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Imaging and Therapy of Pancreatic Cancer with Phosphatidylserine-Targeted Nanovesicles[1]

Victor M. Blanco*, Tahir Latif*, Zhengtao Chu*,† and Xiaoyang Qi*,†

*Division of Hematology and Oncology, Department of Internal Medicine, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA; †Division of Human Genetics, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Abstract

Pancreatic cancer remains one of the most intractable cancers, with a dismal prognosis reflected by a 5-year survival of ~6%. Since early disease symptoms are undefined and specific biomarkers are lacking, about 80% of patients present with advanced, inoperable tumors that represent a daunting challenge. Despite many clinical trials, no single chemotherapy agent has been reliably associated with objective response rates above 10% or median survival longer than 5 to 7 months. Although combination chemotherapy regimens have in recent years provided some improvement, overall survival (8-11 months) remains very poor. There is therefore a critical need for novel therapies that can improve outcomes for pancreatic cancer patients. Here, we present a summary of the current therapies used in the management of advanced pancreatic cancer and review novel therapeutic strategies that target tumor biomarkers. We also describe our recent research using phosphatidylserine-targeted saposin C–coupled dioleoylphosphatidylserine nanovesicles for imaging and therapy of pancreatic cancer.

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Introduction

Although it ranks as the 12th most frequent cancer worldwide, pancreatic cancer is the 4th leading cause of cancer-related deaths and carries the highest mortality rate (~94% at 5 years) of all major cancers [1,2]. In the United States, where currently about 127 people are diagnosed with pancreatic cancer and 108 die of the disease each day, a recent analysis predicts that pancreatic cancer will become the second leading cause of cancer deaths by 2030 [3,4]. Its most prevalent form at diagnosis, pancreatic ductal adenocarcinoma (PDAC), is usually asymptomatic in its early stages but progresses rapidly. Thus, the majority of the pancreatic cancer cases are detected when the tumor has already metastasized and about 70% of these patients die of the disease in less than 1 year. A minority of patients, around 15% to 20%, are eligible for potentially curative resection, and yet, in spite of adjuvant, post-resection chemotherapy or chemoradiation, the 5-year survival for these patients is only 20%, with death resulting from metastatic disease and/or locoregional recurrences [5,6].

Etiology and Pathogenesis of Pancreatic Cancer

The etiology of pancreatic cancer remains largely unknown. It is believed that PDAC arises not from ductal cells but through a process known as acinar to ductal metaplasia, in which mature acinar cells transdifferentiate into ductal-like cells [7]. The risk increases with age (>50 years), obesity, and type 2 diabetes. Smoking is the most common risk factor, responsible for ~25% of PDAC cases [8,9]. Genetic predisposition, involved in 5% to 10% of cases, has been

Address all correspondence to: Xiaoyang Qi, PhD, Division of Hematology and Oncology, Department of Internal Medicine, College of Medicine, University of Cincinnati, The Vontz Center for Molecular Studies, 3125 Eden Ave, Cincinnati OH 45267, USA.
E-mail: xiaoyang.qi@uc.edu

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associated with several germline mutations in the *BRCA2*, *STK11/LKB1*, *p16/CDKN2A*, and *PRSS1* genes [10]. Inflammation, associated with chronic pancreatitis (commonly triggered by heavy alcohol consumption), also increases the risk of pancreatic cancer [11,12].

A signature molecular profile has emerged from genetic studies, identifying activating mutations in the oncogene *KRAS* and inactivating mutations in the tumor suppressors *CDKN2A*, *TP53*, and *SMAD4/DPC4*, as the main drivers of pancreatic carcinogenesis [13,14]. Analysis of early-, middle-, and late-stage disease samples revealed that mutations in these genes arise sequentially and contribute to increased malignancy [15]. Propelling the search for novel therapies, further insights highlighting the complexity of PDAC have come from genetic studies showing that advanced pancreatic cancers contain an average of 63 genetic alterations, defining 12 core signaling pathways represented in two thirds of all tumors analyzed [13]. Another significant step forward was provided by a recent analysis of clinical data sets and human pancreatic cancer cell lines, which allowed the molecular characterization of three subtypes of PDAC, namely, classic, quasimesenchymal, and exocrine-like, with distinct progression rates and differential therapeutic responses [16].

Current Treatment Strategies

Early-Stage Resectable PDAC

Complete surgical resection with negative surgical margins (R0 resection) is the only treatment that can potentially result in long-term survival for some patients with early-stage pancreatic cancer (5-year survival around 20%). However, only 15% to 20% of patients are eligible for surgical resection, and many of these patients develop recurrent and metastatic disease soon after resection. Several studies have shown negative surgical margin and nodal status as important prognostic factors. In fact, some studies have demonstrated similar survival of early-stage PDAC patients who had positive surgical margins and locally advanced unresectable PDAC patients treated with chemoradiotherapy or chemotherapy only [17]. Adjuvant chemotherapy improves the outcomes for these patients, as shown in the European Charité Onkologie CONKO-001 trial where 368 patients were randomly assigned to gemcitabine *versus* observation after surgical resection. This study showed significant and persistent improvement in overall survival with 6 months of gemcitabine therapy (21% *vs* 10%, 5-year survival and 12.2% *vs* 7.7%, 10-year survival) [18].

Neoadjuvant chemotherapy or chemoradiotherapy is gaining popularity in an attempt to achieve R0 resection in more patients. Phase I/II studies have demonstrated that neoadjuvant chemoradiotherapy can be safely delivered to patients with localized pancreatic cancer; however due to the lack of a surgery-alone arm, it is not clear if this approach improves resectability or survival, and benefits are not inferior to adjuvant therapy. Advancements in imaging and surgical techniques have made the distinction between resectable and unresectable locally advanced tumors somewhat blurry, and more patients are classified as borderline resectable. Neoadjuvant therapy may be especially useful in these patients, and clinical trial participation is strongly encouraged in this group of patients to determine the most appropriate preoperative therapy.

Unresectable Locally Advanced PDAC

For almost 40% of PDAC patients with unresectable non-metastatic disease, there is no known best treatment strategy, and options include radiotherapy, chemotherapy, or chemoradiotherapy [19]. Most patients undergo chemotherapy initially, with single agent gemcitabine still considered standard treatment in this setting [20,21]. Many centers however are using FOLFIRINOX, a combination of 5-fluorouracil (5-FU), leucovorin, irinotecan, and oxaliplatin, for patients with excellent performance status and normal liver function or gemcitabine with nab-paclitaxel, citing higher response rates for these combination chemotherapy regimens in the metastatic setting (10% with gemcitabine alone, 23% with gemcitabine plus nab-paclitaxel (albumin-bound paclitaxel) i.e. GEM/NAB-P, and 32% with FOLFIRINOX), making it more likely to convert these patients into resectable disease [22,23]. However, evidence from prospective trials in favor of this theory is still lacking, and no randomized trials have been conducted comparing neoadjuvant *versus* adjuvant therapy. Most patients will also undergo chemoradiotherapy if no progression is noted on interval staging. Best concomitant chemotherapy with external beam radiotherapy is also not well established and could include infusional 5-FU, capecitabine, or gemcitabine. Many centers are also evaluating stereotactic body radiation as an alternative to conventional external beam radiotherapy. Unfortunately, even with all these therapies, the prognosis, rate of resection, and long-term survival remain dismal for patients who initially have categorically unresectable tumors at diagnosis.

