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## Current Status of Biomarker and Targeted Nanoparticle Development: The Precision Oncology Approach for Pancreatic Cancer Therapy

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### Abstract

Pancreatic cancer remains one of the major causes of cancer-related mortality. The majority of pancreatic cancer patients are diagnosed at the advanced stage with unresectable and drug resistant tumors. The new treatments with the combination of chemotherapy, molecular targeted therapy, and immunotherapy have shown modest effects on therapeutic efficacy and survival of the patients. Therefore, there is an urgent need to develop effective therapeutic approaches targeting highly heterogeneous pancreatic cancer cells and tumor microenvironments. Recent advances in biomarker targeted cancer therapy and image-guided drug delivery and monitoring treatment response using multifunctional nanoparticles, also referred to as theranostic nanoparticles, offer a new opportunity of effective detection and treatment of pancreatic cancer. Increasing evidence from preclinical studies has shown the potential of applications of theranostic nanoparticles for designing precision oncology approaches for pancreatic cancer therapy. In this review, we provide an update on the current understanding and strategies for the development of targeted therapy for pancreatic cancer using nanoparticle drug carriers. We address issues concerning drug delivery barriers in stroma rich pancreatic cancer and the potential approaches to improve drug delivery efficiency, therapeutic responses and tumor imaging. Research results presented in this review suggest the development of an integrated therapy protocol through image-guided and targeted drug delivery and therapeutic effect monitoring as a promising precision oncology strategy for pancreatic cancer treatment.

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## Keywords

pancreatic cancer; molecular imaging; targeted therapy; image-guided cancer therapy; theranostic nanoparticles

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## Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States [1, 2]. In 2016, it is estimated that 53,070 new cases of pancreatic cancer will be diagnosed in the US, and 41,789 pancreatic cancer patients will die as a result of this disease [1]. Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer type (over 95%) [2]. Because of its aggressive biological nature and the ineffectiveness of current treatments, PDAC has a mortality rate almost equal to its incidence with a five-year survival rate of 5%. One important reason for the poor survival is that the majority (over 85%) of the patients are diagnosed with advanced diseases and have a poor prognosis [1]. Only few patients (approximately 15%) are diagnosed when they are at the earliest-stage of PDAC and are candidates for the potentially curative surgery [3].

A challenge for screening and early detection of PDAC is that patients lack specific clinical symptoms at the early stage and the risk factors are not well known except for smoking and family history [4]. A major problem is that human pancreatic cancer is highly heterogeneous with a tumor mass from a single patient containing 63 genetic alterations and 12 core signal pathway abnormalities. There are also heterogeneity in tumor biomarker expression among different cancer patients [5]. Currently, blood-test based on biomarkers and imaging methods are clinical standard due to the ease of operation, and relatively noninvasive nature [4, 6]. Tumor biomarkers that allow for the reliable diagnosis of pancreatic cancer have yet to be identified. However, investigations are ongoing to evaluate the effect of several biomarkers for pancreatic cancer detection. So far, the levels of serum carbohydrate antigen (CA 19-9), CA-125, ICAM-1, CEA, mutant Kras DNA, miRNAs, and glypican-1 in exosomes [7, 8] have been evaluated as serum biomarkers for the detection of pancreatic cancer. However, many of those biomarkers are not specific for pancreatic cancer since they are also expressed in other tumor types and some of them on normal and premalignant tissues. It is likely that the combination of serum biomarker detection with the biomarker targeted molecular imaging to localize and characterize pancreatic cancer lesions will improve sensitivity and specificity of the early detection of pancreatic cancer.

At present, various targeted imaging probes have been developed for non-invasive tumor imaging using different imaging modalities. For example, nanoparticle imaging probes targeting plectin-1, mesothelin, uPAR, EGFR, and IGF-1R are under investigations for the detection of pancreatic cancer [9–12]. With the development of nanomedicine, it is expected that nanomaterials modified with PDAC targeting ligands can facilitate the early diagnosis of PDAC so that personalized therapeutic strategy can be designed and applied timely to the patients. Additionally, biomarker targeted imaging nanoparticles are not only promising imaging contrasts for tumor detection, but also for the evaluation of the biomarker

expression in primary and metastatic pancreatic cancers for stratifying the patients with biomarker positive tumors for targeted therapy.

Currently, therapeutic options applicable to PDAC are still limited to surgery, chemotherapy and radiotherapy. Most PDAC patients have advanced diseases and are treated by chemotherapy. However, most chemotherapeutics are not very effective with minimal impact on survival in most cases. For example, advanced PDAC patients receiving gemcitabine (2'-2'-difluorodeoxycytidine) or the combination of fluorouracil, oxaliplatin, irinotecan (CPT-11) and folinic acid (FOLFIRINOX) treatment, still have median survival less than 12 months [13]. One reason for this unfavorable treatment outcome is the presence of “desmoplasia” in PDAC, which is known as tumor stroma with characteristics of abnormal and poorly functioning vasculatures, altered extracellular matrix, infiltrating macrophages and proliferation of active fibroblasts. Intense tumor stroma consisting of 50–80% of the PDAC tissues creates a drug delivery barrier for many therapeutic agents, including small molecules, higher molecular weight antibody-based drugs, and nanoparticle formulations. In addition, PDAC cells are highly resistant to chemotherapy drugs [14], because of abnormal levels of gene expression, genetic mutations, activation or inhibition of cellular signal pathways, tumor hypoxia, and the stroma-rich tumor microenvironment [15]. Therefore, new and more potent therapeutic agents and novel treatment protocols will improve the clinical outcomes of pancreatic cancer. Since pancreatic tumor microenvironment plays critical roles in tumor biology, drug delivery and response to therapy, it is necessary to consider the stroma effect for designing and evaluation of new therapeutic agents [16].

In this article, we will provide an overview of the current research regarding potential biomarkers or molecular targets in pancreatic cancer for the development of targeted therapy and imaging for pancreatic cancer PDAC with a particular focus on nanoparticle or theranostic nanoparticle drug carriers.

## Pancreatic cancer biomarkers and molecular targets

Over the years, intensive efforts have been devoted to identify biomarkers and molecular targets for the development of targeted molecular imaging and therapeutic approaches [17]. In comparison with other solid tumors, PDAC has unique pathological characteristics to consider when developing imaging and drug delivery agents. Several molecules that are currently under evaluation for PDAC targeted treatment based on their roles in tumorigenesis and progressions will be reviewed here (Figure 1). Although these biomarkers may not be specific for PDAC, the detection of the presence of high levels of the biomarkers in suspected pancreatic lesions should not only provide supportive information for diagnosis of pancreatic cancer, but also identify the cell surface molecular targets for the development of biomarker targeted nanoparticle drug carriers, or theranostic nanoparticles. The following are examples of cell surface biomarkers that are highly expressed in pancreatic cancer for the development of tumor targeted imaging and therapeutic agents:

