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Cutting-Edge Approach to Targeted Therapy: Repositioning of Old Drugs in Combination with Standard Clinical Chemotherapeutics Potentiates a Propitious Novel Targeted Therapy for Human Pancreatic Cancer

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ABSTRACT: Metastatic pancreatic cancer leads to a fatal outcome, with a median progression-free survival of approximately six months when utilizing the most successful combination of chemotherapeutic regimens. When drug resistance develops, it facilitates an increase in primary tumor growth with new and growing metastases. Patients inevitably and quickly succumb to their disease and die. Notably, chemotherapy has an unintended impact on the development of drug resistance through the enhancement of EMT development and the enrichment of cancer stem cells (CSC). A recent report discovered that neuraminidase-1 (Neu-1) regulates EMT induction, angiogenesis, and cellular proliferation by the activation of several receptor tyrosine kinases. Here, the continual therapeutic inhibition of Neu-1 through intravenous administration of oseltamivir phosphate (OP) and aspirin (ASA) alongside GEM treatment significantly inhibits tumor progression, crucial compensatory signaling pathways, EMT program, CSC, and metastasis progression in a preclinical RAG2xCy double mutant BALB/c mouse model of human PANC-1 pancreatic cancer. The tumorigenic and metastatic potential of the xenotumors from the animals treated with the experimental protocols were significantly ablated when transferred into the mammary fat pads of NSG (NOD SCID gamma) branded mice.

Keywords: Pancreatic cancer; Chemoresistance; Drug repurposing; EMT.

النهج المتطور للعلاج المستهدف: إعادة توظيف الأدوية القديمة بالاشتراك مع العلاجات الكيميائية السريرية القياسية يعزز علاجاً مستهدفاً جديداً واعداً لسرطان البنكرياس البشري

راشيل الدبعي، ليلي باغايي، ديفيد أ. بونسِك، إيميلين أوكوين، يونفان لي، دانييلا غوكاسيان، ومايرون ر. سزيوتشوك

الملخص: إن إعادة تموضع الأدوية القديمة بالاشتراك مع العلاج الكيميائي القياسي السريري يفتح نهجًا علاجيًا سريريًا واعدًا للمرضى الذين يعانون من سرطان البنكرياس. تسلط هذه المقالة الضوء على علاج جيمسيتابين جنبًا إلى جنب مع إعادة التموضع العلاجي للتروية المستمرة للأسبرين وفوسفات الأوسيلتاميفير كخيار علاج فعال للأفراد المصابين بسرطان البنكرياس. تشير النتائج إلى أن إعادة استخدام هذه الأدوية مع التروية المستمرة مع جيمسيتابين

يعيق المقاومة الكيميائية، تطور الورم، برنامج الانتقال الظهاري الوسيطي، وكذلك النقائل، والخلايا الجذعية السرطانية في نموذج فأر لسرطان البنكرياس البشري. وتستحق هذه النتائج المشجعة المزيد من الاستكشاف لتقييم إمكانية تطبيق هذه العلاجات في سياق سريري، بهدف تعزيز تشخيص مرضى السرطان الذين يواجهون مرضًا مميتًا.

الكلمات المفتاحية: ألياف البلورة الفوتونية ،طريقة الخطوة المنفصلة لفوريه ، نبضة سوليتون، المسافة بين الفجوات الهوائية، التوسع الطيفي الفائق.

Simple Summary: Repositioning old drugs in combination with clinical standard chemotherapeutics opens a promising clinical treatment approach for patients with pancreatic cancer. This review highlights gemcitabine treatment along with the therapeutic repositioning of continuous perfusion of aspirin and oseltamivir phosphate as an efficacious treatment option for individuals affected by pancreatic cancer. The data suggest that repurposing these drugs with continuous perfusion with gemcitabine (GEM) hinders chemoresistance, tumor progression, epithelial-mesenchymal transition (EMT) program, metastases, and cancer stem cells in a mouse model of human pancreatic cancer. These encouraging findings merit further exploration to evaluate the possibility of applying these treatments in a clinical context, aiming to enhance the prognosis of cancer patients facing an otherwise fatal disease.