Metastatic PDAC

Pancreatic cancer is strikingly unresponsive to most conventional chemotherapies [24]. The nucleoside analog gemcitabine (2′,2′-difluorodeoxycytidine), adopted in the mid-1990s as first-line chemotherapy, provides only modest survival benefits (<6 months) to pancreatic cancer patients [25] and has been combined with many other drugs, including cisplatin, [26,27] oxaliplatin [28,29], irinotecan [21,30], exatecan [31], 5-FU [32], and pemetrexed [33], in phase III trials without significant improvements [2]. Although gemcitabine in combination with erlotinib (an inhibitor of the epidermal growth factor receptor type 1) demonstrated a statistically significant improvement in overall survival (6.2 *vs* 5.9 months) in a phase III study, the difference was not clinically meaningful [34]. In 2011, FOLFIRINOX showed significant survival benefit (11.1 *vs* 6.8 months) compared with gemcitabine alone in a phase III study of metastatic PDAC [22]. This trial also demonstrated a significant increase in toxicity, limiting the use of FOLFIRINOX to patients with good performance status [35]. Another phase III study evaluated GEM/NAB-P *versus* gemcitabine alone in patients with untreated metastatic pancreatic cancer after early studies showed promising activity of this combination [23]. The combined chemotherapy yielded a modest but significant survival benefit (8.5 vs 6.7 months), and due to its milder toxicity, it may be a better option for older patients with poorer performance status [36]. A recent network meta-analysis of chemotherapy regimens for advanced pancreatic cancer provided a comprehensive assessment of the efficacy and tolerability of combined therapies *versus* gemcitabine alone [37].

Molecular Targets for Pancreatic Cancer Therapy

On the basis of our increasing knowledge of the genetic and molecular alterations in pancreatic cancer, numerous trials combining gemcitabine and one or more tumor-targeted agents are currently under way [38]. These efforts are largely driven by preclinical data generated in animal models, including heterotopic and orthotopic human pancreatic cancer xenografts as well as genetically engineered mouse models that closely resemble the molecular alterations encountered in the clinic [39,40]. Among the latter, the most enticing target is the

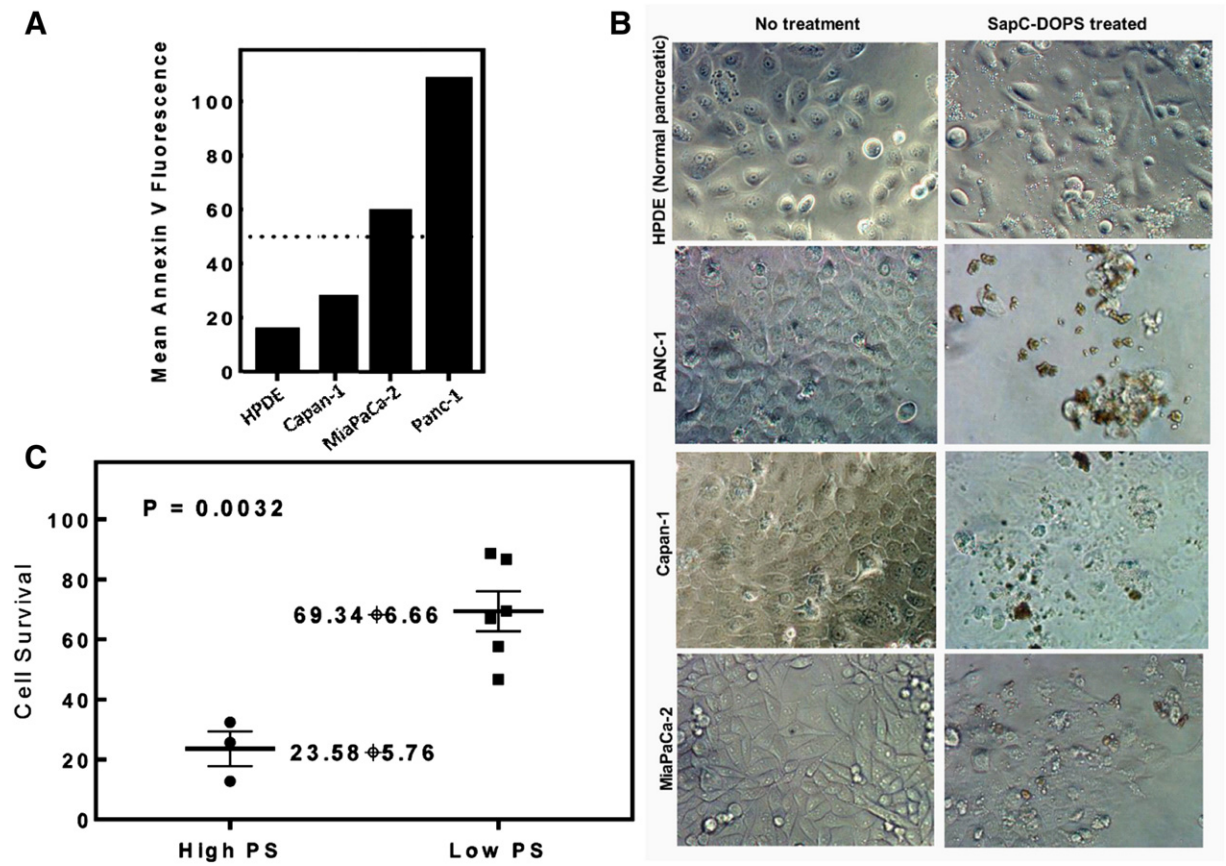

Figure 1. PS levels and cytotoxic effects of SapC-DOPS on human pancreatic cancer cells. (A) Measurement of PS exposure levels (annexin V binding assay) in human, untransformed pancreatic ductal epithelium (HPDE), and pancreatic cancer cell lines. (B) Microscopy images of untreated and SapC-DOPS–treated cells show preferential killing of high surface PS cancer cells. (C) Cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay shows increased killing ability of SapC-DOPS toward human pancreatic cancer cells with high surface PS.