### Mesothelin

Mesothelin is a membrane type protein that is normally expressed in mesothelial cells of pleura, peritoneum and pericardium. A high level of mesothelin is detected in various types

of cancers, including mesothelioma, non-small cell lung, ovarian and pancreatic cancers [18]. Studies so far have reported that mesothelin is related to cell survival, migration, invasion and tumor progression [19, 20]. It was also uncovered that silencing the expression of mesothelin suppressed tumor growth [21], implying that mesothelin can be a potential marker for cancers, including PDAC [22]. Indeed, mesothelin was detected in PDAC tissues but not in normal pancreas and chronic pancreatitis [23]. It was reported that significantly elevated levels of circulating mesothelin protein were detected in 73 of the 74 patients with pancreatic adenocarcinoma, and in all five patients with benign pancreatic disease, but not in the healthy controls [24]. As the most of the other PDAC biomarkers, mesothelin is not a PDAC specific biomarker but can be combined with other biomarkers for confirming diagnosis of PDAC [25]. Recent studies have shed light on the possible role of mesothelin as an antigen for immunotherapy. A positive correlation between prognosis and the presence of a humoral response to mesothelin has been shown in pancreatic cancer patients [26]. It has been reported that modification of imaging agents and drug-delivery systems with anti-mesothelin antibody would improve diagnostic and therapeutic efficiency in mesothelin overexpressing PDAC [27]. Mesothelin antibody conjugated liposomes loaded with iron oxides and doxorubicin (DOX) has shown the ability of simultaneous detection and therapy of PDAC in a pancreatic cancer xenograft animal model [28], suggesting that the role of targeting mesothelin in imaging and therapy of pancreatic cancer. In addition, several other mesothelin targeted complexes are under clinical evaluations as therapeutics, such as SS1(dsFv)-PE38 (a recombinant single chain anti-mesothelin and immunotoxin fusion protein), anti-mesothelin antibody drug conjugates (BAY-94 9343), mesothelin tumor vaccine (CRS-207), and mesothelin-specific chimeric antigen receptors (CAR)[29]. Currently, mesothelin is considered as a cell surface target of pancreatic cancer cells. Therefore, mesothelin targeted therapeutic agents and nanoparticle drug carriers have to overcome the tumor stromal barriers to be delivered into tumor cells.

### **Urokinase Plasminogen Activator/Urokinase Plasminogen Activator Receptor**

Urokinase plasminogen activator (uPA) is a serine proteinase that activates plasminogen into functional plasmin to promote protease activity. Its function involves tumor growth, invasion metastasis, and angiogenesis by activation of matrix metalloproteinases and degradation of extracellular matrix. A high level of uPA, therefore, has been associated with a poor prognostic biomarker for cancer patients [30]. Cellular receptor of uPA (uPAR) is anchored on plasma membrane *via* a glycosylphosphatidylinositol (GPI) and controls uPA mediated plasminogen activation [31]. It is overexpressed in tumor cells, especially invasive tumor cells, and several stromal cell types in tumor microenvironment, including angiogenic tumor endothelial cells, active fibroblasts, and active macrophages [32]. In addition, the soluble form of uPAR (suPAR) in urine was also reported as a useful marker for the identification of a subset of patients with poorer outcome [30]. A marked pathological characteristic of pancreatic cancer is the presence of an extensive tumor stroma that consists of 50-85% of a tumor mass. It has been shown that about 80 to 98% of human pancreatic cancer tissues have a high level of uPAR expression and 58% of them have uPAR gene amplification [33]. The level of uPAR mRNA in pancreatic tumor tissue is 9.6 folds higher than that of the adjacent normal tissues. Among 15 biomarkers examined, uPAR was placed as the first biomarker that could distinguish cancer from normal tissues. Results of another study also showed that

uPAR is the most accurate biomarker among 29 pancreatic cancer biomarkers to discriminate between PDAC and chronic pancreatitis [34].

Since high levels of uPAR expression are found in pancreatic cancer and tumor stromal fibroblasts and macrophages, targeting uPAR for the development of tumor imaging and therapy agents has the potential to improve intratumoral delivery and distribution by binding to multiple cell types, increasing retention of small nanoparticles in the tumor tissue, and improving intratumoral cell drug delivery efficiency by receptor-mediated internalization. Therefore, uPAR is an excellent molecular target for the development of targeted imaging and therapy agents for stroma rich pancreatic cancer. Several groups have utilized uPAR or uPA-targeted nanoparticle for tumor imaging and treatment in preclinical studies using pancreatic animal tumor models. Results of our studies also showed that systemic delivery of uPAR targeted magnetic iron oxide nanoparticles (IONPs) led to the selective accumulation of the nanoparticles in orthotopic pancreatic tumors and detection of tumor lesions as small as 1 mm<sup>3</sup> in diameter in a human pancreatic cancer xenograft model by non-invasive NIR optical and MR imaging [12].

Recent studies have demonstrated the ability of overcoming drug resistant mechanisms by receptor-mediated internalization of nanoparticle-drug complexes. It has been shown that endocytosis of uPA-uPAR complex led to nuclear translocation of the complex, suggesting the presence of an endosomal escape mechanism for targeted nanoparticles and its therapeutic payload [12, 35]. In addition to be a pair of attractive PDAC target and targeting ligand, uPA/uPAR has been reported related to aggressive and drug-resistant phenotype [36]. Downregulation of uPA expression in pancreatic cancer cells sensitizes to drug treatment [30, 37]. To overcome the physical barrier of the stroma in drug delivery, theranostic nanoparticles targeting uPAR have been developed by our group and their anti-tumor effects have been examined in a human pancreatic cancer xenograft model in nude mice. Amino-terminal fragment (ATF) peptides of uPA were conjugated onto IONPs carrying a conditional release chemotherapy drug, gemcitabine (ATF-IONP-Gem) [38]. Systemic delivery of uPAR-targeted ATF-IONP-Gem resulted in significant growth inhibition of pancreatic tumors (Figure 2).

## Growth factor receptors

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

IGF-1R is a cell receptor with tyrosine kinase activity. It is overexpressed in many tumors including PDAC [39]. IGF-1R signal pathway is well known to be associated with cancer cell growth, invasion and metastasis. Detection of a high level of IGF-1R in a lesion in the pancreas by molecular imaging should assist in detection of pancreatic cancer as well as selection of IGF-1R targeted therapeutic agents. Recently, IGF-1R targeted imaging probes have been shown to be able to detect PDAC using different imaging modalities such as fluorescent imaging [40], MRI [9] and PET imaging [41]. It has been shown that cancers overexpressing IGF-1R are more resistant to drug- and radiation-induced apoptosis. Following chemotherapy, drug resistant cancer cells further increase the level of IGF-1R expression [42]. Therefore, targeting IGF-1R may allow for overcoming drug resistance when treating pancreatic tumors [43]. Additionally, targeting IGF-1R that is highly

expressed in tumor cells as well as tumor stromal fibroblasts and macrophages is a suitable approach for stroma-rich pancreatic cancer [9, 44]. Currently, a humanized monoclonal antibody specific to IGF-1R, named Dalotuzumab or MK-0646, has been developed for advanced pancreatic cancer treatment. MK-0646 specifically binds with IGF-1R and blocks its interaction with the IGF-I/ II ligands, which enhances gemcitabine-induced apoptosis and inhibits the MEK/Erk and the PI3-kinase/Akt signaling [45]. A randomized phase II study of MK-0646 is being conducted in combination with gemcitabine or gemcitabine plus erlotinib for advanced pancreatic cancer (NCT00769483) [45].

