVE

Previous investigations in basic research and clinical trials of targeted therapy of pancreatic cancer have built a good platform for further study to treat this dismal disease. Genomic and proteomic studies will be essential in generating useful approaches towards effective targeted therapies. This challenge needs to be accomplished by interdisciplinary efforts from cell biologists, pathologists, oncologists, geneticists, molecular biologists, proteomics investigators, and pharmacologists. Information obtained needs to be analyzed from multiple angles and levels, in order to better understand the network of pancreatic cancer pathogenesis, maintenance and progression. Importantly, the genomic studies need to be correlated with pathological characteristics of pancreatic lesions at different stages of development. An intensive investigation in genomics and proteomics using biopsy or surgical samples may supply valuable information for the design of molecular targeted strategies. In pre-clinical investigations, a cancer environment resembling human pancreatic cancer needs to be created and used to test different therapeutic methods. These efforts might help to establish an effective, combined targeted therapy with acceptable side effects, and eventually to establish a tailored, well tolerated and successful therapeutic regimen to improve the prognosis of pancreatic cancer.

Acknowledgments

Supported by NIH grant 2 P50 CA101955.

ABBREVIATIONS

ADCC	Antibody-dependent cellular cytotoxicity
AuNP	Gold nanoparticles
CEA	Carcinoembryonic antigen
DFS	Disease-free survival
DISC	Death-inducing signaling complex
DR	Death receptor
GPCR	G protein-coupled receptor
Hh	Hedgehog
HSG	Histamine-succinyl-glycine
IGF-1	Insulin/insulin-like growth factor 1
IGF-1R	Insulin-like growth factor-1 receptor
IR	Insulin receptor
MSLN	Mesothelin gene
PD	Progressive disease
PFS	Progression free survival

PLGF	Placenta growth factor
PR	Partial response
PSC	Pancreatic stellate cells
Ptc	Patched
RAAG12	N-linked carbohydrate antigen
SD	stable disease
SPARC	Secreted protein, acidic and rich in cysteine
SS1P	Single-chain Fv against mesothelin isolated from a phage display library
STAT3	Signal transducer and activator of transcription 3
TNFR	Tumor necrosis factor receptor
TRAIL	TNF-related apoptosis-inducing ligand

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Fig. 1. Hedgehog signaling pathway

Hedgehog ligand receptor (Ptc) is a transmembrane protein. **(A)** When there is no binding of Hh ligand to Ptc, Ptc represses the downstream protein Smo. Smo represses the downstream Gli family. **(B)** When Hh ligand binds to Ptc, Ptc activates Smo, which subsequently activates Gli family and induces transcription of Hh targeted genes.

Fig. 2. Delivery of nab-paclitaxel to cancer cells from blood stream

Injected nab-paclitaxel drug binds to albumin receptor, gp 60, through its albumin molecules which are transported into endothelial cells. The drugs are then transported across cells through formation of caveolae and released into extracellular space. The drugs selectively bind to SPARC, which are expressed on the stromal cells in pancreatic cancer tissue.

Fig. 3. Inhibition of HSP70 expression is highly effective as a therapeutic strategy in an animal model of pancreatic cancer

(A) Inhibition of HSP70 expression by triptolide markedly reduced the growth of MIA PaCa-2 tumors in an orthotopic model of pancreatic cancer in nude mice. Triptolide was administered at a dose of 0.2 mg/kg/day for 60 days. Animals were sacrificed at the 60th day and the tumor volumes in the triptolide group were compared with the control group (treated with vehicle alone). $n=8$ in each group, $*=p<0.05$. (B) Representative pictures of tumor tissue in the control and triptolide treatment group. (C) Table demonstrating reduced loco-regional spread in the triptolide treatment group. ($p < 0.001$, $n = 8$, χ^2 analysis) [167].

Fig. 4. T cell activation in immunosurveillance

(A) Tumor antigens are expressed by antigen-presenting cells (APCs). T cells are activated by the binding of expressed tumor antigens to T cell receptors. The activation requires the binding of CD80/CD86 from APCs to CD28 which is expressed on T cell surface. (B) CTLA-4 is a homolog of CD28 expressed on T cells. CTLA-4 can bind to CD80/CD86 with a high affinity. This binding prevents the activation of T cells even in the presence of tumor antigen on APCs. Blocking the binding between CTLA-4 and CD80/CD86 may resume T cell activation and enhance immunosurveillance.