KRAS oncogene, a critical driver of tumorigenesis that is mutated in ~95% of pancreatic cancers [41–43]. Thirty years after this realization, however, attempts to target activating, mutant KRAS proteins have been largely unsuccessful [20,44]. New approaches have focused instead on targeting RAS effector pathways such as RAF → mitogen-activated protein kinase kinase → extracellular signal-regulated kinase and phosphatidylinositol 3-kinase → AKT, with synergistic antitumor effects observed upon simultaneous inhibition of these pathways in human cell lines and pancreatic cancer mouse models [45]. Recent studies also advanced potential therapies that target gene transcription mediated by the proto-oncogene *c-Myc* [46] and mitochondrial respiration in stem cells resistant to *KRas* ablation [47].

Tumor Stroma

Advanced pancreatic tumors have a dense, fibrotic, hypovascular stroma with low cellularity and pro-inflammatory infiltrating cells (i.e., desmoplastic reaction) that contributes to tumor progression and reduces therapeutic success by hampering the penetration of drugs [48,49]. In recent years, several studies sought to target the cellular components (pancreatic stellate cells, fibroblasts, and immune cells) of the desmoplastic matrix and their tumor-promoting molecular mediators, such as cytokines, growth factors, and metalloproteases (MMPs) [50]. Two animal model studies in particular shed light on the importance of the PDAC stroma in the resistance to antitumor therapies. The first, conducted in a genetically engineered mouse model, showed that inhibition of the Hedgehog signaling pathway, involved in tumor-stromal crosstalk, depleted the tumor stroma, restored vascularity, enhanced delivery of gemcitabine, and produced a modest extension in survival, although the stromal reaction ultimately returned [51]. Unfortunately, a clinical trial prompted by this research was stopped before conclusion due to better responses in the control arm. The second preclinical study tested gemcitabine with enzymatic therapy to degrade hyaluronic acid, a main determinant of the barrier properties of PDAC stroma; this treatment normalized interstitial pressure and vascularization and doubled survival in mice [52]. MMPs play a fundamental role in tumor stroma remodeling and promotion of tumor growth [53,54]. Building on promising preclinical data, anti-MMP therapies using marismat and BAY-12-9566 were tested in clinical trials without positive results [48]. Likewise, attempts to block angiogenesis, a critical mechanism facilitating the expansion of most solid tumors, also yielded disappointing results in clinical trials [55–57]. However, animal studies suggest that novel antiangiogenic therapies targeting the C-X-C chemokine receptor type 2 may prove beneficial [58].

Tumor Immunity and Inflammation

Inflammation and immunosuppression collaborate to create a permissive environment for tumor growth [50,59]. Highlighting the role of immunosuppression in pancreatic cancer, preclinical studies showed that ablation of *KRAS*-driven granulocyte-macrophage

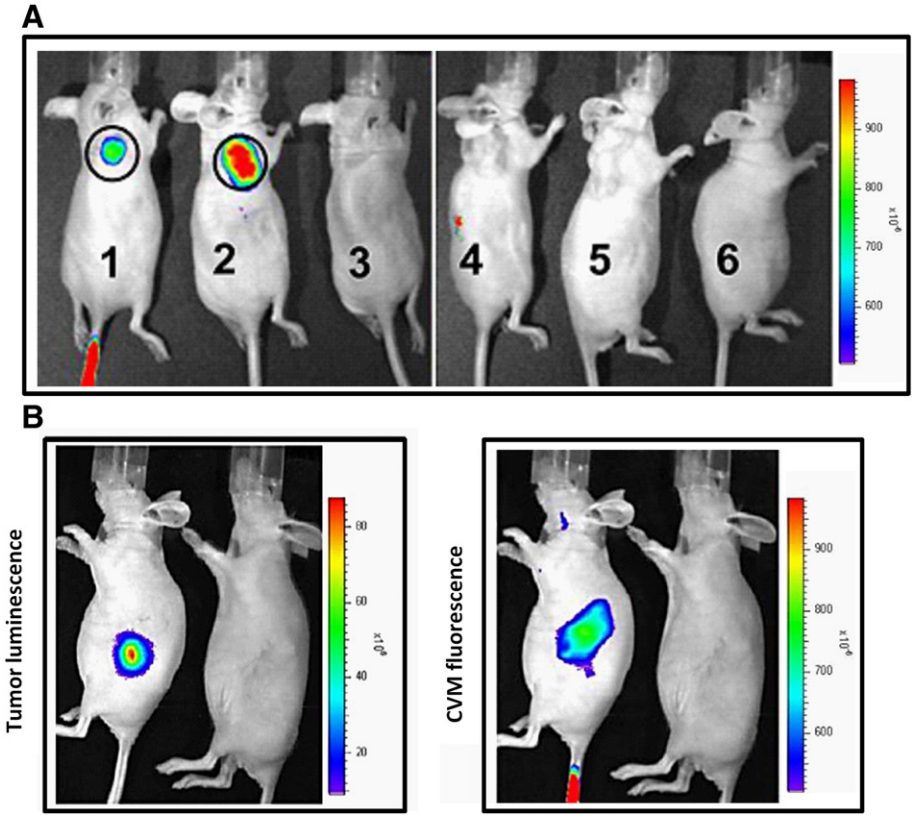

Figure 2. *In vivo* imaging of pancreatic cancer xenografts with fluorescently labeled SapC-DOPS. (A) Optical imaging of mice bearing subcutaneous pancreatic tumors (MiaPaCa-2 human cancer cells) after injection (i.v.) with SapC-DOPS–CVM (mice 1 and 2), non-complexed SapC plus fluorescently labeled DOPS (mouse 4), DOPS-CVM (mouse 5), and phosphate-buffered saline (PBS; mouse 6). Mouse 3 bore no tumor and was injected with PBS. Mice 1 to 3 were imaged at 24 hours and mice 4 to 6 at 48 hours after tail vein injection. Transient accumulation in liver was also observed, although it dissipated by 24 hours, while SapC-DOPS–CVM fluorescence persisted for up to 4 days. (B) Tumor bioluminescence (left) and optical imaging (SapC-DOPS–CVM; right) of mice bearing orthotopic pancreatic tumors induced by implantation of luciferase-expressing human pancreatic cancer cfPac1-Luc3 cells. Note specific tumor targeting by SapC-DOPS–CVM 48 hours after tail vein injection. A control mouse (non-tumor; PBS injected) is shown on the right.

