Sonoporation-Enhanced Chemotherapy Significantly Reduces Primary Tumour Burden in an Orthotopic Pancreatic Cancer Xenograft

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Abstract

Purpose: Adenocarcinoma of the pancreas remains one of the most lethal human cancers. The high mortality rates associated with this form of cancer are subsequent to late-stage clinical presentation and diagnosis, when surgery is rarely possible and of modest chemotherapeutic impact. Survival rates following diagnosis with advanced pancreatic cancer are very low; typical mortality rates of 50 % are expected within 3 months of diagnosis. However, adjuvant chemotherapy improves the prognosis of patients even after palliative surgery, and successful newer neoadjuvant chemotherapeutical modalities have recently been reported. For patients whose tumours appear unresectable, chemotherapy remains the only option. During the past two decades, the nucleoside analogue gemcitabine has become the first-line chemotherapy for pancreatic adenocarcinoma. In this study, we aim to increase the delivery of gemcitabine to pancreatic tumours by exploring the effect of sonoporation for localised drug delivery of gemcitabine in an orthotopic xenograft mouse model of pancreatic cancer.

Experimental Design: An orthotopic xenograft mouse model of luciferase expressing MIA PaCa-2 cells was developed, exhibiting disease development similar to human pancreatic adenocarcinoma. Subsequently, two groups of mice were treated with gemcitabine alone and gemcitabine combined with sonoporation; saline-treated mice were used as a control group. A custom-made focused ultrasound transducer using clinically safe acoustic conditions in combination with SonoVue® ultrasound contrast agent was used to induce sonoporation in the localised region of the primary tumour only. Whole-body disease development was measured using bioluminescence imaging, and primary tumour development was measured using 3D ultrasound.

Results: Following just two treatments combining sonoporation and gemcitabine, primary tumour volumes were significantly lower than control groups. Additional therapy dramatically inhibited primary tumour growth throughout the course of the disease, with median survival increases of up to 10 % demonstrated in comparison to the control groups.

Anthony Delalande and Mihaela Popa contributed equally.

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Introduction

Pancreatic adenocarcinoma (PA) morbidity and mortality remain almost unchanged over the past two decades. Available statistical predictive data estimate 45,220 new cases and 38,460 deaths due to PA in 2013 [1]. This type of cancer can rarely be treated radically, and the overall survival rate is less than 4% [2]. When surgical intervention is possible, survival rates can increase to a median of 36 months and surpass 5 years [3]. However, adjuvant chemotherapy improves the prognosis of the patients even after palliative surgery, and successful newer neoadjuvant chemotherapeutical modalities have recently been reported [4]. Most of the patients with advanced PA have about a year to live after diagnosis [5, 6], and even an increase in survival of a few months matters in the pancreatic cancer field. Gemcitabine, a nucleoside analogue, is considered as the most effective chemotherapeutic agent, is microbubbles [28–30] filled lipid-shelled microbubbles in the size range of 2–10 μm [12]. When excited by ultrasound, microbubbles scatter the incoming ultrasound and volumetrically oscillate, generating their own ultrasonic pulse. This results in various complex and controllable actions that can be taken advantage of for therapeutic purposes [13], e.g. sonoporation-enhanced therapy [14–19]. Sonoporation is the transient formation of nanometre- to micrometre-sized pores in cell membranes by the use of ultrasound or ultrasound and microbubbles, allowing for intracellular uptake of drugs or genes [19].

Previous research has elaborated six common mechanistic effects of sonoporation-mediated drug and gene delivery [19–25], including push, pull, shear, jetting, inertial cavitation, and translation. While “push”, “pull”, “shearing” and “translation” occur at all acoustic amplitudes resulting in perturbation of the cell membrane, “jetting” [26] and “inertial cavitation” [27] only occur at high acoustic amplitudes, surpassing current ultrasound safety guidelines with the introduction of a cavitation nucleation agent, i.e., microbubbles [28–30]. Furthermore, a large majority of studies only evaluate the effect of sonoporation in vitro [16, 31–33] in subcutaneous models [34] or for the efficacy of gene delivery [15, 31, 32, 35]. Ultimately, in a sonoporation-enhanced therapeutic approach, the goal must be to employ acoustically safe amplitudes but still impact translation of microbubbles and therapeutic across the cell membrane. Thus, “translation” sonoporation offers the preferred clinical modality, resulting in microbubbles being forced inside a cell, leaving a small pore that re-seals itself, occurring at low, clinically safe acoustic amplitudes.