Table 1

List of Small Molecule Kinase Inhibitors in Ongoing Pancreatic Cancer Clinical Trials

Targets	Inhibitors	Drugs Combined in Trials	References
EGFR, ErbB2	Lapatinib (GW572016)	Gemcitabine	*
		Gemcitabine/oxaliplatin	[96]
		Oxaliplatin/folinic acid/5-fluorouracil	[97]
		Oxaliplatin, cetuximab	*
	Erlotinib	Capecitabine	*
		MK-0646 (IGF-1R antibody), gemcitabine	[68]
		Isoflavones, gemcitabine	[98]
		Gemcitabine, IMC-A12	[21]
		Apricoxib, gemcitabine	[99]
		Gemcitabine, capecitabine	[100]
		Bevacizumab	[101]
		Bevacizumab, gemcitabine, capecitabine	[102]
		Gemcitabine	[103–105]
		Gemcitabine vs. gemcitabine/docetaxel	[106]
		Capecitabine, radiation	[107]
		Rapamycin	[108]
		Oxaliplatin	*
		GDC-0449±gemcitabine	*
		Gemcitabine, cisplatin	*
		Pertuzumab	*
		Gemcitabine+trastuzumab	*
		ASD6244 (selumetinib)	*
		Capecitabine, bevacizumab	*
		Gemcitabine, nab-paclitaxel	*
VEGFR	Pazopanib		[109]
		Paclitaxel	[110]
	Axitinib (AG013736)	Gemcitabine	[111]
PDGFR	Imatinib (glivec)	Gemcitabine, oxaliplatin	[112]
IGF-1R	BMS-754807		[69]
mTOR	Temsirolimus (CCI-779)		[108, 113, 114]
	Everolimus (RAD001)	Gemcitabine	*
		Irinotecan, cetuximab	*
		Cetuximab, capecitabine	*
MEK 1/2	AS70326 (MSC1936369B)		*
Multiple kinases	Sorafenib (Bay43-9006)	Tipifarnib	[115]
		Gemcitabine	*
		Gemcitabine, radiation	*

Targets	Inhibitors	Drugs Combined in Trials	References
		Everolimus	*
		Erlotinib	*
	GSK 1363089 (foretinib)	Gemcitabine	[116]
	Sunitinib		*
Src family	Saracatinib (AZD 0530)		[117]
	Dasatinib		*

*
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Table 2

Clinical Trial Results of GDC-0449, Nab-paclitaxel and Bevacizumab

Investigator	Phase	Patient numbers	Drug combined	Results	References
GDC-0449					
Lorusso <i>et al.</i>	I/II	68 solid tumor (8 PC)	Monotherapy	20 response, 14 SD (no PC), 8 PD	[159]
Nab-paclitaxel					
Chien <i>et al.</i>	I	25 solid tumors(2 PC)	Lapatinib	65% PR or SD (no pancreatic cancer patients)	[160]
Hosein <i>et al.</i>	II	19 PC (progressed on gemcitabine)	Monotherapy	6 mo OS 58%, median OS 7.3 mo, median PFS 1.6 mo, 1 pt PR, 6 pts SD, 12 PD	[152]
Isacoff <i>et al.</i>	Pilot	28 PC (advanced)	5-FU (low dose), leucovorin, oxaliplatin, bevacizumab	1 CR, 13 PR, OS>8 months	[154]
Thapliya	I/II	13 PC (unresectable/borderline resectable)	Gemcitabine	9 PD, 3 SD, 1 PD, EORTC PET response: 7/10 PR, 3/10 SD	[153]
Bevacizumab					
Berlin <i>et al.</i>	II	25 PC (resected tumor)	Gemcitabine, capecitabine, radiation	At 2 years: DFS 22%, OS 37%	[22]
Watkins <i>et al.</i>	I/II	39 PC (advanced)	Gemcitabine, capecitabine, erlotinib	Radiological response 23%, median PFS 8.5 mo, median OS 12.8 mo Median & 1 year survival 11.1 mo & 49% (pts with metastasis), 14.8 mo & 89% (pts with local advanced)	[102]
Martin <i>et al.</i>	II	39 PC (advanced)	Gemcitabine, 5-FU	Freedom from progression in 53% pts (24 weeks), 24% PR, 47% SD, mean time to progression 8.1 mo, median survival 10.7 mo	[161]

Abbreviations: PC, pancreatic cancer, PR, partial response, SD, stable disease, OS, overall survival, DFS, disease-free survival, PD, progressive disease, PFS, progression free survival.

Table 3

List of Factors Related to Chemotherapy Resistance in Pancreatic Cancer

Factors	Description	Antagonist	References
CXCL12-CXCR4 (chemokine receptor)	Activation of FAK, ERK, Akt, β -catenin and NF- κ B	AMD3100	[181]
Multidrug resistance protein 5 (MRP5)	Gemcitabine and 5-FU upregulate MRP5	SiRNA	[182–184]
MUC1	Overexpressed MUC1 decrease intracellular drug concentration	Genzyl- α , GalNAC	[185]
14-3-3 σ	Increases cell cycle arrest upon DNA damage, resistant to drug induced apoptosis	SiRNA	[186]
TMS1	TMS1, a pro-apoptotic gene was silenced by methylation	Azacitidine, decitabine	[187, 188]
Notch signaling	Anti-apoptosis through activation of PI3K/Akt	MK0752, RO4929097	[177, 189–191]