colony-stimulating factor production reduced myeloid cell infiltration, unleashing T cell ($CD8^+$)–dependent immune responses and causing tumor growth arrest [60,61]. Further efforts to evoke intrinsic antitumor responses are exemplified by the development of an α-enolase DNA vaccine, which halted tumor progression by activating humoral and cellular responses [62], and vaccination with *Listeria* monocytes engineered to express Kras(G12D) after depletion of regulatory T cells, which triggered T cell–dependent cytotoxicity and blocked tumor progression at early stages [63]. Tumor-associated macrophages are another relevant therapeutic target, since they contribute to gemcitabine resistance by upregulating cytidine deaminase, the enzyme that metabolizes gemcitabine, rendering it inactive [64]. In a small study of patients with inoperable PDAC, administration of an agonist CD40 antibody in combination with gemcitabine caused tumor regression in some patients [65]. Modeling this study in mice showed that pancreatic tumor-associated macrophages became activated on CD40 antibody ligation and elicited T cell–independent antitumor actions leading to a tumor regression rate (~30%) that reproduced that in human patients [66]. Other appealing therapeutic targets are the signaling hubs represented by the Stat3 and nuclear factor kappa-light-chain-enhancer of activated B cells pathways, which determine the release of proinflammatory cytokines such as tumor necrosis factor alpha and interleukins 6 and 1 [50].

Phosphatidylserine-Targeted Imaging and Therapy of Pancreatic Cancer with SapC-DOPS Nanovesicles

Phosphatidylserine (PS) is an anionic phospholipid with important structural and signaling properties [67]. In animal cell membranes, it localizes in the internal aspect of the cell membrane, but it is externalized on induction of apoptosis and at sites of injury, where it stimulates hemostasis and activates the complement cascade [68,69]. Notably, viable cancer cells and tumor-associated vascular cells usually present elevated levels of PS on the surface of their membranes [70,71]. It is not clear whether this is advantageous for tumor cells, although evidence seems to indicate that tumor immunity and metastatic potential may be counteracted and favored, respectively, by increased surface PS levels [72]. In the last decade, our group and others have worked to exploit this distinctive feature of cancer cells to develop PS-targeted therapies. What follows is a summary of our work using PS-binding lipid-protein nanovesicles for imaging and treatment of pancreatic cancer.

Saposin C (SapC) is a small, thermostable lysosomal protein that binds to PS and acts as a co-factor in the activation of acid β-glucosidase, acid sphingomyelinase, and acid β-galactosylceramidase [73,74]; the catalytic action of these enzymes results in the formation of ceramide, a well-established pro-apoptotic mediator [75]. Given the strong affinity of SapC toward PS, and its role

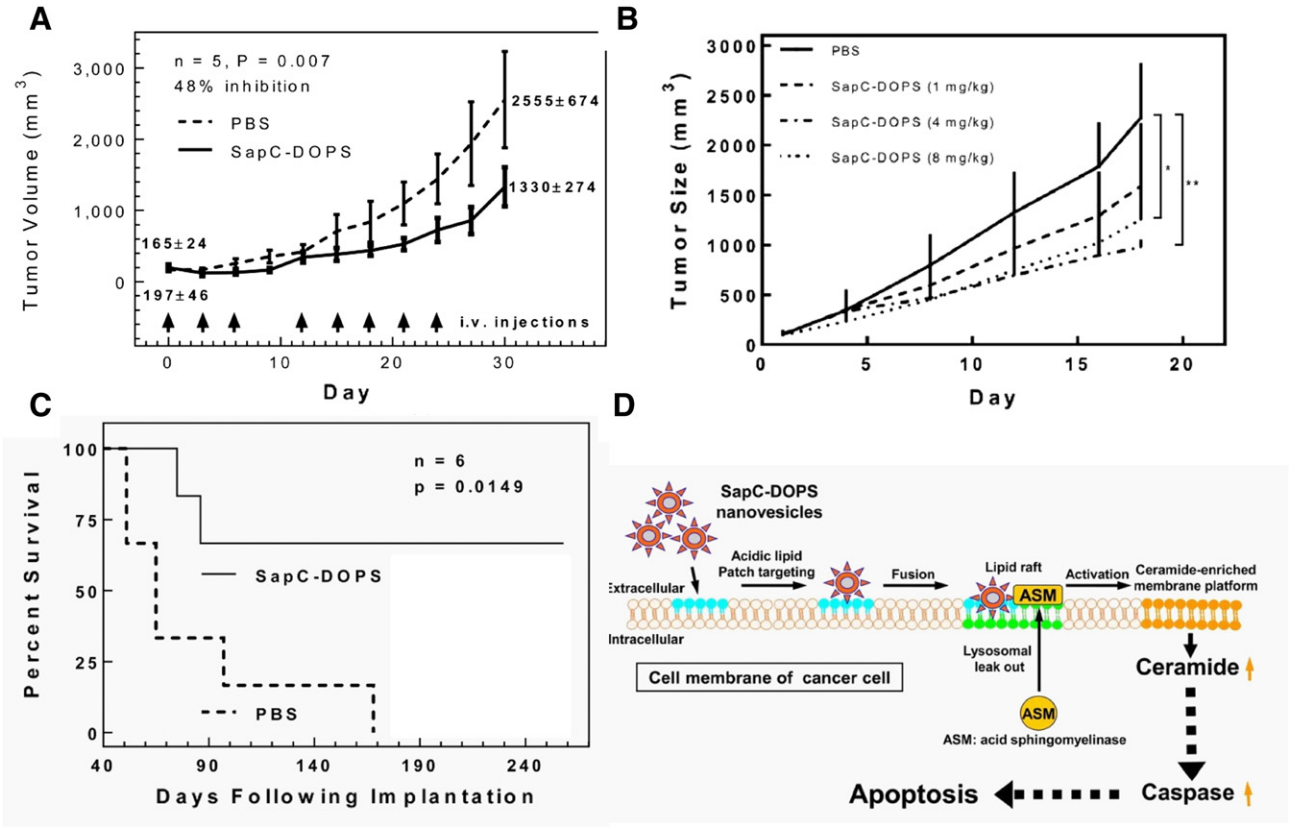

Figure 3. *In vivo* antitumor actions of SapC-DOPS on pancreatic cancer mouse models. Tumor size measurements in subcutaneous xenografts of MiaPaCa-2 cells (A) or Panc-1 cells (B). After tumor establishment, mice were treated with SapC-DOPS or PBS through tail vein injections as described in detail in [77]. (C) Kaplan-Meier survival curves of mice bearing orthotopic pancreatic tumors (human cfPac1-Luc3 cells) treated with PBS or SapC-DOPS as described in [77]. SapC-DOPS treatment reduced the growth of s.c. tumors and eliminated pancreatic tumors in four of six mice. (D) Hypothetical mechanism mediating the selective targeting and toxicity exerted by SapC-DOPS against cancer cells [77].