Pre-clinical drug development in xenograft models of PA has historically been reliant upon subcutaneous inoculation of PA cells in the flanks of immunodeficient mice. However, subcutaneous models are not sufficiently representative of the clinical paradigm of PA, particularly with respect to metastasis development and drug response [36, 37]. Using an orthotopic model provides many advantages such as the potential to target local invasion at a much more clinically relevant site, while blood flow and vascularisation of the tumours more closely mimic the human model. Taking into account the importance of an orthotopic model, in this study we aimed to investigate the potential of sonoporation for enhanced localised drug delivery of a clinical chemotherapeutic (gemcitabine) to the primary tumour of PA in an orthotopic model.

Materials and Methods

Ultrasonic Treatment Conditions

A custom-made single-element ultrasound transducer consisting of a 25-mm spherically focused element with a geometric focus of 44 mm (Precision Acoustics Ltd., Dorchester, UK) was used as the treatment probe. To ensure correct acoustic alignment with the pancreas’ depth and location, a custom adaptor was designed and built based on the transducer dimensions and beam profile. The adaptor was filled with distilled water, and an 80-μm-thick nitrile membrane was used at the contact surface to ensure maximum beam propagation. Figure 1a shows the design of the transducer and adaptor.

In order to drive the ultrasound transducer, a 40 % duty cycle sine wave was generated by an AFG3102 function generator (Tektronix, Inc., Beaverton, OR, USA) and amplified by a 2100 L amplifier (Electronics & Innovation Ltd., Rochester, NY, USA). The acoustic field was calibrated with the acoustic adaptor in place using an automated 3D scanning chamber with a 50-μm resolution and a calibrated HGL-200 hydrophone (Onda, Sunnyvale, CA, USA) in degassed water.

Based on our previous in vitro findings [19, 24], the acoustic settings shown in Table 1 were used to treat the pancreatic tumour.
Ultrasound Contrast Agent/Microbubbles

A clinically employed ultrasound contrast agent (SonoVue®, Bracco Imaging S.p.A, Milan, Italy) was prepared according to the manufacturer's instructions. This results in phospholipid-shelled microbubbles containing an SF₆ gas core with a mean diameter of 2.5 μm at concentrations between 1×10⁸–5×10⁸ microbubbles per millilitre [38]. The microbubbles were prepared immediately prior to treatment of the first mouse, and the vial was gently agitated prior to each treatment to ensure a homogenous concentration.

Cell Lines and Cell Culture

The human pancreatic adenocarcinoma MIA PaCa-2 cell line was kindly provided by Dr. Anders Molven (The University of Bergen, Norway). The cells were retrovirally transfected using Phoenix cells (LGC Standards AB, Boras, Sweden) and a luciferase-encoding plasmid. High luciferase-expressing cells were selected by puromycin (2.5 μg/ml). Cells were cultured in DMEM (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) complemented by 10 % FBS, 2.5 % FHS, 100 μg/ml penicillin-streptomycin and 2 mM L-glutamine (Sigma-Aldrich).

Animals

All experiments were approved by The Norwegian Animal Research Authority and conducted according to The European Convention for the Protection of Vertebrates Used for Scientific Purposes. NOD-scid IL2rγ−/− mice (Gades Institute, University of Bergen; originally a generous gift of Prof. Leonard D. Shultz, Jackson Laboratories, Bar Harbour, ME, USA) were used. During depilation and imaging, mice

Table 1. Acoustic settings used to enhance drug delivery

<table>
<thead>
<tr>
<th>Centre frequency (MHz)</th>
<th>Duty cycle (%)</th>
<th>Mechanical index</th>
<th>Acoustic intensity, $I_{SPTA}$ (mW/cm²)</th>
<th>Peak negative acoustic pressure (MPa)</th>
</tr>
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<tr>
<td>1.00</td>
<td>40 (40 μs of ultrasound every 100 μs)</td>
<td>0.2</td>
<td>688</td>
<td>0.20</td>
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were anaesthetized with 2 % isoflurane (Isoba® vet, Intervet, Kenilworth, NJ, USA).