### **Epidermal growth factor receptor (EGFR and HER-2/neu)**

EGFR is a membrane of epidermal growth receptor family that regulates cell growth and proliferation in response to the binding of growth factor [46]. Upon ligand binding, EGFR forms homo- or heterodimeric complexes (usually with HER2) and in turn activates intracellular signaling cascades, resulting in cell proliferation, production of proangiogenic factors, increase in cell invasion, and resistance to the apoptotic cell death [47]. EGFR is highly expressed in 45–95% of human pancreatic cancer tissues [48]. Therefore, targeting EGFR is an appealing approach for selective cancer therapy. Single-chain anti-EGFR antibody conjugated nanoparticles have been shown to target to orthotopic pancreatic tumors in a human pancreatic cancer xenograft model, implying the potential of EGFR-targeted delivery of therapeutic agents for PDAC using nanoparticle drug carriers [49]. Anti-EGFR monoclonal antibody was covalently conjugated onto Poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulated with gemcitabine for selective PDAC chemotherapy with improved treatment response [50], demonstrating that this polymeric drug delivery system offers an excellent targeted drug delivery potency with translational potential by targeting of EGFR. It has been shown that another EGFR family cellular surface protein, Her-2/neu, is overexpressed in 30 to 52% of human pancreatic cancer tissues [51, 52]. Although anti-Her2 antibody treatment alone has not shown significant anti-tumor growth effect, the combination of antibodies for EGFR and Her-2/neu has demonstrated synergistic effect in human pancreatic cancer xenograft models in nude mice [53]. Anti-Her-2 antibody (Herceptin) or Her-2 affibody have been used as targeting ligands to develop Her-2 targeted imaging and therapy nanoparticles [54, 55].

For the development of nanoparticle based imaging and therapeutic agents for pancreatic cancer, EGFR and Her-2 targeted nanoparticles are delivered into tumor tissues by the enhanced permeability and retention effect (EPR) effect mediated by leaky tumor vessels. The majority of EGFR or Her2 targeted nanoparticle drug carriers may be trapped in the tumor stroma area before reaching EGFR or HER-2 overexpressing tumor cells.

### **Plectin-1**

Plectin-1 is a multidomain protein with versatile binding properties[56]. Plectin-1 has been shown to interact with intermediate filament proteins of various types and to physically link intermediate filament proteins with microfilaments and microtubules [57, 58]. Plectin-1 expression is low in normal pancreatic ductal cells but is overexpressed in PDAC, indicating that plectin plays important roles in PDAC progression and would be a good target for

PDAC [56]. A peptide named Plectin-1 Targeting Peptide (PTP) with the sequence of KTLTP was identified and attached to a magnetofluorescent nanoparticle for imaging of PDAC *in vivo* by intravital confocal microscopy [56]. In a later report, PTP peptide was labeled with  $^{111}\text{In}$  for single photon emission computed tomography (SPECT) detection of PDAC in an orthotopic and liver metastasis murine model [59]. More recently, an engineered adeno-associated virus (AAV) vector fusing with PTP (AAV-PTP) was developed for specifically targeting PDAC cells *in vitro* and *in vivo* [60]. Results from this study demonstrated a 37-fold preference of AAV-PTP for PDAC tumor over normal organs, confirming that plectin-1 is a promising target for PDAC. Current studies focus on the development of plectin-1 targeted imaging and therapeutic agents for the detection PDAC and ultimately therapy of PDAC in human patients.

## Mucin-1

Mucin is a family of high-molecular-weight glycoproteins with a heavily O-glycosylated tandem repeat region (TRR). On the basis of their structural characteristics, mucins can be divided into membrane type (MUC1, MUC3A/B, MUC4, MUC11–13) and secreted type (MUC2, MUC5AC, MUC5B, MUC6, MUC19 and MUC7) [61]. Specifically, mucin 1 is abnormally expressed in almost all human epithelial cell adenocarcinomas, such as human breast, ovarian, colorectal and pancreatic carcinomas. Besides, the fact that mucin-1 is heavily glycosylated in the health tissue but underglycosylated in neoplastic tissues makes mucin a cellular target for imaging and possible therapeutics. At present, investigations have also been focused on the potential to use MUC-1 as a target for immunotherapy. Several MUC-1 monoclonal antibodies have been reported for cancer imaging and therapy [62]. By taking advantages of nanotechnology, mucin-1 targeted multifunctional magnetic IONOs were produced for monitoring treatment response of 5-FU to PDAC [63]. A decreased MRI signal was observed in 5-FU treated PDAC mouse model (Figure 3). Another study reported that about 75–80% of mucin-1 protein was downregulated when HPAF-II pancreatic cells were treated by curcumin or curcumin encapsulated nanoformulations, a naturally polyphenol with significant cancer prevention activity [64], implying the potency of tracking the PDAC chemotherapy response by targeting mucin-1.

## Zinc Transporter 4 (ZIP4)

Zinc is an essential trace element and catalytic/structural component required by many metalloenzymes, such as carbonic anhydrase and matrix metalloproteinases (MMPs) that overexpressed in tumor. One of the zinc transporters, Zrt-, Irt-like proteins 4 (ZIP4), is overexpressed in pancreatic cancer cells but not in normal ductal epithelium cells in human pancreatic cancer tissues [65, 66]. A recent study from examination of fine needle aspiration (FNA) and surgical specimens revealed that ZIP4 was significantly overexpressed in pancreatic cancer cells. Interestingly, ZIP4 level in FNA samples was significantly associated with tumor differentiation and patient survival, indicating that targeting of ZIP4 has the potential to direct detection and targeted therapy of PDAC [67]. A study utilized RNA interference technique to specifically silence ZIP4 expression successfully inhibited pancreatic cancer growth and significantly increased the survival rate of pancreatic cancer xenografts[68].

## CURRENT ADVANCES IN CANCER NANOTECHNOLOGY FOR DETECTION AND THERAPY of PANCREATIC CANCER

Conventional chemotherapy drugs for PDAC have low efficiency in intratumoral delivery and distribution due to the presence of drug delivery barriers in pancreatic cancer tissues. Relative high levels of combination of several potent drugs, such as FOLFIRINOX, are used for the treatment of pancreatic cancer and result in severe systemic toxicity but only modestly improved the therapeutic response. In recent years, nanoparticle drug carriers have attracted great attention for improving drug formulation, enhancing tumor accumulation, and reducing side effects of anticancer drugs. Various types of nanoparticles with large surface area, biocompatibility and high drug loading capacity have been engineered for drug delivery into pancreatic cancer. Some nanoparticle drug carriers are also imaging contrasts agents, also termed as theranostic nanoparticles for their capacity in both therapy delivery and non-invasive imaging, which allow monitoring nanoparticle drug delivery. This function is important for selecting the most efficiency drug delivery systems for pancreatic cancer patients. Heterogeneous distributions of tumor vasculatures, dense stroma components, and ductal cancer cells in pancreatic cancer tissues will require non-invasive imaging to evaluate targeted drug delivery in tumors in a timely manner.