in lysosomal hydrolase activation, we hypothesized that SapC-containing nanovesicles may be useful agents to selectively target and kill tumor cells. To this end, we combined recombinant human SapC and dioleoylphosphatidylserine (DOPS) to generate stably assembled proteoliposomal nanovesicles (SapC-DOPS) [76] and tested their targeting and antitumor capabilities against pancreatic cancer cells [77]. We first examined the correlation between PS levels and the antitumor efficacy of SapC-DOPS in a panel of eight human pancreatic cancer cell lines. As shown for glioblastoma [78] and lung cancer cells [79], a higher killing capacity (i.e., lower half maximal inhibitory concentration; IC_{50}) was observed for high PS-expressing cells (Figure 1). To evaluate the tumor-targeting potential of SapC-DOPS, we attached a far-red, lipophilic, fluorescent dye (CellVue Maroon, CVM) and analyzed the biodistribution of SapC-DOPS–CVM in subcutaneous and orthotopic xenografts of human pancreatic cancer cells in nude mice. As shown in Figure 2, specific tumor fluorescence was observed in both models after intravenous injection of SapC-DOPS–CVM. The PS selectivity of SapC-DOPS was confirmed by showing that blocking surface PS residues in cancer cells before subcutaneous implantation abolished targeting by CVM-labeled nanovesicles [77]. The antitumor actions of SapC-DOPS were evaluated in mouse models of pancreatic cancer (Figure 3, *A*–*C*). These experiments showed that SapC-DOPS treatment significantly suppressed subcutaneous tumor growth and eradicated orthotopic tumors in four of six mice with pancreatic xenografts. Molecular studies suggested that caspase-mediated apoptosis is involved in SapC-DOPS cytotoxicity against pancreatic cancer cells (Figure 3*D*) [77].

Other investigators have also exploited the ubiquitous expression of PS in tumor cells and tumor vasculature to design and test the tumor-targeting and therapeutic efficacy of anti-PS antibodies. For instance, a recent study used liposomes functionalized with a PS-targeted human monoclonal antibody that contained both a near-infrared dye and superparamagnetic iron oxide nanoparticles to perform selective, bimodal (magnetic resonance and optical) imaging of breast cancer xenografts [80]. Interestingly, another study has shown that anti-PS antibodies can elicit immune antitumor responses by converting myeloid-derived suppressive cells into tumoricidal M1 macrophages or dendritic cells capable of engaging cytotoxic T cell–dependent cytotoxicity [81]. Preclinical studies showed good targeting efficacy of PS-directed antibodies in several tumor models [82–86]. In orthotopic mouse models of pancreatic cancer, Beck et al. showed that gemcitabine plus the PS-targeting antibody 3G4 had additive antitumor activity and significantly reduced metastases [87]. PS-targeted antibodies have shown, so far, modest therapeutic effciacy in clinical trials [88,89]. A randomized, open-label phase II trial evaluated an anti-PS antibody, bavituximab, plus gemcitabine *versus* gemcitabine alone in patients with advanced PDAC. The combined regimen was well tolerated and showed moderate activity with 28% tumor response rate *versus* 13% in the gemcitabine arm. Median

survival was 5.6 months *versus* 5.2 months for the control arm (hazard ratio = 0.75) [90].

Conclusions

Pancreatic cancer is a devastating disease for which there are no effective therapies. While concerted efforts to reduce risk factors such as obesity and tobacco and alcohol abuse are clearly paramount to prevent pancreatic cancer, its incidence is only expected to increase with a larger aging population sustained by constant medical advances. Two factors contribute to the high mortality of pancreatic cancer: the difficulty in early detection, due to unspecific symptomatology and a lack of robust biomarkers, and the resistance of advanced tumors to conventional chemotoxic agents and radiation therapy. Targeted therapies are poised to revolutionize cancer treatment by providing increasing efficacy while avoiding or reducing the adverse side effects characteristic of conventional cancer treatments. A formidable challenge, however, is presented by the complex nature of most tumors, in which multiple redundant and compensatory mechanisms virtually guarantee that tumor eradication cannot be achieved by silencing any individual molecule or signaling pathway. Rather, strategies that target broadly expressed tumor-specific antigens, while concurrently triggering tumor autolysis, may provide a breakthrough in the treatment of pancreatic and other cancers.

Our recent work has shown that PS targeting and tumor toxicity can be effectively achieved in mouse models of pancreatic cancer with PS-targeted nanovesicles [77]. Preclinical studies from our laboratory have also shown the potential of PS-targeted SapC-DOPS nanovesicles as imaging and therapeutic agents in a number of primary and metastatic tumors [76,78,91–95]. Importantly, the affinity of SapC for PS is greatly enhanced at acidic pH [96,97], a condition encountered in the majority of solid tumors that is known to stimulate drug resistance and create a propitious environment for tumor stem cells [78,79,98]. This evidence, along with the favorable safety profile of SapC-DOPS [76], strongly supports testing its applicability as a diagnostic and therapeutic agent for pancreatic cancer patients in clinical studies [99].

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References

[1] Hariharan D, Saied A, and Kocher H (2008). Analysis of mortality rates for pancreatic cancer across the world. *HPB* **10**(1), 58–62.

[2] Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, and Hruban RH (2013). Recent progress in pancreatic cancer. *CA Cancer J Clin* **63**(5), 318–348.

[3] Siegel R, Ma J, Zou Z, and Jemal A (2014). Cancer statistics, 2014. *CA Cancer J Clin* **64**(1), 9–29.

[4] Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, and Matrisian LM (2014). Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* **74**(11), 2913–2921.

[5] Matsuoka L, Selby R, and Genyk Y (2012). The surgical management of pancreatic cancer. *Gastroenterol Clin North Am* **41**(1), 211–221.

[6] Hidalgo M (2010). Pancreatic cancer. *N Engl J Med* **362**(17), 1605–1617.

[7] Maitra A and Leach Steven D (2012). Disputed paternity: the uncertain ancestry of pancreatic ductal neoplasia. *Cancer Cell* **22**(6), 701–703.

[8] Bosetti C, Lucenteforte E, Silverman DT, Petersen G, Bracci PM, Ji BT, Negri E, Li D, Risch HA, and Olson SH, et al (2012). Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). *Ann Oncol* **23**(7), 1880–1888.

[9] Blackford A, Parmigiani G, Kensler TW, Wolfgang C, Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, and Eshleman JR, et al (2009). Genetic mutations associated with cigarette smoking in pancreatic cancer. *Cancer Res* **69**(8), 3681–3688.

[10] Shi C, Hruban RH, and Klein AP (2009). Familial pancreatic cancer. *Arch Pathol Lab Med* **133**(3), 365–374.

[11] Lucenteforte E, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, Bosetti C, Li D, Gallinger S, and Miller AB, et al (2012). Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* **23**(2), 374–382.