1. Introduction

Pancreatic cancer continues to be a problematic therapeutic challenge due to intrinsic chemoresistance and the rapid onset of acquired resistance during treatment. The new therapeutic targets and molecular pathogenesis focusing on pancreatic cancer have been reviewed by Wong and Lemoine [1,2], Maitra and Hruban [3], and Javadrashid *et al*. [4]. These reports suggest that there is a select number of signaling cascades and processes involved in the development of pancreatic tumors that are affected by alterations of genetic profiles, involving epidermal growth factor receptor (EGFR), rat sarcoma (Ras), vascular endothelial growth factor (VEGF), gastrin hormone and matrix metalloproteinase (MMP). Unfortunately, these molecules have been targeted with clinical therapeutic intent but collectively have proven to be less effective in specifically targeting and killing tumor cells.

Intrinsic resistance of pancreatic cancer cells to chemotherapy treatment is well-recognized clinically and in the laboratory [5-9]. However, combining first-line chemotherapies in treating metastatic pancreatic cancer, including Abraxane with gemcitabine (GEM) or 5-FU with Oxaliplatin and Irinotecan, can control the disease process for a median length of time of approximately six months in patients [3,4,10,11]. An estimated 20-30% of patients will experience an initial positive response with these treatments, indicated by a reduction of the primary tumor and metastases. However, there is often the development of treatment resistance. As a result, patients typically die rapidly from their diseases. Pancreatic cancer has a high lethality and a small window of opportunity to utilize current treatments due to intrinsic and acquired resistance. Moreover, the use of novel treatments is required to overcome this obstacle in treating pancreatic cancer.

With the unknown nature of treatment resistance, there are conflicting theories on why it occurs in response to chemotherapy treatment. However, current studies postulate that cancer treatments, including chemotherapy, develop treatment resistance through upregulating epithelial-mesenchymal transition (EMT) [12-16]. EMT is a highly conserved process where epithelial cells with distinct structural and functional features change into a less specialized state, assuming a mesenchymal phenotype associated with the ability to migrate, invade tissue, and resist apoptosis [17,18]. Interestingly, in experimental settings, specialized cancer cells with differentiated properties that undergo EMT develop characteristics associated with stem cells [19]. Cancer stem cells (CSC) are more resistant to chemotherapy and are more effective in promoting angiogenesis and micro-metastasis formation [20]. As cells undergo EMT, more differentiated epithelial cells acquire stem cell properties. There is evidence that experimental induction of EMT in cancer cells promotes cell invasion, metastasis and resistance to chemotherapy [21]. For example, cancer cells that underwent EMT have an increase in the half-maximal inhibitory concentration (IC50) dose of a chemotherapy drug GEM by ~10-fold. Even a partial EMT can see a similar increase.

The relationship between pancreatic ductal adenocarcinoma (PDAC) EMT and metastasis is poorly understood because the metastatic genetic composition resembles the complementary primary tumors [22-24]. The promoters of metastasis in PDAC are tumor protein P53 (TP53) [25], suppressor of mothers against decapentaplegic 4 (SMAD4) [26], wingless-related integration site (Wnt) [27], and extracellular matrix (ECM) gene expression [28]. The transforming growth factor beta (TGF-β) promotes invasion and migration by initiating EMT [29,30]. In normal pre-cancerous states, the p53 protein is known to inhibit the genomic and phenotypic alterations associated with cancer development. This complex interplay signals critical roles in critical cellular processes such as cell division, maintenance of genomic stability, apoptosis, autophagy, immunity [31].

The SMAD4 protein is a transcription factor and a tumor suppressor. This transcription factor helps to control the activity of particular genes and keeps cells from growing and dividing too fast or in an uncontrolled way. The Wnt

proteins are lipid-modified secreted glycoproteins that facilitate communication between cells, by regulating cell growth, their function, differentiation, and cell death. Wnt proteins also have a central role in bone development, modeling, and remodeling [32]. The extracellular matrix (ECM) is a dynamic noncellular structure involved in the maintenance of normal tissue architecture and homeostasis. The ECM can undergo constant remodeling in response to stressors, tissue needs, and biochemical signals, primarily mediated by matrix metalloproteinases (MMPs) [33] .