To address clinical challenges in PDAC treatment, new nanotherapeutics have been developed using various targeting ligands that bind to cell receptors upregulated in pancreatic cancer cells and tumor microenvironment. In this aspect, anti-tumor agents, such as, small molecular drugs, small interfering RNAs (siRNAs), antisense nucleotides, toxins, antibodies are all potential agents for the development of nanotheranostics for PDAC [13, 69, 70], presenting a new perspective on PDAC treatment using molecular therapy approaches. Currently, targeted theranostic nanoparticles are mostly in preclinical studies. Clinical trials using various nanoparticle drug carriers are focused on safety and therapeutic efficacy (Table 1). Clinical protocols for image-guided drug delivery and monitoring therapeutic responses using theranostic nanoparticles have yet to be developed and approved by the FDA. In the following sections, we discuss about current status and examples of the development of theranostic nanoparticles using different nanomaterials.

### Chemotherapy and small molecular drugs

Nanoparticles have been investigated as key drug carriers for the development of targeted therapeutic agents for efficient delivery of conventional chemotherapeutic drugs [71]. Abraxane® or nab-Paclitaxel (130 nm size), is human albumin nanoparticles encapsulated with paclitaxel. The FDA has recently approved Abraxane in combination with gemcitabine for the treatment of metastatic pancreatic cancer as the results of clinical trials that showed improved overall survival by 1.8 months compared to gemcitabine alone. The delivery mechanisms of Abraxane are thought to be mediated by passive targeting tumors by the EPR effect through the leaky tumor vessels and active targeting to the serum protein acidic and rich in cysteine [72]. The observed efficacy in pancreatic cancer therapy is also considered to be mediated by disrupting the tumor stroma from the initial nanoparticle-drug delivery that further increases drug delivery into the tumor [73–76]. Preclinical studies showed that modification of the human albumin-paclitaxel nanoparticles by conjugating tumor necrosis



factor-related apoptosis-inducing ligand [77] or RGD integrin targeting ligand [76] enhanced the therapeutic effect. It was also reported that albumin-bound formulation of paclitaxel nanoparticles in the combination with other chemotherapy drugs, such as gemcitabine, significantly increased overall survival of pancreatic cancer patients with metastatic diseases [78]. Another promising clinical trial for pancreatic cancer therapy involved co-delivering chemotherapy agents with Peglyated hyaluronidase (PEGrHuPH20) [73]. Delivery of PEGrHuPH20 into pancreatic cancer tissues leads to the degradation of extracellular matrix, hyaluronic acid, in the tumor stroma and reduction of the interstitial pressure, allowing improved drug delivery into the tumor bed and enhanced therapeutic responses. However, PEGrHuPH20 only acts on hyaluronic acid and has no effect on other stromal components, especially stromal cell barrier and dense fibrotic collagen.

Gemcitabine is a nucleoside analog commonly used as standard chemotherapy for pancreatic cancer therapy. Although it enhances the effects in treating patients with advanced PDAC and improving survival of the patients' efficiently [79], systemic toxicity is associated with relatively high doses (1000 mg/m<sup>2</sup>) applied. Moreover, gemcitabine (Gem) can be rapidly inactivated by deoxycytidine deaminase after *in vivo* injection (half-life is about 17 min), which reduces the effectiveness of the drug. Various nanocomplexes, such as liposome/Gem [80], Silica Nanoparticle/Gem[81], biopolymer/Gem[82] and iron oxide nanoparticle/Gem [38], were designed to increase gemcitabine *in vivo* half-life, enhance therapeutic effects and reduce systemic toxicity in PDAC patients. Another concern for gemcitabine is drug resistance induced by deficiency or inhibition of nucleoside transporters in tumor cells that facilitate gemcitabine cellular internalization and intracellular phosphorylation for activation. Receptor targeted nanoparticle drug carriers that facilitate internalization of nanoparticle-drug to tumor cells should increase drug concentration in tumor cells and bypass the g-glycoprotein mediated multidrug resistance.

Paclitaxel was used in several studies as a chemotherapeutic agent due to its effect on the treatment of gemcitabine-resistant PDAC. For example under magnetic resonance imaging (MRI) guidance, systemic delivery of paclitaxel encapsulated in poly(ethyleneoxide)copoly(D,L-lactide) in combination with ultrasound enhanced intratumoral paclitaxel accumulation by increasing tumor perfusion and blood vessel and cell membrane permeability, leading to improved tumor growth inhibition [83].

One feature in drug resistant PDAC tumor is the dense tumor stroma that blocks drug or drug complex reaching cancer cells after extravasation from tumor blood vessels. Nanoparticle drug carriers targeting tumor stromal fibroblasts and macrophages offer the opportunity for enhanced intratumoral retention and distribution of nanoparticle-drug and drug delivery into tumor cells. In addition to uPAR-targeted theranostic nanoparticles, IGF-1R that expresses at a high level in pancreatic cancer cells and tumor stromal fibroblasts and macrophages is another cellular receptor for the development of targeted nanoparticles that have the potential of improving drug delivery in stroma rich pancreatic cancer tissues. For example, IGF-1R targeted IONPs carrying anthracycline doxorubicin (Dox) have been developed and have shown to deliver nanoparticle-drug into IGF1R-expressing tumor cells and tumor associated stromal cells [84]. The effect of the theranostic IONPs was demonstrated in an orthotopic human pancreatic cancer patient tissue derived xenograft

(PDX) model that recapitulated heterogeneous tumor cells and enriched tumor stroma in human pancreatic cancer. Results of this study demonstrated that systemic delivery of IGF1R targeted IGF1-INOP-Dox theranostic nanoparticles efficiently delivered to pancreatic tumors and were detectable by optical and MR imaging. Repeated delivery of IGF1-IONP-Dox led to breaking tumor stromal drug delivery barriers and significant tumor growth inhibition in this pancreatic PDX model in nude mice (Figure 4). Therefore, the IGF-1R-targeted theranostic IONP is a promising drug delivery system for the treatment of drug resistant pancreatic cancer.

In addition to the single drug treatment, many groups are investigating the potency of combination therapies of PDAC to improve treatment response and capitalize on the drug's initial promises. For example, FOLFIRINOX (Leucovorin Calcium, Fluorouracil, Irinotecan Hydrochloride and Oxaliplatin), OFF (Oxaliplatin, Fluorouracil and Leucovorin Calcium), Gem-Cisplatin and Gem-Oxaliplatin are four FDA approved drug combinations for PDAC. The other combinations, such as chemotherapy-radiation therapy [85], phototherapy-chemotherapy [69] and gene therapy-chemotherapy [81], have also been investigated. Because each drug kill cancer cells through different mechanisms, these combination therapies usually show improved therapeutic effects compared to the single drug treatment. Unfortunately, systemic toxicity often becomes worse in combination therapy. Thus, it is important to develop nanoparticles simultaneously carrying multiple drugs to enhance therapeutic response and overcome drug resistance while reducing side effects in PDAC therapy.