[12] Yadav D and Lowenfels AB (2013). The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* **144**(6), 1252–1261.

[13] Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, and Jimeno A, et al (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* **321**(5897), 1801–1806.

[14] Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, and DePinho RA (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes Dev* **17**(24), 3112–3126.

[15] Bardeesy N and DePinho RA (2002). Pancreatic cancer biology and genetics. *Nat Rev Cancer* **2**(12), 897–909.

[16] Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, Cooc J, Weinkle J, Kim GE, and Jakkula L, et al (2011). Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. *Nat Med* **17**(4), 500–503.

[17] Varadhachary GR, Tamm EP, Abbruzzese JL, Xiong HQ, Crane CH, Wang H, Lee JE, Pisters PW, Evans DB, and Wolff RA (2006). Borderline resectable pancreatic cancer: definitions, management, and role of preoperative therapy. *Ann Surg Oncol* **13**(8), 1035–1046.

[18] Oettle H, Neuhaus P, Hochhaus A, Hartmann JT, Gellert K, Ridwelski K, Niedergethmann M, Zulke C, Fahlke J, and Arning MB, et al (2013). Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA* **310**(14), 1473–1481.

[19] Seufferlein T, Bachet JB, Van Cutsem E, and Rougier P, on behalf of the ESMO Guidelines Working Group (2012). Pancreatic adenocarcinoma: ESMO-ESDO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **23**(Suppl. 7), vii33–vii40.

[20] Van Cutsem E, Van De Velde H, Karasek P, Oettle H, Vervenne W, Szawlowski A, Schoffski P, Post S, Verslype C, and Neumann H (2004). Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* **22**(8), 1430–1438.

[21] Lima CMR, Green MR, Rotche R, Miller WH, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, and Miller LL (2004). Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. *J Clin Oncol* **22**(18), 3776–3783.

[22] Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul J-L, Gourgou-Bourgade S, and de la Fouchardière C (2011). FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* **364**(19), 1817–1825.

[23] Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, and Saleh MN, et al (2013). Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* **369**(18), 1691–1703.

[24] Gresham GK, Wells GA, Gill S, Cameron C, and Jonker DJ (2014). Chemotherapy regimens for advanced pancreatic cancer: a systematic review and network meta-analysis. *BMC Cancer* **14**(471), 1471–2407.

[25] Burris Hr, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, and Tarassoff P (1997). Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* **15**(6), 2403–2413.

[26] Colucci G, Giuliani F, Gebbia V, Biglietto M, Rabitti P, Uomo G, Cigolari S, Testa A, Maiello E, and Lopez M (2002). Gemcitabine alone or with cisplatin for the treatment of patients with locally advanced and/or metastatic pancreatic carcinoma. *Cancer* **94**(4), 902–910.

[27] Heinemann V, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, and Heinrich B (2006). Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* **24**(24), 3946–3952.

[28] Louvet C, Labianca R, Hammel P, Lledo G, Zampino M, Andre T, Zaniboni A, Ducreux M, Aitini E, and Taieb J (2005). Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. *J Clin Oncol* **23**(15), 3509–3516.

[29] Poplin E, Feng Y, Berlin J, Rothenberg ML, Hochster H, Mitchell E, Alberts S, O'Dwyer P, Haller D, and Catalano P (2009). Phase III, randomized study of gemcitabine and oxaliplatin versus gemcitabine (fixed-dose rate infusion) compared with gemcitabine (30-minute infusion) in patients with pancreatic carcinoma E6201: a trial of the Eastern Cooperative Oncology Group. *J Clin Oncol* **27**(23), 3778–3785.

[30] Stathopoulos GP, Syrigos K, Aravantinos G, Polyzos A, Papakotoulas P, Fountzilas G, Potamianou A, Ziras N, Boukovinas J, and Varthalitis J, et al (2006). A multicenter phase III trial comparing irinotecan-gemcitabine (IG) with gemcitabine (G) monotherapy as first-line treatment in patients with locally advanced or metastatic pancreatic cancer. *Br J Cancer* **95**(5), 587–592.

[31] Abou-Alfa GK, Letourneau R, Harker G, Modiano M, Hurwitz H, Tchekmedyian NS, Feit K, Ackerman J, De Jager RL, and Eckhardt SG (2006). Randomized phase III study of exatecan and gemcitabine compared with gemcitabine alone in untreated advanced pancreatic cancer. *J Clin Oncol* **24**(27), 4441–4447.

[32] Berlin JD, Catalano P, Thomas JP, Kugler JW, and Haller DG (2002). Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* **20**(15), 3270–3275.

[33] Oettle H, Richards D, Ramanathan R, Van Laethem J-L, Peeters M, Fuchs M, Zimmermann A, John W, Von Hoff D, and Arning M (2005). A phase III trial of pemetrexed plus gemcitabine versus gemcitabine in patients with unresectable or metastatic pancreatic cancer. *Ann Oncol* **16**(10), 1639–1645.

[34] Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, and Wolff RA (2007). Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* **25**(15), 1960–1966.

[35] Valsecchi ME, Díaz-Cantón E, de la Vega M, and Littman SJ (2014). Recent treatment advances and novel therapies in pancreas cancer: a review. *J Gastrointest Cancer* **45**(2), 190–201.

[36] Abbruzzese JL and Hess KR (2014). New option for the initial management of metastatic pancreatic cancer? *J Clin Oncol* **32**(23), 2405–2407.

[37] Gresham GK, Wells GA, Gill S, Cameron C, and Jonker DJ (2014). Chemotherapy regimens for advanced pancreatic cancer: a systematic review and network meta-analysis. *BMC Cancer* **14**(1), 471.

[38] Arslan C and Yalcin S (2014). Current and future systemic treatment options in metastatic pancreatic cancer. *J Gastrointest Oncol* **5**(4), 280–295.

[39] Guerra C and Barbacid M (2013). Genetically engineered mouse models of pancreatic adenocarcinoma. *Mol Oncol* **7**(2), 232–247.

[40] Herreros-Villanueva M, Hijona E, Cosme A, and Bujanda L (2012). Mouse models of pancreatic cancer. *World J Gastroenterol* **18**(12), 1286–1294.

[41] Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, and Perucho M (1988). Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell* **53**(4), 549–554.

[42] Hruban R, Van Mansfeld A, Offerhaus G, Van Weering D, Allison D, Goodman S, Kensler T, Bose K, Cameron J, and Bos J (1993). K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol* **143**(2), 545.

[43] Wang Y, Kaiser CE, Frett B, and Li H-y (2013). Targeting mutant KRAS for anticancer therapeutics: a review of novel small molecule modulators. *J Med Chem* **56**(13), 5219–5230.

[44] Thompson H (2013). US National Cancer Institute's new Ras project targets an old foe. *Nat Med* **19**(8), 949–950.