Therapies that target a single oncogenic pathway may not be enough to inhibit the growth of pancreatic cancer. Future studies must suppress pancreatic tumor cells' multiple enabling hallmark(s) capabilities. Despite the therapeutic interventions, the cancer cell survival program is adaptive and invasive, allowing more aggressive phenotypes to survive and metastasize. The clinical evidence is that repeat instability emerges first, followed by more significant aberrations, with compensatory effects leading to robust tumor fitness maintained throughout the progression. Here, a novel treatment approach targeting Neu-1 was hypothesized to ablate these compensatory effects of pancreatic cancer, as depicted in Figure 1 [34,35]. Neuraminidase-1 and matrix metalloproteinase-9 (MMP9) crosstalk tethered with G protein-coupled receptor(s) (GPCR) regulates receptor tyrosine kinases (RTKs) and TOLL-like (TLR) receptors in cancer cells and tumor microenvironment, setting the multistage for tumorigenesis.

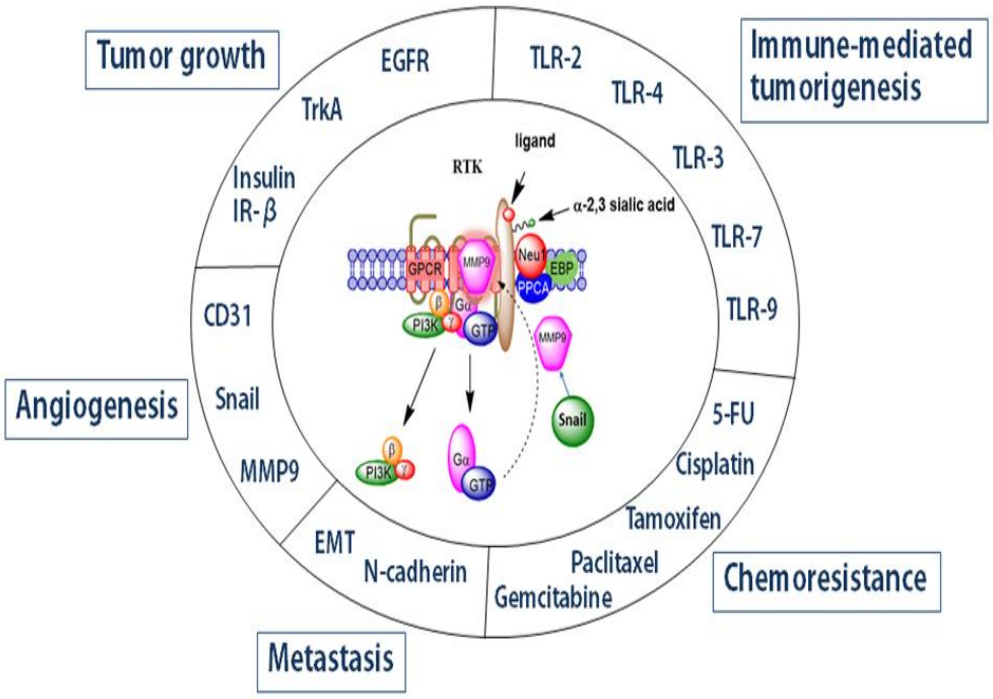

Figure 1. Neu1-MMP9-GPCR signaling platform in regulating receptor tyrosine kinases (RTK) and the molecular targeting of multistage tumorigenesis. Neuraminidase-1 (Neu1) and matrix metalloproteinase-9 (MMP9) crosstalk tethered with G protein-coupled receptor(s) (GPCR) regulates RTKs and TOLL-like (TLR) receptors in cancer cells, setting the stage for multistage tumorigenesis. **Abbreviations:** Neu1, neuraminidase-1; MMP, matrix metalloproteinase; IRβ, insulin receptor β; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; 5-FU, 5-fluorouracil; PI3K, phosphatidylinositol 3-kinase; GTP, guanine triphosphate; GPCR, G protein-coupled receptor; EBP, elastin binding protein; PPCA, protective protein cathepsin A. **Citation:** Taken from ©Abdulkhalek et al. Research and Reports in Biochemistry 2013:3,17–30, and ©Abdulkhalek et al. Clinical and Translational Medicine 2014:3,28. Publisher and licensee Dove Medical Press Ltd. This is an open-access article permitting unrestricted non-commercial use, provided the original work is properly cited.