## siRNA

RNA interference, or gene silencing, is referring to utilization of small RNA (siRNA) molecules to specifically turn off the expression of a target gene. It has great therapeutic potential for diseases caused by abnormal gene expression and genetic mutation. The main challenges in applying this approach are the poor stability of RNA *in vivo* and its inability to pass the cell membrane. In this regard, various nanomaterials provide possible carriers for delivery siRNAs [86]. Polymer/calcium phosphate/siRNA hybrid nanoparticles carrying siRNA for vascular endothelial growth factor (VEGF) siRNA was delivered into subcutaneous BxPC3 tumor following systemic delivery and significant VEGF gene silencing was achieved [87] (Figure 5). Recently, another micelle with poly(ethylene glycol)-block-charge-conversional polymer /calcium phosphate from the same group was used to transferred VEGF siRNA into spontaneous pancreatic tumors and found obvious tumor ablation [70]. Due to the unique shape, single wall carbon nanotube was also reported for delivery of siRNA into pancreatic cells for potential therapeutic application [88]. Interestingly, it has been shown that knocking-down expression of critical genes not only suppresses the tumor growth, but also enhances efficacy of chemotherapy. For example, silencing *AURKA* genes can induce apoptosis and increase cytotoxicity of taxanes in pancreatic cancer cells [89]. In another study, ubiquitin ligase ITCH was specifically silenced by anti-ITCH siRNA and poly(propyleneimine) dendrimers complex. It was reported that down-regulation of ITCH would sensitize cells to chemotherapeutic agents and that RNAi based therapy would act as a booster for conventional chemotherapeutic agents. Indeed, the growth of PDAC co-administered with gemcitabine and ITCH-shRNA [90].

However, the *in vivo* safety and biocompatibility of these siRNA carriers have to be clarified. Further, studies are also necessary before drawing any conclusions regarding the efficacy of the siRNA targeted therapy for PDAC treatment.

### Photodynamic therapy

In comparison with conventional chemotherapy, laser-triggered photodynamic therapy (PDT) is an alternative tumor ablation regimen, especially for those chemo/radio resistant cancers. It utilizes light at a specific wavelength to excite nontoxic photosensitizers to produce toxic reactive oxygen species (ROS) to destroy tissues and vasculatures for both benign and malignant tumors [91, 92]. PDT can be a noninvasive therapy approach with minimal systemic effects, due to its killing of cancer cells by local light activation [93]. The first report on the application of PDT in clinic was reported in 2002 and 16 patients were received PDT after intravenously injection of photosensitizer (PS), meso-tetrahydroxyphenyl chlorin (mTHPC) [94]. Substantial tumor necrosis was observed and the median survival time after PDT is 9.5 months (range 3–40). Although further studies about using PDT in PDAC are required to confirm the therapeutic efficacy, this study encouraged the ablation of PDAC by PDT. More recently, the second-generation PS, named verteporfin, was evaluated by the same group in 15 patients with advanced PDAC. Tumor necrosis was induced with lesser side effects compared to their previous study [95]. In addition to PDT single treatment, photofrin and gemcitabine combined therapy is now under phase I clinic trial (NCT01770132). Overall, PDT presents an attractive alternative to the traditional therapeutic approaches. Accumulating studies on applications of PDT have been widely reported in recent years. Yu et al. encapsulated PS into amphiphilic sodium alginate-derivative nanoparticles and tested their PDT effects in Panc-1 human pancreatic cancer cells [96]. Under ultraviolet irradiation, the high level of reactive oxygen species generated by the treatment resulted in strong phototoxicity and apoptosis. However, reduction of side effects of PDT, optimization of the light penetration, the pre-existing hypoxic tumor microenvironment and the potential toxicity of PS are still challenging for applications of PDT in clinic, especially for PDAC treatment. Therefore, combination of PDT with other PDAC treatment approaches, development of new PS and drug delivery using novel nanomaterials may address those problems.

### Conclusion and perspectives

The combination of high doses of several chemotherapeutic drugs has been used for the treatment of pancreatic cancer patients. Despite aggressive chemotherapy, there is only modest improvement in therapeutic response and survival of the patients, however, with drawback of severe systemic toxicity. Targeted nanoparticle drug carriers offer promising approaches for selective delivery of highly potent chemotherapy drugs into pancreatic cancer tissues while markedly reducing systemic toxicity. Through appropriate surface modifications, nanoparticle drug carriers or theranostic nanoparticles offer a possibility of reducing or overcoming the therapeutic limitations of conventional chemotherapy by targeted delivery to cancer cells without obvious toxicity to healthy tissue. The ability of high capacity of loading one or multiple therapeutic agents of the nanoparticle drug carriers make them suitable drug delivery system for targeted therapy of drug resistant pancreatic cancer using high doses of potent chemotherapeutic drugs. The large surface of the

nanoparticles allows conjugating agents that act upon tumor stroma to break the drug delivery barrier to improve drug delivery into pancreatic cancer cells. Due to the presence of a physical barrier limiting the amount of therapeutic agents delivered into pancreatic tumor tissues and tumor cells, chemotherapy agents have to be highly toxic to effectively kill tumor cells. Since nanoparticle drug carriers are not able to pass the tight junctions of normal endothelial cells to enter most normal tissues and organs, the toxicity can be reduced. For macrophages in the spleen and Kupffer cells in the liver, those tissue macrophages are mostly post mitotic cells and are relatively resistant to chemotherapy drugs acting on proliferating cell populations. Furthermore, highly potent but also highly cytotoxic drugs could be encapsulated in the targeted nanoparticles to be delivered into pancreatic cancer tissues.

In summary, research progresses of the development of PDAC biomarkers and treatment strategies discussed in this review paved the way for further development of targeted cancer therapeutic agents, and multifunctional theranostic nanoparticle drug carriers for effective treatment of pancreatic cancer. Theranostic nanoparticles are promising new therapeutic agents for the development and translation of integrated image-guided and targeted therapeutic agents for personalized treatment of pancreatic cancer patients. The ability of non-invasive imaging to monitor nanoparticle-drug delivery is essential for maximizing the effect of biomarker-targeted therapy. Additionally, the development of novel stroma breaking or stroma penetrating nanoparticles, should allow therapeutic agents to overcome stroma barriers to reach to pancreatic cancer cells, which is highly likely to improve the therapeutic effect of current treatment agents. At the same time, nanoparticles as promising carriers provide new opportunities for some highly potent drugs that previously failed in clinical development due to low solubility, inappropriate pharmacokinetics, lack of bioavailability, or severe systemic toxicity. Through multidisciplinary cooperations among biologists, chemists, engineers, and clinicians, we believe that more powerful nanotherapeutic agents will be developed to address critical clinical issues on pancreatic cancer detection and therapy and to significantly improve the outcome of current therapy of pancreatic cancer.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Highlight**

- Early diagnosis and effective treatment of pancreatic cancer still present challenges.
- Cell surface biomarkers have been identified for the development of targeted imaging and therapeutic agents for detection and treatment of pancreatic cancer.
- Theranostic nanoparticles are promising drug delivery platforms for the development of targeted and image-guided therapy for stroma rich pancreatic cancer.
- Major challenges for translation of targeted nanoparticle drug carriers into clinical applications for detection and treatment of pancreatic cancer have been discussed.