[45] Collisson EA, Trejo CL, Silva JM, Gu S, Korkola JE, Heiser LM, Charles R-P, Rabinovich BA, Hann B, and Dankort D (2012). A central role for RAF → MEK → ERK signaling in the genesis of pancreatic ductal adenocarcinoma. *Cancer Discov* **2**(8), 685–693.

[46] Stellas D, Szabolcs M, Koul S, Li Z, Polyzos A, Anagnostopoulos C, Cournia Z, Tamvakopoulos C, Klinakis A, and Efstratiadis A (2014). Therapeutic effects of an anti-myc drug on mouse pancreatic cancer. *J Natl Cancer Inst* **106**(12), dju320.

[47] Viale A, Pettazzoni P, Lyssiotis CA, Ying H, Sanchez N, Marchesini M, Carugo A, Green T, Seth S, and Giuliani V, et al (2014). Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* **514**(7524), 628–632.

[48] Neesse A, Michl P, Frese KK, Feig C, Cook N, Jacobetz MA, Lolkema MP, Buchholz M, Olive KP, and Gress TM, et al (2011). Stromal biology and therapy in pancreatic cancer. *Gut* **60**(6), 861–868.

[49] Provenzano P and Hingorani S (2013). Hyaluronan, fluid pressure, and stromal resistance in pancreas cancer. *Br J Cancer* **108**(1), 1–8.

[50] Steele CW, Jamieson NB, Evans TRJ, McKay CJ, Sansom OJ, Morton JP, and Carter CR (2013). Exploiting inflammation for therapeutic gain in pancreatic cancer. *Br J Cancer* **108**(5), 997–1003.

[51] Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, and Allard D (2009). Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* **324**(5933), 1457–1461.

[52] Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, and Hingorani SR (2012). Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* **21**(3), 418–429.

[53] Ardito CM, Briggs CD, and Crawford HC (2008). Targeting of extracellular proteases required for the progression of pancreatic cancer. *Expert Opin Ther Targets* **12**(5), 605–619.

[54] Mehner C, Miller E, Khauv D, Nassar A, Oberg AL, Bamlet WR, Zhang L, Waldmann J, Radisky ES, and Crawford HC (2014). Tumor cell-derived MMP3 orchestrates Rac1b and tissue alterations that promote pancreatic adenocarcinoma. *Mol Cancer Res* **12**(10), 1430–1439.

[55] Paulson AS, Tran Cao HS, Tempero MA, and Lowy AM (2013). Therapeutic advances in pancreatic cancer. *Gastroenterology* **144**(6), 1316–1326.

[56] Kindler HL, Niedzwiecki D, Hollis D, Sutherland S, Schrag D, Hurwitz H, Innocenti F, Mulcahy MF, O'Reilly E, and Wozniak TF (2010). Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol* **28**(22), 3617–3622.

[57] Spano J-P, Chodkiewicz C, Maurel J, Wong R, Wasan H, Barone C, Létourneau R, Bajetta E, Pithavala Y, and Bycott P (2008). Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study. *Lancet* **371**(9630), 2101–2108.

[58] Hertzer KM, Donald GW, and Hines OJ (2013). CXCR2: a target for pancreatic cancer treatment? *Expert Opin Ther Targets* **17**(6), 667–680.

[59] Clark CE, Hingorani SR, Mick R, Combs C, Tuveson DA, and Vonderheide RH (2007). Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res* **67**(19), 9518–9527.

[60] Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G, and Bar-Sagi D (2012). Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* **21**(6), 836–847.

[61] Bayne LJ, Beatty GL, Jhala N, Clark CE, Rhim AD, Stanger BZ, and Vonderheide RH (2012). Tumor-derived granulocyte-macrophage colony-stimulating factor regulates myeloid inflammation and T cell immunity in pancreatic cancer. *Cancer Cell* **21**(6), 822–835.

[62] Cappello P, Rolla S, Chiarle R, Principe M, Cavallo F, Perconti G, Feo S, Giovarelli M, and Novelli F (2013). Vaccination with ENO1 DNA prolongs survival of genetically engineered mice with pancreatic cancer. *Gastroenterology* **144**(5), 1098–1106.

[63] Keenan BP, Saenger Y, Kafrouni MI, Leubner A, Lauer P, Maitra A, Rucki AA, Gunderson AJ, Coussens LM, and Brockstedt DG, et al (2014). A Listeria vaccine and depletion of T-regulatory cells activate immunity against early stage pancreatic intraepithelial neoplasms and prolong survival of mice. *Gastroenterology* **146**(7), 1784–1794.

[64] Weizman N, Krelin Y, Shabtay-Orbach A, Amit M, Binenbaum Y, Wong RJ, and Gil Z (2014). Macrophages mediate gemcitabine resistance of pancreatic adenocarcinoma by upregulating cytidine deaminase. *Oncogene* **33**(29), 3812–3819.

[65] Beatty GL, Torigian DA, Chiorean EG, Saboury B, Brothers A, Alavi A, Troxel AB, Sun W, Teitelbaum UR, and Vonderheide RH, et al (2013). A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin Cancer Res* **19**(22), 6286–6295.

[66] Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, and Sharp LL, et al (2011). CD40 agonists alter tumor

stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* **331**(6024), 1612–1616.
[67] Fadeel B and Xue D (2009). The ins and outs of phospholipid asymmetry in the plasma membrane: roles in health and disease. *Crit Rev Biochem Mol Biol* **44**(5), 264–277.
[68] Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, and Henson PM (1992). Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J Immunol* **148**(7), 2207–2216.
[69] Versteeg HH, Heemskerk JWM, Levi M, and Reitsma PH (2013). New fundamentals in hemostasis. *Physiol Rev* **93**(1), 327–358.
[70] Ran S and Thorpe PE (2002). Phosphatidylserine is a marker of tumor vasculature and a potential target for cancer imaging and therapy. *Int J Radiat Oncol Biol Phys* **54**(5), 1479–1484.
[71] Utsugi T, Schroit AJ, Connor J, Bucana CD, and Fidler IJ (1991). Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. *Cancer Res* **51**(11), 3062–3066.
[72] Riedl S, Rinner B, Asslaber M, Schaider H, Walzer S, Novak A, Lohner K, and Zweytick D (2011). In search of a novel target—phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. *Biochim Biophys Acta* **1808**(11), 2638–2645.
[73] Kolter T and Sandhoff K (2005). Principles of lysosomal membrane digestion: stimulation of sphingolipid degradation by sphingolipid activator proteins and anionic lysosomal lipids. *Annu Rev Cell Dev Biol* **21**, 81–103.
[74] Qi X and Grabowski GA (2001). Differential membrane interactions of saposins A and C: implications for the functional specificity. *J Biol Chem* **276**(29), 27010–27017.
[75] Taha TA, Mullen TD, and Obeid LM (2006). A house divided: Ceramide, sphingosine, and sphingosine-1-phosphate in programmed cell death. *Biochim Biophys Acta* **1758**(12), 2027–2036.
[76] Qi X, Chu Z, Mahller YY, Stringer KF, Witte DP, and Cripe TP (2009). Cancer-selective targeting and cytotoxicity by liposomal-coupled lysosomal saposin C protein. *Clin Cancer Res* **15**(18), 5840–5851.
[77] Chu Z, Abu-Baker S, Palascak MB, Ahmad SA, Franco RS, and Qi X (2013). Targeting and cytotoxicity of SapC-DOPS nanovesicles in pancreatic cancer. *PLoS One* **8**(10), e75507.
[78] Wojton J, Chu Z, Mathsyaraja H, Meisen WH, Denton N, Kwon CH, Chow LM, Palascak M, Franco R, and Bourdeau T, et al (2013). Systemic delivery of SapC-DOPS has antiangiogenic and antitumor effects against glioblastoma. *Mol Ther* **21**(8), 1517–1525.
[79] Zhao S, Chu Z, Blanco VM, Nie Y, Hou Y, and Qi X (2015). SapC-DOPS nanovesicles as targeted therapy for lung cancer. *Mol Cancer Ther* **14**(2), 491–498.
[80] Zhang L, Zhou H, Belzile O, Thorpe P, and Zhao D (2014). Phosphatidylserine-targeted bimodal liposomal nanoparticles for in vivo imaging of breast cancer in mice. *J Control Release* **183**, 114–123.
[81] Yin Y, Huang X, Lynn KD, and Thorpe PE (2013). Phosphatidylserine-targeting antibody induces M1 macrophage polarization and promotes myeloid-derived suppressor cell differentiation. *Cancer Immunol Res* **1**(4), 256–268.
[82] Jennewein M, Lewis MA, Zhao D, Tsyganov E, Slavine N, He J, Watkins L, Kodibagkar VD, O'Kelly S, and Kulkarni P, et al (2008). Vascular imaging of solid tumors in rats with a radioactive arsenic-labeled antibody that binds exposed phosphatidylserine. *Clin Cancer Res* **14**(5), 1377–1385.
[83] He J, Luster TA, and Thorpe PE (2007). Radiation-enhanced vascular targeting of human lung cancers in mice with a monoclonal antibody that binds anionic phospholipids. *Clin Cancer Res* **13**(17), 5211–5218.
[84] He J, Yin Y, Luster TA, Watkins L, and Thorpe PE (2009). Antiphosphatidylserine antibody combined with irradiation damages tumor blood vessels and induces tumor immunity in a rat model of glioblastoma. *Clin Cancer Res* **15**(22), 6871–6880.
[85] Zhou H, Stafford JH, Hallac RR, Zhang L, Huang G, Mason RP, Gao J, Thorpe PE, and Zhao D (2014). Phosphatidylserine-targeted molecular imaging of tumor vasculature by magnetic resonance imaging. *J Biomed Nanotechnol* **10**(5), 846–855.
[86] Huang X, Ye D, and Thorpe PE (2011). Enhancing the potency of a whole-cell breast cancer vaccine in mice with an antibody-IL-2 immunocytokine that targets exposed phosphatidylserine. *Vaccine* **29**(29–30), 4785–4793.
[87] Beck AW, Luster TA, Miller AF, Holloway SE, Conner CR, Barnett CC, Thorpe PE, Fleming JB, and Brekken RA (2006). Combination of a monoclonal anti-phosphatidylserine antibody with gemcitabine strongly inhibits the growth and metastasis of orthotopic pancreatic tumors in mice. *Int J Cancer* **118**(10), 2639–2643.
[88] DeRose P, Thorpe PE, and Gerber DE (2011). Development of bavituximab, a vascular targeting agent with immune-modulating properties, for lung cancer treatment. *Immunotherapy* **3**(8), 933–944.
[89] Reichert JM (2014). Antibodies to watch in 2014: mid-year update. *mAbs* **6**(4), 799–802.
[90] Shuchi Sumant Pandya LW, Bullock Andrea J, Grabelsky Stephen A, Kingman Shum Merrill, Shan Joseph, Menander Kerstin B, and Reid Tony R (2013). Randomized, open-label, phase II trial of gemcitabine with or without bavituximab in patients with nonresectable stage IV pancreatic adenocarcinoma. *J Clin Oncol* **31**, 4054 [Suppl.].
[91] Abu-Baker S, Stevens AM, Li J, and Qi X (2012). Cytotoxicity and selectivity in skin cancer by SapC-DOPS nanovesicles. *J Cancer Ther* **3**, 321–326.
[92] Blanco VM, Chu Z, Vallabhapurapu SD, Sulaiman MK, Kendler A, Rixe O, Warnick RE, Franco RS, and Qi X (2014). Phosphatidylserine-selective targeting and anticancer effects of SapC-DOPS nanovesicles on brain tumors. *Oncotarget* **5**(16), 7105–7118.
[93] Kaimal V, Chu Z, Mahller YY, Papahadjopoulos-Sternberg B, Cripe TP, Holland SK, and Qi X (2011). Saposin C coupled lipid nanovesicles enable cancer-selective optical and magnetic resonance imaging. *Mol Imaging Biol* **13**(5), 886–897.
[94] Winter PM, Pearce J, Chu Z, McPherson CM, Takigiku R, Lee JH, and Qi X (2014). Imaging of brain tumors with paramagnetic vesicles targeted to phosphatidylserine. *J Magn Reson Imaging* **41**(4), 1079–1087.
[95] Wojton J, Meisen WH, Jacob NK, Thorne AH, Hardcastle J, Denton N, Chu Z, Dmitrieva N, Marsh R, and Van Meir EG (2014). SapC-DOPS-induced lysosomal cell death synergizes with TMZ in glioblastoma. *Oncotarget* **5**(20), 9703–9709.
[96] de Alba E, Weiler S, and Tjandra N (2003). Solution structure of human saposin C: pH-dependent interaction with phospholipid vesicles. *Biochemistry* **42**(50), 14729–14740.
[97] Liu A and Qi X (2008). Molecular dynamics simulation of saposin C-membrane binding. *Open Struct Biol J* **2**, 21–30.
[98] Keith B and Simon MC (2007). Hypoxia-inducible factors, stem cells, and cancer. *Cell* **129**(3), 465–472.
[99] Olowokure O and Qi X (2014). Pancreatic cancer: current standards, working towards a new therapeutic approach. *Expert Rev Anticancer Ther* **14**(5), 495–497.