A study by O'Shea et al. [36] found that OP disrupted the chemoresistance of PANC-1 using cisplatin or gemcitabine or when used in combination in a dose-dependent manner. Also, OP reversed the EMT characteristic of N-cadherin to E-cadherin changes that occur when cisplatin and gemcitabine resistance develops. The EGFR (epidermal growth factor receptor) plays a vital role in the EMT process in pancreatic cancer [37]. Gilmour et al. [38] uncovered a novel neuraminidase-1 (Neu1) and matrix metalloproteinase-9 (MMP-9) crosstalk in conjunction with neuromedin B, a GPCR that regulated EGF-induced receptor activation and cellular signaling, as depicted in Figure 1.

Acetylsalicylic acid (ASA) targets the Neu-1-MMP-9-GPCR signaling platform, inhibiting ligand-induced receptor tyrosine kinase activation involved in key cancer hallmarks of malignancy.

Over the past five decades, there have been minimal improvements in treatment outcomes due to ineffective screening and early detection methods capable of identifying pancreatic cancer in a pre-malignant stage [39]. Clinical treatment options include surgical resection, neoadjuvant and adjuvant chemotherapy, radiation, and immunotherapy

[40-42]. Unfortunately, the presence of diverse cancer cell populations in primary tumors and secondary micro-metastases results in resistance to cytotoxic therapies [43,44]. Despite pancreatic cancer initially responding to chemotherapies, including gemcitabine, drug resistance will still develop in patients. One potential mechanism(s) could be the tissue-damaging effects of chemotherapy, initiating the release of tissue repair molecules and EMT induction in the remaining cancer cell population, fostering the enhanced growth of cancer stem cell populations [13,45]. The proposed explanation for drug resistance development and progression during chemotherapy has been shown in many diseases, including bladder and ovarian cancer. Despite this, therapeutic intervention may solve these obstacles in cancer treatment [16,46]. Novel therapies must target and inhibit the biological mechanisms responsible for PDAC progression and metastasis to overcome the limitations in current pancreatic cancer treatment. Examples of these mechanisms include the inflammatory and immune-derived promoters of tumor growth and development, mechanisms of acquired drug resistance, and the pro-metastatic signaling pathways in the tumor microenvironment (TME), as depicted in Figure 1, that potentiate cancer cell distribution to distant organs [47].

There is no single signaling pathway that regulates the key cancer hallmarks of malignancy [47-49]. Drugs that have mono- or multi-hallmark-targeting provide therapeutic advantages in targeting several pharmacological pathways. Moreover, these approaches partially avoid drug resistance development [5,9,47,50]. A recent review by Zhang *et al*. [51] describes the current challenges in discovering new drugs for cancer therapy. They propose the need for an alternative strategy of drug repurposing, which is the use of old drugs for new therapeutic targets. For example, Zhang and colleagues [52] studied the effect of the anti-inflammatory drug ASA on PDAC cell lines. They found that ASA (a) enhances the therapeutic effectiveness of GEM by overcoming cancer resistance and modifying the expression of reprogramming factors, (b) suppresses cancer cell self-renewal and amplifies gemcitabine efficacy, (c) inhibits the spheroid formation and the activity of aldehyde dehydrogenase isoform 1 (ALDH1), a marker for self-renewal capacity, (d) disrupts the development of primary CSC spheroids, (e) reduces in-vivo tumor growth and invasion, and (f) diminishes the deposition of ECM components, including fibronectin and collagen.

Studies have increasingly focused on targeting the tumor-promoting inflammation using non-steroidal anti-inflammatory drugs (NSAIDs) as anti-cancer agents. Notably, NSAIDs are known to target cyclooxygenase (COX) enzymes which have been shown to promote a chemotherapy-sensitizing role [53]. The COX enzymes catalyze prostaglandin (PG) synthesis, converting arachidonic acid to prostaglandin E2 (PGE2) [54]. Elevated PG levels have been reported in breast, colon, lung, and pancreatic cancer, involved in cell proliferation, adhesion, metastasis, apoptosis, and immune surveillance [55,56]. Of the two COX enzymes, COX-1 is constitutively expressed in most tissues, while COX-2 is induced by pro-inflammatory mediators and mitogenic stimuli. This increased PG synthesis in inflamed and neoplastic tissues can activate nuclear factor kappa-light-chain- enhancer of activated B-cells (NF-κB) to promote angiogenic factors and induce COX-2 expression, thus playing a role in malignant cell proliferation, oncogenesis, and apoptotic resistance [55]. COX-2 overexpression in malignant cells has also been linked to reduced levels of E-cadherin that is required for cellular adhesion, increasing inflammation and promoting EMT development [57]. Qorri et al. [7] recently reported on the missing link connecting ASA's anti-cancer efficacy to glycosylation in inflammation and tumorigenesis. Here, ASA was shown to exert anti-cancer effects by targeting and inhibiting mammalian neuraminidase-1 (Neu-1), as depicted in Figure 2.

