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Evolution of novel therapeutic options for pancreatic cancer

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Abstract

Purpose of Review—Pancreatic cancer is the most devastating of all cancers with an extremely poor prognosis. In US alone over 50,000 new cases of pancreatic cancer are reported annually and about the same number succumb to it, making pancreatic cancer the 3rd most common cause of cancer deaths. Most patients with pancreatic cancer present with advanced disease, which cannot be resected surgically and for these patients chemotherapy is the only option. Even patients who undergo resection require adjuvant therapy to decrease the risk of recurrence. Since 1950's a variety of different agents, like anti-metabolites, nucleoside analogs and DNA intercalating compounds have been used against pancreatic cancer, alone or in combination, with little improvement in the survival statistics. The current article reviews the evolution of chemotherapy for pancreatic cancer and discusses some novel therapeutic options that are emerging in recent times, with special emphasis on Minnelide, a novel HSP70 inhibitor which is currently in clinical trials.

Recent Findings—Approaches towards developing therapies for pancreatic cancer have evolved tremendously over last decade. Research has shown that apart from the inherent drug resistance, drug delivery to pancreatic cancer has also posed a major challenge. The extensive desmoplastic stroma of pancreatic cancer is believed to create inordinately high interstitial fluid pressures leading to vascular collapse and substantial barrier to perfusion of chemotherapeutics, thus creating an additional layer of protection for pancreatic cancer. Recent research thus is focused not only on understanding the biology of and developing strategies to target cancer cells, but also targeted towards depletion of stroma in order to ensure better delivery of chemotherapeutic compounds to the tumor.

Summary—The current article describes the novel therapies that are constantly being evaluated to address and overcome the challenges that make pancreatic cancer a difficult disease to treat.

Keywords

Pancreatic Cancer; Chemotherapy; Minnelide; HSP70; Gemcitabine; 5-fluorouracil

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Conflict of Interest: University of Minnesota has a patent for Minnelide, which has been licensed to Minneamrita Therapeutics, LLC. AKS is the co-founder and the Chief Scientific Officer of this company. Dr. Banerjee is a compensated consultant with Minneamrita Therapeutics LLC, the licensee of the intellectual property being used in the study and this relationship is managed by University of Miami.

Conflicts of interest: None

Introduction

Pancreatic cancer is the most devastating of all cancers with a dismal survival rate. In US alone over 50,000 new cases are reported for this disease annually and about a same number succumb to it, making pancreatic cancer the 3rd most common cause of cancer related deaths. 5-year survival rate of all patients with pancreatic cancer is about 5% and this figure has remained relatively unchanged over the past 25 years [1,2]. Diagnosis at advanced stages and overall aggressive biology are the two major challenges in the field of pancreatic cancer. The majority of patients present with locally advanced or metastatic disease, and such individuals have median survivals of 6–10 months and 3–6 months, respectively [3]. Even when diagnosed at an early and potentially resectable stage, the outcome continues to be poor as most patients who undergo resection of pancreatic cancer experience recurrence of disease [4]. Since 1950's a variety of different agents, like anti-metabolites, nucleoside analogs and DNA intercalating compounds have been used against pancreatic cancer, alone or in combination with other agents, with little improvement in the survival statistics. Focused research on the biology of pancreatic cancer and its microenvironment is needed to better understand the disease and develop effective therapies.

Adjuvant Therapy for Pancreatic Cancer

Over the decades cancer therapy has been primarily directed against the rapidly dividing cancer cells. One of the common group of drugs targeting the rapidly dividing cells are the anti-metabolites like 5-fluorouracil and Gemcitabine. Fluorinated pyrimidine, 5-fluorouracil or 5'FU was one of the first chemotherapeutic agents to be used against pancreatic cancer and has continued to be employed for almost 40 years [5]. Given the poor outcomes of patients with even resectable pancreatic cancer, clinicians sought better therapies. The 'Gastrointestinal Tumor Study Group' trial was the earliest to demonstrate a benefit and generated interest in adjuvant therapy for patients with pancreatic cancer. Though it included less than 50 resected pancreatic cancer patients who were randomized into observation only or 5'FU based chemoradiation, the study found that the receiving adjuvant therapy had the longest median survival [6]. The case for adjuvant therapy was further strengthened by a landmark European trial, ESPAC-1 [7]. This multi-institutional study had a complex trial design, which evaluated the role of chemotherapy, with or without chemoradiotherapy for the treatment of pancreatic cancer. This landmark trial clearly demonstrated that adjuvant chemotherapy had a significant survival benefit in patients with resected pancreatic cancer. Intriguingly, this trial also demonstrated that chemoradiotherapy may actually be deleterious. Based on this trial, chemoradiotherapy is not used in Europe for adjuvant treatment of patients with pancreatic cancer, even though it continues to be used in North America.