Orthotopic Xenograft Model

Animals were fully anaesthetized with 250 mg/kg tribromoethanol diluted in 2 methyl-2 butanol and 12.5 mg/ml (Sigma-Aldrich) and placed on a heating pad in dorsal recumbency. Hair was removed by shaving, and the abdomen was washed with isobetadine and 70 % alcohol. A small incision (0.5 cm) in the abdomen was made below the last rib on the left side, parallel to the linea alba. The pancreas was exteriorized, and the cells (1×10⁶ MIA PaCa-2luc) suspended in 20-µL phosphate-buffered saline were injected using a 30-gauge needle. After placing the pancreas back in the original position, the muscles and the skin were sutured with Ethilon II 5–0 polyamide sutures (Ethicon, Inc., Somerville, NJ, USA). Prior to return to their holding cages, the animals were placed under heat lamps for approximately 30 min and monitored for any postoperative complications. All animals were euthanized following institutional guidelines.

Optical Imaging

The mice were anaesthetised and injected intraperitoneally (IP) with 150 mg/kg d-luciferin (Biosynth AG, Staad, Switzerland) 10 min prior to ventral and dorsal imaging using an In-Vivo FX PRO molecular imaging system (Carestream Health, Inc., Rochester, NY, USA). Total bioluminescence values were measured using manual ROIs in the Carestream MI software (Standard Edition, v.5.0.6.20, Carestream Health, Inc.). Two types of ROIs were used, one whole abdomen and one locally in the primary tumour area.

High-Resolution Ultrasonic Imaging

The mice were briefly re-anaesthetised and placed on the mouse handling table set at 40 °C in dorsal recumbency. The abdominal hair on the mice was removed using depilatory cream. A Vevo2100 ultrasound scanner combined with a MS250 (13–24 MHz) probe and 1D stage (VisualSonics Inc., Ontario, Canada) was used to capture 3D images of the primary tumour. Enhanced abdominal imaging B-mode and 3D mode was used. Doppler imaging was also used to aid primary tumour identification. Respiration gating was used to capture the 3D images. The tumours were measured manually using parallel segmentation in the Vevo2100 software (v.1.3.0, VisualSonics Inc.).

Treatment Protocol

Three weeks following xenotransplantation, mice were randomly separated into three groups: control (n=3), gemcitabine alone (125 mg/kg, Q7D, n=3) and combined treatment group, employing Sonovue® (50 µL) and gemcitabine (125 mg/kg, Q7D, n=4). Therapy was initiated on week 3 and continued weekly for a total of 8 cycles. Mice were euthanized when they started to demonstrate progressive weight loss below 10 % from initial body weight, jaundice, and lethargy.

Control mice received 200-µL saline (IP) post-imaging procedures. The gemcitabine group was treated with gemcitabine (125 mg/mg, i.p.) once weekly, whilst combination-treated animals received gemcitabine (125 mg/mg, i.p.) and Sonovue® ultrasound contrast agent (50 µL, i.v.) 30 min later. Immediately after Sonovue® injection, the mouse was placed on a heated table at 37 °C, and ultrasound was applied for a total of 10 min (4 min total cumulated ultrasound time). Figure 1b shows how the mice and therapeutic transducer were positioned for the treatment.

Histopathology

Organ samples collected following euthanasia were placed in 4 % paraformaldehyde for paraffin embedding. Sections were stained with hematoxylin and eosin (H&E), and results were analysed by standard light microscopy (Nikon Eclipse Ti, Nikon Instruments Europe BV, Amsterdam, Netherlands) by an experienced pathologist.

Statistics

Three different observers measured the primary tumour volume where two were blind to the groups. Results were expressed as the mean values±SEM. Comparisons between groups were made using a two-tailed unpaired Student t-test. Survival data were analysed using a log-rank (Mantel–Cox) test. Differences where p<0.05 were considered as statistically significant. Statistics were analysed using GraphPad PRISM® v5.0a (GraphPad Software Inc., La Jolla, CA, USA) software.

Results

Transducer Validation and Characterisation

The therapeutic transducer was fabricated in order to induce sonoporation in a very localised region, generating a very precise and sharp focal (treatment) zone. The 3D field scan validated the beam profile, indicating a focus of 4.0 mm in diameter and 22 mm in length (Fig. 1c). A small focus was chosen to ensure that only the primary pancreatic tumour was treated as only this region would receive the ideal dose of ultrasound. The non-linearity ratio was 1.00, ensuring there were no shockwaves generated in the acoustic field prohibiting any other adverse effects seen by high-intensity ultrasound treatments and preventing microbubble destruction. Furthermore, the adaptor reduced the side lobe intensity, allowing for a Gaussian sound intensity profile [39].