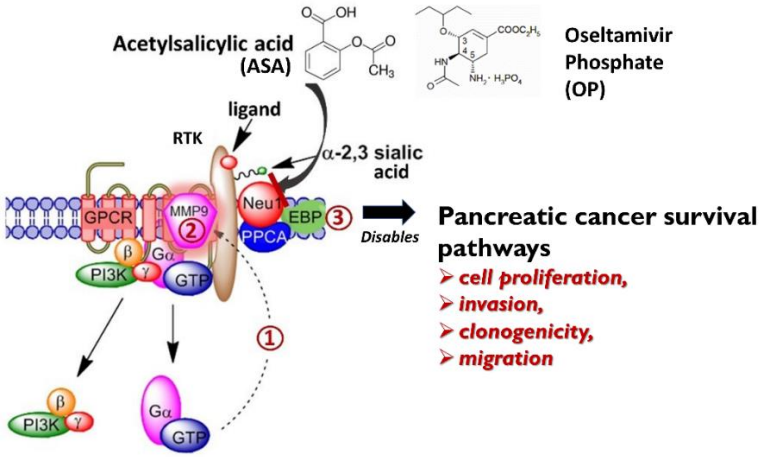

Figure 2. Acetylsalicylic acid targets the Neu-1-MMP-9-GPCR signaling platform, inhibiting receptor tyrosine kinase (RTK) activation [6]. When the ligand binds to its RTK, a conformational change in the associated G protein-coupled receptor (GPCR) is activated. (1) Gαi subunit signaling activates matrix metalloprotease-9 (MMP-9). (2) Via its elastin-degrading properties, MMP-9 removes elastin-binding protein (EBP) in a complex with neuraminidase-1 (Neu-1) and protective protein cathepsin A (PPCA) to activate Neu-1. (3) Activated Neu-1 cleaves terminal α-2,3 sialic acid residues on the ectodomain of RTKs to relieve steric hindrance and allow for receptor dimerization, phosphorylation, and downstream signal activation. Acetylsalicylic acid targets Neu-1 in complex with the MMP-9-GPCR, inhibiting ligand-induced receptor tyrosine kinase activation. **Abbreviations:** RTK, receptor tyrosine kinase; GPCR, G-protein coupled receptor; MMP-9, matrix metalloprotease-9; Neu-1, neuraminidase-1; PPCA, protective protein cathepsin A. **Citations:** Modified in part from © 2013 Abdulkhalek et al Research and Reports in Biochemistry 2013:3 17–30,

Drug repurposing in combination with current chemotherapy treatments can introduce a new clinical treatment avenue for individuals diagnosed with pancreatic cancer [6]. [6] reported a positive therapeutic effect of combining aspirin and oseltamivir phosphate (OP) with gemcitabine, offering a strong therapeutic alternative for pancreatic cancer treatment [6], as depicted in Figure 2. Cancer resistance to chemotherapeutics and high rates of metastasis have a significant contribution to pancreatic cancer's abysmal survival rate. A novel approach for treating human pancreatic cancer is the repurposing of the anti-inflammatory drug ASA with OP in conjunction with gemcitabine. Here, ASA, in combination with OP, was found to significantly improve the effectiveness of GEM in the treatment of pancreatic cancer and to disable key survival pathways critical to disease progression [6,7]. The synergistic combination efficacies of ASA, OP, and GEM were found to be concentration-dependent and have sensitivity differences for different cancer cells, enabling their enhanced therapeutic efficacies [6]. Here, the combination index (CI) was determined for the combination degree of ASA and OP interactions with GEM on MiaPaCa-2, PANC-1, and HEK 293 cells. Synergistic drug combinations have been extensively explored for enhanced drug therapeutic efficacies. In investigating synergistic drug combinations, the level of synergism is typically measured and quantified by the drug combination index, CI, a quantitative measure of drug combination effects using the Chou and Talalay's CI formula equation from dose-response data. The sum of the ratio of the drug concentration (mM) in the compound to the dose when used alone is used to the combination of drugs to produce 50%, 75%, and 95% efficacies. The results showed that, for 50% treatment efficacies of drug combinations, the calculated combination index (CI) for GEM greater than 1 (CI>1) at 0.8 µM plus 0.12 mM OP or 0.8 mM ASA indicated antagonism for both MiaPaCa-2 and PANC-1. The calculated CI equal to 1 was 1.6 mM for both OP and ASA at 0.8 µM GEM, indicating an additive effect for both MiaPaCa-2 and PANC-1 for a 75% treatment efficacies of drug combinations. The calculated CI less than 1 was 4.8 mM for OP and 3.2 mM for ASA at 0.8 µM GEM, indicating a synergistic effect for both MiaPaCa-2 and PANC-1 [6]. Interestingly and notably, the 95% treatment efficacies of drug combinations revealed the calculated CI less than 1 was 4.8 mM for OP and 0.6 mM for ASA at 0.2, 0.4 and 0.8 µM GEM, indicating a synergistic effect for both MiaPaCa-2 and PANC-1.