In a trial by Charite' Onkologie investigators (CONKO-001), role of single-agent gemcitabine was compared to observation alone following surgical resection of pancreatic cancer. In this trial gemcitabine was found to be effective in doubling the disease free survival, though only with a modest improvement in overall survival. The results of CONKO-001 have provided level-1 evidence in support of gemcitabine as a single agent for adjuvant therapy. Though these trials demonstrated that both 5'FU and Gemcitabine have modest efficacy against pancreatic cancer in an adjuvant setting, whether one is better than

the other remained un-answered. This was addressed in ESPAC-3 trial of over 1000 patients with pancreatic cancer randomized to gemcitabine or 5'FU with folinic acid after curative resection[8]. In this trial, median survival for patients in gemcitabine and 5'FU with folinic acid group was 23 and 23.6 months, respectively. While detailed discussion of other major adjuvant therapy trials is beyond the scope of current review, few general principles have emerged.

1. Adjuvant systemic therapy, either gemcitabine or 5'FU/leucovorin for 6 months represents standard of care.
2. Adjuvant chemoradiation has not been shown to have clear advantage over chemotherapy, though it may lead to better local tumor control.

In coming years, we should see extrapolation and evaluation of some of the regimens found to be effective in metastatic situation e.g. FOLFIRINOX in adjuvant setting.

Evolution of therapeutic regimens for metastatic and locally advanced pancreatic cancer

Similar to role of gemcitabine in adjuvant therapy, gemcitabine continued to be the standard of care for treatment of patients with metastatic and locally advanced pancreatic cancer for almost two decades. The role of gemcitabine for treatment of metastatic and locally advanced disease was established by a North American study where Burris et al randomized one hundred twenty-six patients with advanced pancreatic cancer to receive either gemcitabine 1,000 mg/m² weekly for 7 weeks followed by one week of rest, then weekly for 3 weeks every 4 weeks thereafter (63 patients), or to 5-FU 600 mg/m² once weekly (63 patients). Clinical benefit response, which was a unique composite measure of pain, Karonofsky performance status and weight, was experienced by 23.8% of gemcitabine-treated patients compared with 4.8% of 5'FU-treated patients (P=0.0022). The median survival durations were 5.65 and 4.41 months for gemcitabine-treated and 5'FU-treated patients, respectively (P=0.0025). The survival rate at 12 months was 18% for gemcitabine patients and 2% for 5'FU patients. Since gemcitabine was more effective than 5'FU in alleviating some of the disease-related symptoms in patients with advanced pancreatic cancer (in spite of a modest survival advantage), it was accepted by FDA as well as the oncology community as the new standard of care for advanced pancreatic cancer [9,10].

Poor outlook of patients with advanced pancreatic cancer and lack of substantial improvement with use of the anti-proliferative compounds (as Gemcitabine and 5'FU) alone has led oncology community to develop and evaluate combination therapies. Since the approval of gemcitabine in 1997, many cytotoxic and targeted agents have been pitted against, or combined with gemcitabine in clinical trials for patients with metastatic or locally advanced pancreatic cancer. No drug alone or in combination was shown to make a substantial breakthrough when compared to single-agent gemcitabine, until recently. In 2010, the FOLFIRINOX regimen (bolus and infusional 5-fluorouracil, irinotecan, and oxaliplatin) emerged as a major treatment advance for patients with metastatic pancreatic cancer. In a trial with 342 patients, FOLFIRINOX yielded a longer median overall survival (11.1 vs. 6.8 months, hazard ratio [HR] 0.57, p < 0.001), a superior progression-free survival

(6.4 vs. 3.3 months, HR 0.47, $p < 0.001$), a higher objective response rate (31.6% vs. 9.4%, $p < 0.001$), and a significant increase in time until definitive deterioration in quality of life, compared with gemcitabine [11].

Another combination regimen which has been shown to be more effective than gemcitabine alone is its combination with *nab*-paclitaxel or Abraxane. *Nab*-paclitaxel is nanoparticle albumin bound paclitaxel that allows an increased dosage of paclitaxel without the toxicity of the vehicle. In the study conducted by Von Hoff et al, nab-paclitaxel plus gemcitabine showed increased tumor regression and significantly improved overall survival in treatment-naive patients with metastatic pancreatic cancer, compared with gemcitabine alone [12].