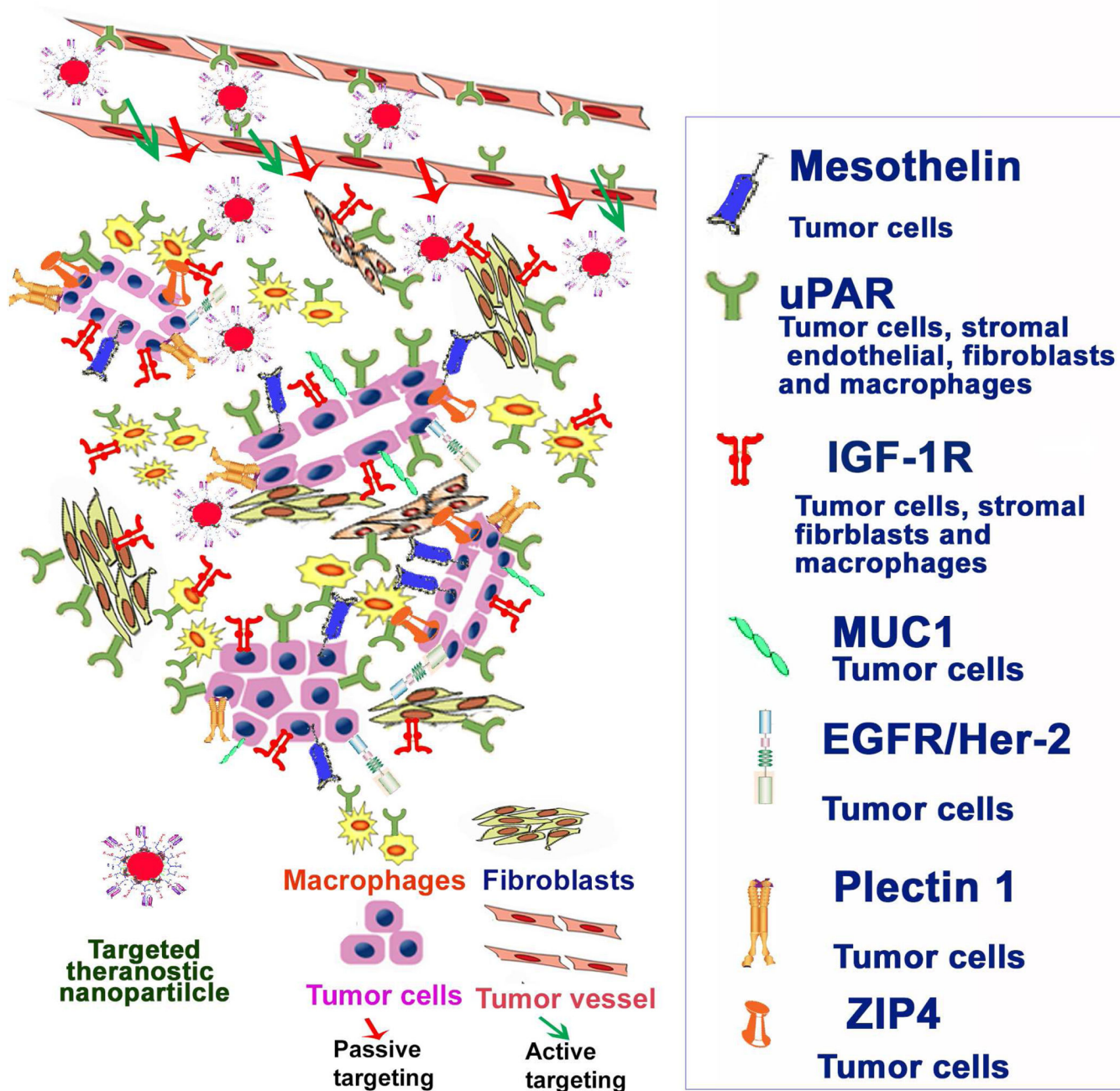
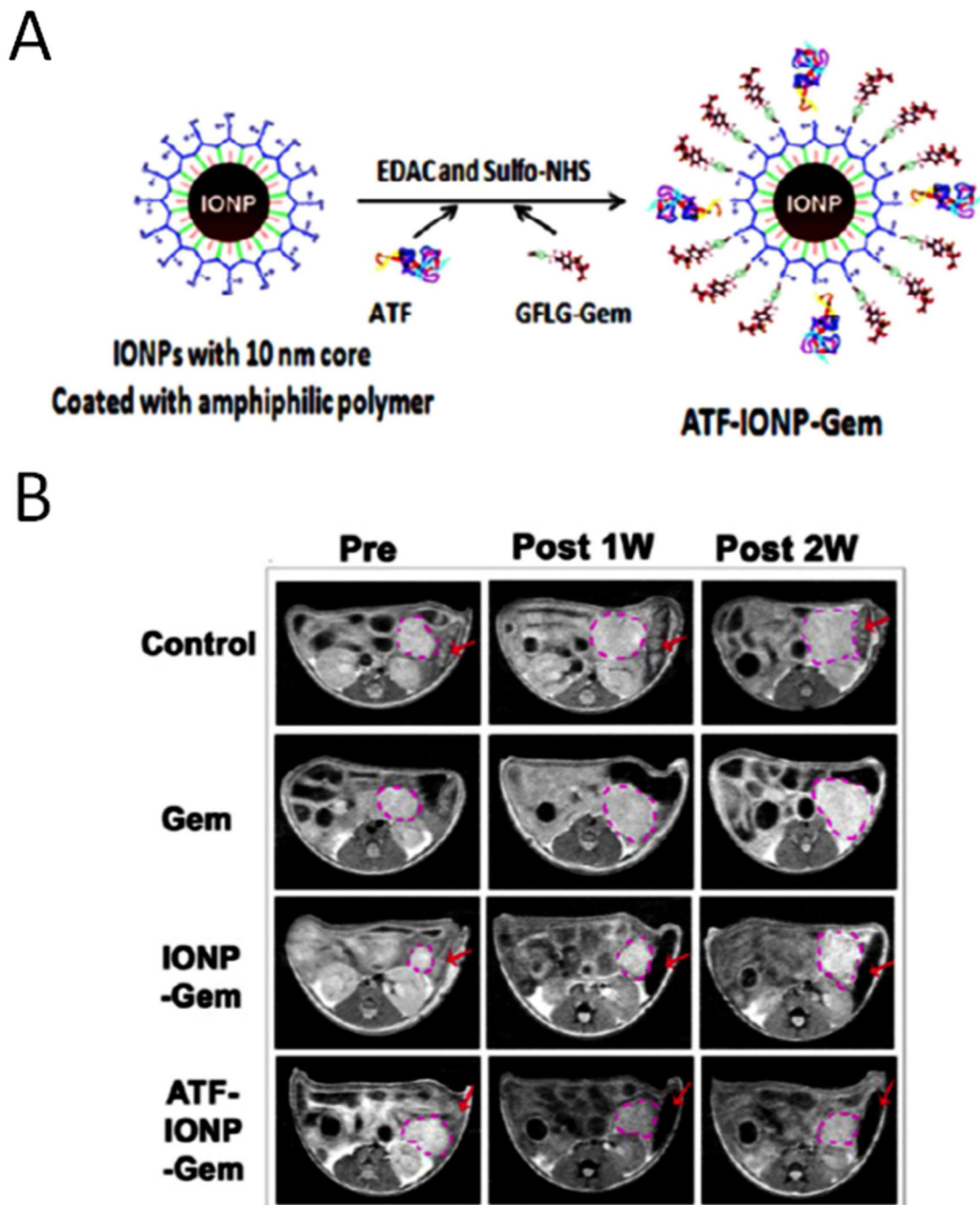
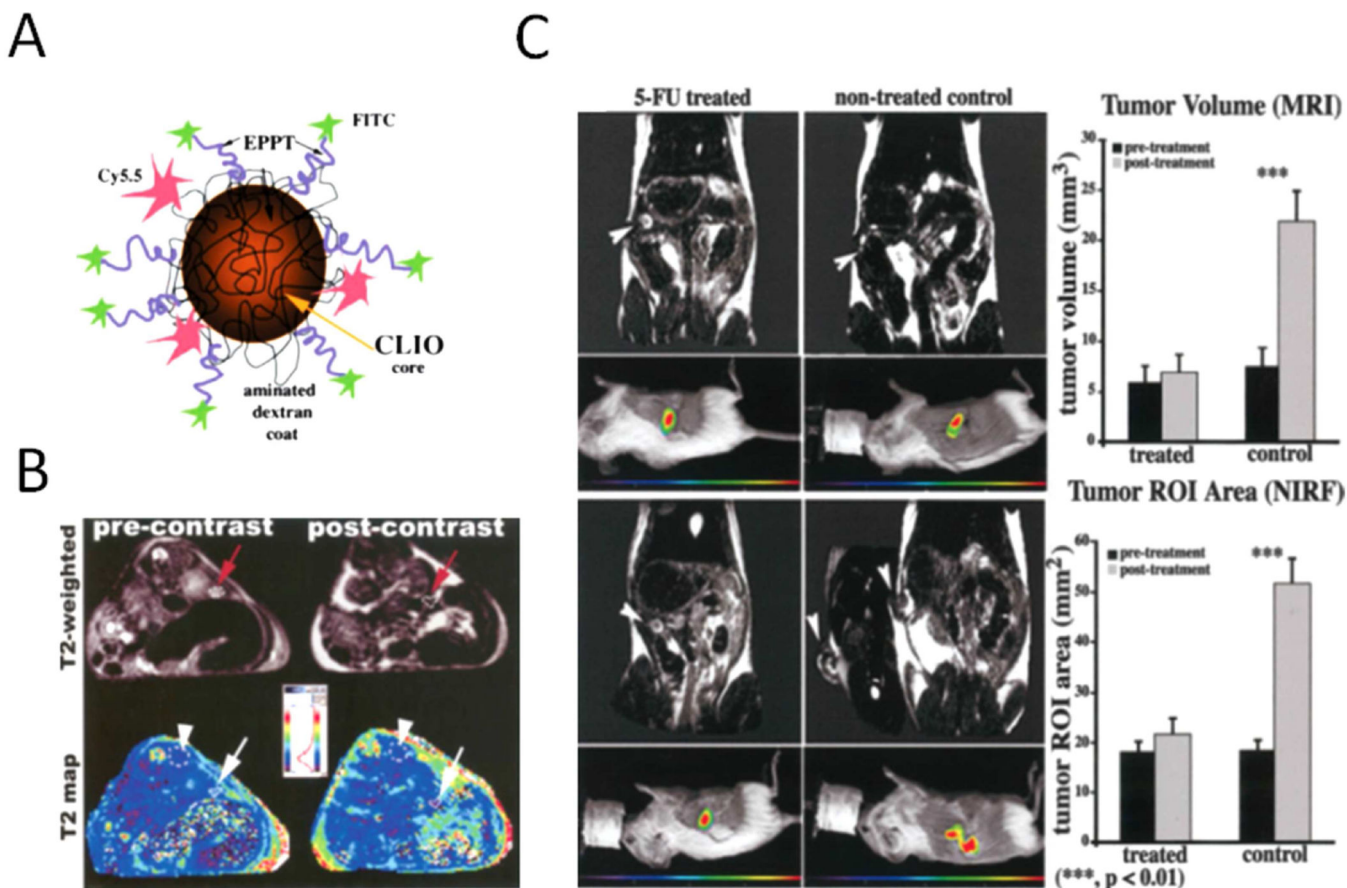