The striking differences in the drug combination indices between PANC-1 and MiaPaCa-2 pancreatic cancer cells may be due to their expression of COX-1 and -2 values through which ASA exerts its therapeutic effect [58]. COX-1 and -2 expressions are absent in MiaPaCa-2 cells and many other pancreatic cancer cells, while PANC-1 cells highly express COX-1 with little expression of COX-2. Overall, the combination of ASA+OP+GEM has the most potent effect in reducing cell viability, expression of critical extracellular matrix proteins, clonogenic potential, migration, and promoting apoptosis, as previously reported [6] and depicted in Figure 2.

Combining ASA and OP also affected several critical hallmarks of cancer as outlined by Hanahan and Weinberg [47,48], including maintaining proliferative signaling, evading growth suppressors, promoting invasion and metastasis, enhancing replicative immortality, triggering angiogenesis, and resisting cell death. Qorri et al. [6] showed that ASA and OP combined with GEM was found to target these emerging hallmarks, such as dysregulating cellular genetics and immune system invasion, genome instability and mutational characteristics, and tumor-promoting inflammation [47]. Zhang et al. [51] highlighted the capabilities of non-oncology drug repurposing for clinical cancer management. Cancer treatment can be enhanced by anti-inflammatory drugs, specifically immunologically cold tumors. However, the underlying mechanism(s) of this treatment remains unclear [59,60].

Despite therapeutic interventions, cancer cells are adaptive and invasive. As a result, more aggressive phenotypes survive and metastasize. Clinical evidence suggests that instability arises, followed by more significant aberrations, resulting in compensatory effects that promote tumor fitness throughout its progression. Qorri at al. [61] identified a novel treatment approach that targets Neu-1, which will negate the increase in tumor fitness effects of pancreatic cancer. Here, a continuous infusion of OP combined with ASA was used with standard treatment with GEM in preclinical RAG2xCy double mutant BALB/c mouse model of human PANC-1 pancreatic cancer. The tumorigenic and metastatic potential of the xenotumors from the animals treated with the experimental protocols were significantly ablated when transferred into the mammary fat pads of NSG (NOD SCID gamma) branded mice. Oral consumption of OP will convert through first-pass metabolism in the liver to form oseltamivir carboxylate, a carboxylate that does not affect mammalian Neu-1 [62]. To avoid this, OP was used in a continuous subcutaneous infusion protocol via an osmotic pump surgically implanted at the tumor site. ASA was incorporated based on previous work showing that ASA specifically targeted and inhibited Neu-1 sialidase activity [7]. Noteworthy, Neu1 was reported to regulate EGF-induced receptor activation in pancreatic PANC-1 cancer cells and its GEM-resistant variant, PANC-1-GemR cells [6,7,38].

Osmotic pumps can deliver drugs by providing sustained, controlled, and slow release. In a preclinical mouse model of human pancreatic cancer, the continuous infusion of OP with ASA effectively disrupted the development of acquired chemoresistance during chemotherapy treatment due to their ability to target several receptor tyrosine kinases implicated in the EMT process [61]. This continuous infusion of OP and ASA through implanted osmotic pumps in combination with GEM therapy hindered tumor growth, reduced chemoresistance, and inhibited metastasis in multiple tumor xenografts in a preclinical mouse model of human pancreatic cancer [61].