Targeted therapy in pancreatic cancer

As summarized by Leach and Sinha in this journal, in recent years we have dramatically increased our understanding of the pathogenesis of pancreatic cancer, but this has not yet translated into development of effective targeted therapies. An example is anti-Epidermal Growth factor receptor (EGFR) therapy. EGFR is a receptor tyrosine kinase of the ERB-B family that is abnormally activated in a number of epithelial tumors. In pancreatic cancer, the co-expression of EGFR and its ligands correlates with the aggressiveness of the tumor [13]. Targeting EGFR has been attempted to achieve therapeutic advantage. Addition of EGFR inhibitor erlotinib to gemcitabine had a trivial effect, only increasing median survival of patients with pancreatic cancer patients from 5.9 months to 6.4 months when compared to gemcitabine alone [14]. Similarly, Ras-Farnesyltransferase and the MMP inhibitors [15], Cetuximab (another EGFR inhibitor) and VEGF inhibitor Bevacizumab [16] have been largely found ineffective in the treatment of pancreatic cancer. Whether individualization of therapies, based on the genetic composition of a patient's specific mutational landscape, will improve outcomes is being evaluated.

Stroma as a barrier to chemotherapy

Pancreatic ductal adenocarcinoma is histologically characterized by the abundance of extracellular matrix (ECM), commonly also referred to as desmoplasia. ECM essentially includes collagen, fibronectin, proteoglycans, and hyaluronic acid, along with catalytically active enzymes and proteinases. As a result of accumulation of ECM components, the normal architecture of the pancreatic tissue is distorted resulting in compression of blood and lymphatic vessels in the tumor [17-21]. Based on this observation, it was hypothesized that the desmoplastic stroma acted as a barrier to drug delivery in the tumor. This was confirmed in a study by Tuveson in which the concentration of 20,2-difluorodeoxycytidine triphosphate (dFdCTP), an active intracellular metabolite of gemcitabine, was high in stroma-poor subcutaneous or orthotopic xenografts/syngenic transplants, but not detectable in stroma-rich PDA tumors in a genetically engineered mouse model [19,21]. Further analysis revealed that transplanted tumors exhibited an increased vascular content and function as compared with primary murine tumors and human PDA. The rigidity of the ECM in the stroma is thus responsible for compressed blood vessels, leading to decreased perfusion that impedes the delivery of drugs to neoplastic pancreas cells.

Sonic hedgehog (SHH) signaling has been shown to be restricted to the stromal compartment [21,22]. Thus, pharmacologic inhibition of the Shh pathway was thought to have a positive impact on gemcitabine delivery, by reducing the desmoplastic stroma. As hypothesized, a study involving combination of the Smoothed inhibitor (IPI-926) and gemcitabine caused depletion of tumor stroma and resulted in increased microvessel density [19]. This tumor microenvironment alteration significantly enhanced intratumoral concentrations of dFdCTP, transient disease stabilization, and a survival benefit [19]. Unfortunately, the phase II trial of the Smoothed inhibitor IPI-926 plus gemcitabine (NCT01130142) was terminated early 2012 because of a lack of benefit.

Another possible strategy to relieve vessel compression and aid drug delivery is to enzymatically degrade the ECM scaffold. Many cancers are rich in hyaluronan (HA), a high molecular weight glycosaminoglycan that retains water due to its high colloid osmotic pressure [23]. This provides elasticity to connective tissue and promotes wound healing in healthy organs, but excessive HA accumulation in solid tumors may raise interstitial fluid pressure and compress blood vessels. Using a spontaneous mouse model of PDA, enzymatic remodeling of the ECM using a hyaluronan degrading enzyme has shown promise. Hyaluronan degradation by hyaluronidase PEGPH20 decreased interstitial fluid pressure in murine PDA tumors. Consequently, increased vessel patency, drug delivery, and survival were also observed [24,25]. PEGPH20 is currently being evaluated for better delivery of gemcitabine in an ongoing phase I/II trial [26,27].

Immune therapy in pancreatic cancer

Pancreatic cancer is often characterized by the early and prominent infiltration of immunosuppressive leukocytes into the tumor stroma. Immunosuppressive cells, including Tumor Associated Macrophages (TAMs), Gr-1+ CD11b+ myeloid cells, and regulatory T cells (Treg), are prominent at the earliest stages of neoplasia and persist through invasive cancer [28,29]. However, intratumoral effector T cells are rare. This pathophysiology is in contrast to many other solid tumors for which infiltration of effector T cells is often prominent and associated with improved clinical outcomes.