Figure 1. Molecular biomarkers for the development of targeted nanoparticle drug carriers and theranostic nanoparticles for pancreatic cancer therapy

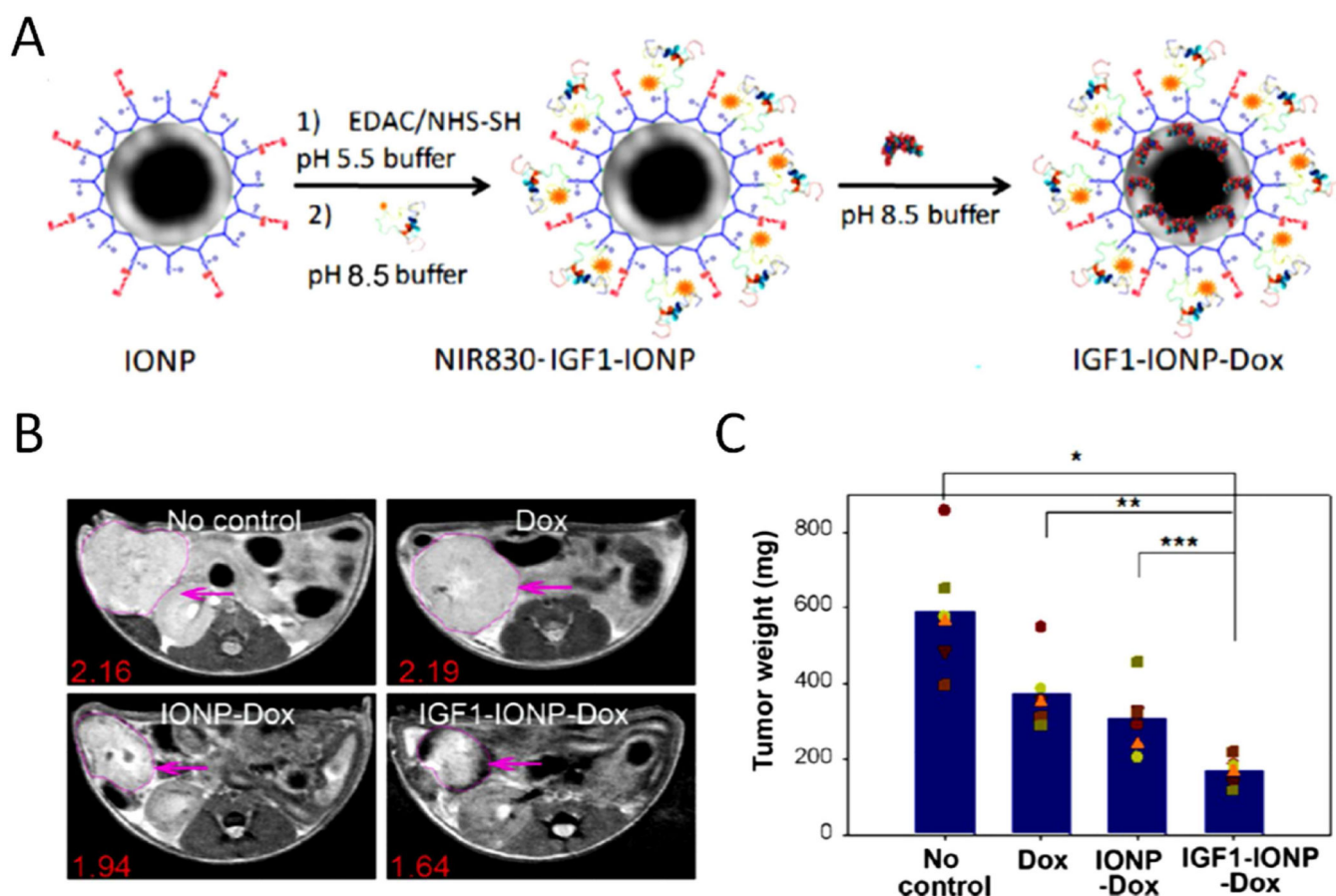


**Figure 2. uPAR targeted theranostic nanoparticles for the treatment of pancreatic cancer**  
 A. Preparation of uPAR targeted iron oxide nanoparticle (IONP) loaded with gemcitabine.  
 B. Axial T<sub>2</sub>-weighted MR images of the tumor-bearing mice before, one week, and two weeks after receiving theranostic nanoparticles. The location and size of the cancer lesions (pink dotted circles) were seen by MR images. Red arrows point out the MRI contrast change (darkening) in the spleen. Reprinted with permission from [38]. Copyright (2016) American Chemical Society.