The tumorigenic and metastatic potential of the residual xenotumors obtained from these animals treated with the experimental combination of drugs revealed a significant ablation when transferred into the mammary fat pads of NSG (NOD SCID gamma) branded mice [61].

A continuous infusion of OP with ASA along with GEM has the potential to impede the emergence of acquired chemoresistance, thereby hindering the rapid disease progression observed in the GEM-only cohort [61]. When combined with current standard chemotherapy treatments, this novel combinational and continuous perfusion therapy may signify a promising therapeutic advancement in this highly lethal and difficult-to-treat malignancy, as depicted in Figure 3.

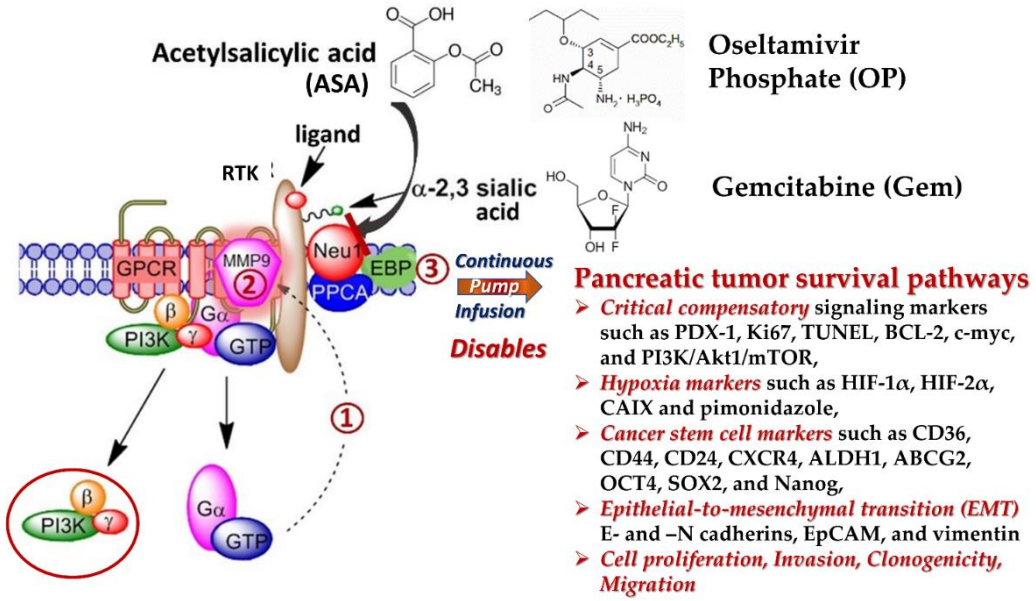

Figure 3. Osmotic pumps can deliver drugs, providing sustained, controlled, and slow release of the drugs for up to 42 days. We hypothesized that the continuous infusion of OP with ASA might effectively disrupt the development of acquired chemoresistance during chemotherapy treatment due to their ability to target several receptor tyrosine kinases implicated in the EMT process. Here, the data indicate that implanted osmotic pumps providing a continuous infusion of OP and ASA with GEM therapy impeded tumor growth, chemoresistance, metastasis, critical compensatory signaling, hypoxia, cancer stem cells, and EMT in tumor xenografts in a mouse model of human pancreatic cancer. **Citation:** Taken in part from: ©Abdulkhalek et al. Research and Reports in Biochemistry 2013:3,17-30, ©Abdulkhalek et al. Clinical and Translational Medicine 2014:3,28. Publisher and licensee Dove Medical Press Ltd, and © Qorri, B. et al. Cancers 2022, 14, 3595. https://doi.org/10.3390/cancers14153595 . This is an open-access article that permits unrestricted non-commercial use, provided the original work is properly cited.

Figure 3 depicts how the implanted osmotic pumps provide a continuous infusion of OP and ASA in combination with GEM therapy, which impeded tumor growth, chemoresistance, metastasis, critical compensatory signaling, hypoxia, cancer stem cells, and EMT in tumor xenografts of human pancreatic cancer [61]. The xenotumors from the PUMP (ASA+OP) treatment exhibited no tumor growth up to day 36, with a slight increase in tumor volume until the study endpoint on day 42 [61]. Notably, the OP- and ASA-treated xenotumors had no macro- and micro-metastases in the liver and lung in comparison to the CTRL and GEM-only xenotumors [61].