Antigens targeted in immunotherapy clinical trials in PDA have included MUC1, mesothelin, KRAS, carcinoembryonic antigen, survivin, and telomerase, as well as whole tumor cells engineered to express granulocyte macrophage colony-stimulating factor [GM-CSF]. In the first phase I clinical trial using irradiated allogeneic GM-CSF-expressing tumor cell vaccines the treatment was well tolerated and found to be safe for use in humans [30]. This result warranted a larger phase II trial to investigate the disease-free and overall survival after surgical resection followed by chemoradiation and vaccination, which reported a median survival of 24.8 months [31]. Another approach is to pulse dendritic cells with tumor antigens ex vivo and reinfuse them into patients.

The PDA tumor microenvironment is predominantly infiltrated with suppressive immune cells and signals that if blocked could allow effective immunotherapy. Single-agent checkpoint inhibitors effective in other human cancers such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death 1 (PD-1), and its ligand PD-L1, have unfortunately

failed to demonstrate objective responses when given as single agents to PDA patients. However, inhibition of the CTLA-4 pathway, when given together with a T cell inducing vaccine gives objective responses in metastatic PDA patients. Combination therapy with vaccine and PD-1 antibody blockade improved murine survival compared with PD-1 antibody monotherapy or GVAX therapy alone. Furthermore, PD-1 blockade increased effector CD8 T lymphocytes and tumor-specific interferon- γ production of CD8 T cells in the tumor microenvironment. Immunosuppressive pathways, including regulatory T cells and CTLA-4 expression on T cells, were overcome by the addition of vaccine and low-dose cyclophosphamide to PD-1 blockade. These indicate that a PD-1 or PD-L1 antibody therapy with a T cell inducing agent for PDA treatment has scope for further evaluation in improving patient survival in pancreatic cancer[32,33].

Novel Therapy for Pancreatic Cancer: Minnelide

Targeting Heat Shock Proteins

Living cells have evolved several protective strategies to ensure survival under stressful conditions. Synthesis of heat shock proteins (HSPs) is one such highly conserved mechanism. Initially, HSPs were believed to be elevated in response to thermal stress; however, now it is known that HSPs are expressed in response to an array of stresses including oxygen-derived free radicals, amino acid analogs, ethanol and heavy metals. Intriguingly, cancer cells also overexpress HSPs and utilize their pro-survival function to their own advantage [34]. We have shown that HSP70, a 70-kDA member of the HSP family, is overexpressed in multiple pancreatic cancer cell lines when compared to normal ductal cells [35]. Similarly, we observed that in human pancreatic cancer tissue HSP70 was overexpressed when compared to surrounding normal pancreatic tissue. That HSP70 overexpression protects cancer cells from cell death was clear from the observation that when we inhibited HSP70 expression by siRNA, it led to apoptotic cell death in cancer cells [35]. This together suggested that HSP70 inhibition could emerge as a therapeutic strategy for pancreatic cancer. We have also evaluated the mechanism by which HSP70 protects cancer cells from cell death and shown that HSP70 stabilizes the lysosomes and attenuates cytosolic calcium. When HSP70 levels decrease, increased cytosolic calcium and lysosomal enzymes released into the cytosol induce apoptotic cascade leading to cell death [36].

Triptolide, an active compound from a Chinese herb, is a potent HSP70 inhibitor. We found that triptolide causes tumor regression, reduces pancreatic tumor growth and prevents metastases in orthotopic models of pancreatic cancer[37]. Triptolide is also effective against colon cancer [38], hepatocellular cancer [39], osteosarcoma [40] and neuroblastoma [41]. Though triptolide was very effective against experimental pancreatic and other cancers, its clinical use has been hampered by its insolubility in water. A water soluble analog of triptolide, named Minnelide overcomes this limitation. This derivative was active against PDA in multiple animal model, simulating multiple clinical scenarios and using orthotopic tumors from cancers of varied aggressiveness [42]. In the aggressive Aspc-1 cell line, tumors were treated with Minnelide showed no recurrence following drug discontinuation. Similar results were observed using Minnelide against human patient derived xenograft model (Figure 1). Minnelide is currently in phase I clinical trial at University of Minnesota and

Honor Health at Scottsdale Arizona, against a variety of advanced gastrointestinal malignancies and preliminary results are very promising.