### Figure 3. Imaging of pancreatic cancer by targeting mucin-1

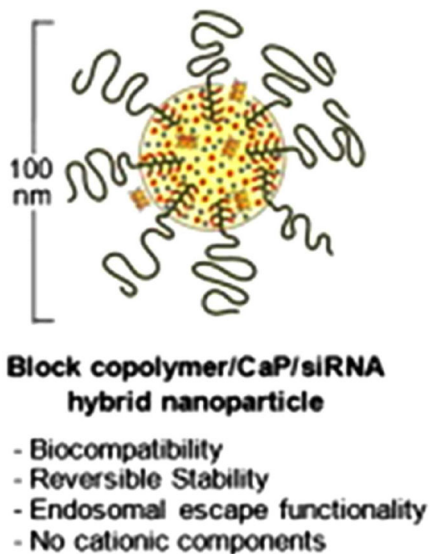
A. Design of pancreatic cancer diagnosis probe, mucin-1 targeted superparamagnetic iron oxide nanoparticles (CLIO-EPPT). B. Representative T<sub>2</sub>-weighted images (top) and corresponding T<sub>2</sub> maps (bottom) of animals bearing PDAC before (left) and 24 h after (right) i.v. injection of CLIO-EPPT. A significant (46.5%  $\pm$  3.2%,  $p < 0.01$ ) reduction in average T<sub>2</sub> relaxation rates was observed in the tumor at 24 h after injection. C. Left, T<sub>2</sub>-weighted MR images and corresponding color-coded NIRF images of mice bearing PDAC before (top) and after (bottom) treatment with 5-FU. Right, Quantitative evaluation of differential tumor growth by MRI and NIRF optical imaging. Tumor growth was inhibited by 5-FU as observed by using CLIO-EPPT with MRI. Reprinted with permission from [62] and [63]. Copyright (2016) Wiley-Liss, Inc.



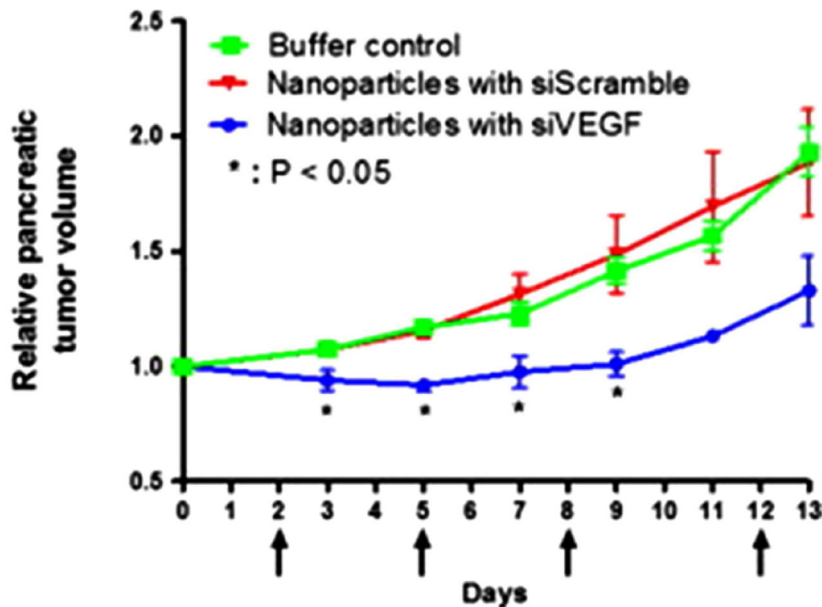
**Figure 4. IGF-1 receptor targeted IONP for imaging guided tumor therapy**

A. Preparation of the DOX capsulated NIR830-IGF1-IONPs. B. T<sub>2</sub>-weighted MRI confirms the accumulation of IGF1-IONP-Dox in the tumor site and tumor growth inhibition in different groups. Pink arrows indicate the locations of pancreatic PDX-tumor lesions. Red numbers are the mean of relative MRI signal intensities of MRI image slices from the entire tumor. A 10.2% MRI signal decrease was detected in nontargeted IONP-Dox-treated tumor, while a 24.1% MRI signal decrease was seen in IGF1-IONP-Dox-treated tumor. C. Tumor growth inhibition. The mean tumor weight (navy bar) and individual tumor weight distributions as color symbols after the treatment are shown. Adapted with permission from [84]. Copyright 2016 American Chemical Society.

A



B



**Figure 5. Polymer hybrid nanoparticles encapsulated with VEGF siRNA for pancreatic cancer therapy**

A. Design of hybrid polymer nanoparticle for siRNA delivery. B. Therapeutic effect of pancreatic cancer by the hybrid nanoparticles with siVEGF. Adapted with permission from [87]. Copyright (2016) Elsevier.



**Table 1**

Selected nanomedicines for pancreatic cancer

| <b>Drug product</b> | <b>Nanocarrier</b>            | <b>Active ingredient</b> | <b>Status</b>             | <b>Conclusion</b>                            |
|---------------------|-------------------------------|--------------------------|---------------------------|--|
| Abraxane            | Albumin                       | paclitaxel               | FDA approved              | Increased overall survival                   |
| MM-398              | liposome                      | irinotecan               | FDA approved              | Increased overall survival                   |
| EndoTAG-I           | cationic liposome             | paclitaxel               | phase II                  | May increase overall survival                |
| Genexol-PM          | micelle                       | paclitaxel               | Marketed in Europe, Korea | Inhibits primary tumor growth and metastases |
| NK105               | micelle                       | paclitaxel               | Phase I                   | Decrease in size of metastatic lesions       |
| NC-6004             | micelle                       | cisplatin                | Phase III                 | May increase overall survival                |
| Lipoplatin          | liposome                      | cisplatin                | Phase II/III              | Symptom relief                               |
| Rexin-G             | Retroviral expression vectors | doxorubicin              | Phase I/II clinical trial | Increased overall survival                   |
| NK911               | Polymeric micelles            | doxorubicin              | Phase 2                   | No update data is available for phase II     |
| SGT 53-01           | Transferrin targeted liposome | p53 gene                 | Phase II                  | May increase overall survival                |
| Atu027              | Liposome                      | anti-PKN3 siRNA          | Phase IIa                 | Safe and well tolerated                      |