Clinical significance

The median progression-free survival in individuals diagnosed with metastatic pancreatic cancer is about six months with either 5FU/Irinotecan/Oxaliplatin or Abraxane with GEM [10,11]. Once disease progression occurs, the patient's cancer becomes more difficult to control. Death occurs at an increased rate if second-line therapies fail. The transition point at which tumors become less responsive to therapy is critical to pancreatic cancer patients. An additional issue to consider is the 'stiffness' of the pancreatic tissue following therapy treatments. Several studies suggest that phenotypic tissue stiffness correlates with therapy responsiveness and resistance to therapy [63,64].

The success of this experimental treatment approach may stem primarily from its capability to suppress compensatory EMT pathways initiated by the chemotherapy treatment. The compensatory EMT mechanism contributing to the development of drug resistance during cancer treatments has been observed in various cancers that have been studied so far [65]. The ability of this experimental treatment to disrupt the EMT process is reflected in increased expression of E-cadherin and reduced N-cadherin expression. Furthermore, stem cell enrichment in the experimental treatment cohort saw a significant reduction compared to the chemotherapy-only cohort and untreated control. The ability to inhibit EMT progression and stem cell enrichment during ongoing ASA and OP treatment with GEM chemotherapy is likely the reason for the effectiveness

of this novel treatment strategy. The results of these studies provide the groundwork to test this therapeutic treatment in a human clinical trial.

Limitations and challenges

One possible limitation to these studies and its potential translational clinical impact is that only single-agent GEM was used as the chemotherapy agent. For a standard of clinical care in the metastatic setting, a combination chemotherapy of both GEM with Abraxane and in combination 5-FU/oxaliplatin/irinotecan is used. These treatments have shown superiority over using a single-agent like gemcitabine in a Phase III clinical trial, monitoring response rates and median progression-free survival in pancreatic cancer This treatment does not detract from the potential translational relevance of this preclinical study. The experimental treatment protocol, when combined with the existing clinical standard of combination chemotherapy, becomes even more compelling due to its capacity to disrupt EMT induced by the chemotherapy treatment.

Summary and Conclusions

In summary, a novel therapeutic strategy targeting Neu-1 in combination with chemotherapy can be used in patients diagnosed with metastatic pancreatic cancer. This treatment approach has the potential to enhance both the response rate and median progression-free survival compared to the existing standard of care for this challenging and often rapidly fatal malignancy. A continuous infusion of OP in conjunction with ASA and GEM can effectively inhibit the progression of chemoresistance and prevent rapid disease progression. This treatment approach, in combination with the current standard chemotherapy treatment, can increase the response rate and median progression-free survival, presenting a potential therapeutic breakthrough in this highly lethal malignancy. Recently, M.R. Szewczuk reports an application to Health Canada seeking approval to test this therapeutic treatment reported in this study in a human clinical trial on patients with pancreatic cancer.

Abbreviations

TP53	tumor protein P53,
SMAD4	suppressor of mothers against decapentaplegic 4,
Wnt	wingless-related integration site,
ALDH1	aldehyde dehydrogenase isoform 1,
EBP	elastin binding protein,
PPCA	protective protein cathepsin A,
ASA	aspirin,
CSC	cancer stem cell,
ECM	extracellular matrix,
EGFR	epidermal growth factor receptor,
EMT	epithelial-mesenchymal transition,
GEM	gemcitabine,
GPCR	G protein-coupled receptor,
CTRL	untreated control cohort,
NSG	NOD SCID gamma mouse,
IC50	half-maximal inhibitory concentration,
INJ	injections of ASA + OP together with GEM
MMP	matrix metalloproteinase,
Neu-1	Neuraminidase-1,
NSAIDs	non-steroidal anti-inflammatory drugs,
OP	oseltamivir phosphate,
PDAC	Pancreatic ductal adenocarcinoma,
PUMP	ALZET Mini Osmotic Pump
Ras	rat sarcoma,
RTK	receptor tyrosine kinase,
TGF-β	transforming growth factor beta,
TME	tumor microenvironment,
VEGF	vascular endothelial growth factor.

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Conflict of interest

The authors declare no conflict of interest.

Ethics statements

This study has been approved by Sultan Qaboos University (SQU) Animal Ethics Committee (SQU-AEC-2019-20/10).

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