Minnelide as combination therapy

Since monotherapy is often unsuccessful against an aggressive disease like pancreatic cancer, we have also evaluated Minnelide in combination with standard of care chemotherapies. Pancreatic cancer cells often develop resistance to platinum based therapy like oxaliplatin by overexpressing DNA Damage repair genes. We have demonstrated that in animal models of pancreatic cancer Minnelide synergizes with oxaliplatin leading to remarkable tumor regression [43]. Such combinations promise to reduced doses of drugs, produce less toxicity, and enhance efficiency over monotherapies. Mechanistically we have demonstrated that Minnelide suppresses DNA damage repair genes induced by oxaliplatin resulting in overcoming resistance to oxaliplatin. Since 2013, the gemcitabine/Abraxane combination has emerged as a new combination therapy regimen for pancreatic cancer. Unpublished and ongoing results from our laboratory show that low dose Minnelide in combination with standard doses of Gemcitabine and Abraxane is significantly better in decreasing tumor burden in animals, improving their survival and decreasing metastasis. These results from combination studies are important as in future phase II trials, Minnelide will be administered in combination with standard of care chemotherapy.

Minnelide as an anti-stromal agent

As discussed above, the presence of reactive fibro-inflammatory stroma is a challenge for PDA therapy and can restrict drug delivery [44,45]. We recently reported that Minnelide interferes with the synthesis of the hyaluronan in the stroma and also prevents crosslinking of the collagen molecules leading to depletion of the stroma. This in turn enhances drug delivery to the tumor [46]. Though it is feared that opening functional tumor blood vessels could increase metastasis, this drug appears to combine enhancement of drug delivery with effectively tumor killing, thus providing a net benefit [46].

Mechanisms of Action of Minnelide

The mechanism of Minnelide, including its inhibition of HSP70, remains unclear. Though high very high concentrations of triptolide can block global transcription by inhibiting subunits of RNA polymerase II. [47], the concentrations of triptolide causing cell death *in vitro* (200nM) and tumor regression *in vivo* (0.42mg/kg body weight of mouse) are far below that concentration. Further, how this drug class inhibits NF- κ B [48] or induces ER-stress [49] leading to cancer cell death remains unclear, but suggests a common master-regulator may be responsible. Our preliminary studies suggest that HSF1, a target of this drug class, may serve this role. HSF1 may be transcriptionally linked to Sp1, a transcription factor that stimulates proliferation and is over-expressed in PDA. Our recent findings show that Triptolide downregulates the transcriptional activity of Sp1, thereby starting a cascade that leads to pancreatic cancer cell death [48]. Our current understanding of the mechanism of action of Minnelide is summarized in Figure 2.

Conclusion

Though pancreatic cancer is a devastating disease with a poor outcome, it is reassuring that extensive research on its oncogenesis and biology has helped us to understand this cancer and change the focus of research. There is a rapidly advancing body of information about pancreatic cancer genetics. The hallmark of pancreatic cancer is the very complex and sequential acquisition of variety of genetic alterations that are involved in most aspects of carcinogenesis, resulting in deregulation of a number of pro-survival pathways. Based on the emerging knowledge, drugs targeting stromal collapse, immune surveillance and many other critical oncogenic pathways are going to be the aspects that need to be followed in developing an effective therapy against this disease.

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Key points in the review

1. Gemcitabine/*nab*-paclitaxel is regarded as the standard of care in pancreatic cancer.
2. Checkpoint inhibitors may be useful in pancreatic cancer when used in combination with other agents
3. Minnelide is a novel therapy that targets cancer epithelial cells and stromal cellos and is currently under Phase I clinical trial for GI malignancies.

Figure 1.

Minnelide increases percent overall survival in a human xenograft model. A de-identified patient pancreatic tumor was implanted into SCID animals. When tumors reached a size of $\sim 300\text{mm}^3$, Animals were randomized into vehicle alone or Minnelide treatment (0.42 mg/kg). In the 300mm^3 group, treatment was terminated on Day 55 and the animals were followed for recurrence of tumor (Minnelide Stop). Also, when tumors in the vehicle alone group reached 1000mm^3 the animals were also started on Minnelide, to see the ability of drug to shrink large tumors. In the 1000mm^3 group, treatment was continued until the termination of the experiment on Day 120[42].

Figure 2.
Proposed potential mechanism of action of Minnelide.