By creating such a controllable sound field, with such a precise focus (treatment zone), we could clearly dictate the location and size of the treatment area, without treating the organs behind or in front of the pancreatic tumour.

Pancreatic Cancer Xenograph Model Development

Following inoculation of 1×10⁶ MIA PaCa-2luc cells in the head of pancreas, recipient mice developed PA with similar characteristics, e.g. tumour vascularisation, encapsulation and metastatic dissemination, as observed in the human counterpart
Three weeks after inoculation, the primary tumour could be visualised by bioluminescence and high-resolution ultrasound (Figs. 2a and 4b, respectively). Metastasis was evident 5–6 weeks after inoculation as demonstrated by the increase of bioluminescence in the whole abdomen when compared to the primary tumour (Fig. 2b). After 8 weeks, metastases have disseminated throughout the whole abdomen, particularly hepatic metastasis, and in 10 weeks it has engulfed the whole abdomen. Disease progression presented with similar features to that observed in human patients with exponential growth of the primary tumour and dense vascular encapsulation. Histology verified that this was a very aggressive form of PA characterised by nuclear polymorphism, atypical mitosis and invasive growth pattern (Fig. 2c).

Sonoporation of Gemcitabine with Microbubbles Is Safe in a Pre-clinical Model of Pancreatic Adenocarcinoma  In order to evaluate the safety and efficacy of combined sonoporation and gemcitabine therapy, mice (n=10) were inoculated, and tumour and metastatic development was monitored by 3D ultrasound and bioluminescent imaging. Primary tumour development was evident at 3 weeks by bioluminescence and 3D ultrasonic imaging when mice were randomised into three groups. Mouse weights were recorded every 2 days to monitor any adverse effects of the treatment. Following treatment, a slight weight loss was recorded in all groups, with subsequent weight recovery following treatment. No significant differences in weight were seen between all three groups (Supplementary Fig. 1), and no additional ill effects were noted, suggesting that sonoporation combined with chemotherapy was safe in this pre-clinical setting.

Combined Gemcitabine Plus Sonoporation Significantly Inhibits Primary Pancreatic Adenocarcinoma Tumour Growth  While it was anticipated that therapy might have an impact upon the metastatic pattern of this very aggressive disease, the ultimate goal remained to evaluate the effect of therapy within the sonoporation field. Thus, we evaluated the primary pancreatic tumours response to therapy by tumoural bioluminescence imaging (Fig. 3) and 3D ultrasonic imaging (Fig. 4). Quantification of bioluminescence output from the primary tumour revealed reduced photonic flux in combination and gemcitabine-treated mice than controls (Fig. 3a, p=0.0003 and 0.0019, respectively); however, scattering of biophotonic light from adjacent metastasis inevitably contaminated the primary tumour photonic quantification. Nevertheless, it was
encouraging to see lower bioluminescence at the primary site compared with controls.

In order to directly quantify primary tumour effects employing 3D ultrasound, we exploited Doppler imaging, which revealed dense vascularisation encapsulating the primary tumour (Fig. 4a, 1 and 2). Under high-frequency ultrasound, the primary tumour was seen as a singular anechoic region (Figs. 4a and 3), indicating a very homogenous cellular structure; this was also

Fig. 3. Primary tumour (a) and whole abdomen (b) bioluminescence count of MIA PaCa-2Luc model treated with gemcitabine, gemcitabine microbubbles and ultrasound or control once a week. Optical bioluminescence imaging of representative mouse of each group (c). No statistical difference between the gemcitabine- and sonoporation-treated group can be seen. The control group had a much higher total bioluminescence count at the final stages of disease progression.

Fig. 4. Primary pancreatic tumour volume measured by 3D ultrasound of MIA PaCa-2Luc model treated with gemcitabine, gemcitabine microbubbles and ultrasound or control once a week. a Steps on how the primary tumour was detected and 3D volume was measured. Using colour Doppler, the feeding arteries of the tumour and kidney can be easily distinguished in both 2D and 3D (1 and 2, respectively). The primary tumour can then be manually contoured throughout the 3D stack (3). The primary tumour can then be visualised within the 3D ultrasound image (4) or on its own (5). 3D tumour volume over time can be visualised and compared. The 3D volumes are visualised in b and mean volume±SEM are shown in c. After two treatments, a statistically significant difference can be seen between the combined treatment group and the gemcitabine alone and/or control group.
evaluated from post-study histology samples (Fig. 6). This anechoic region was used to generate 3D volumes of primary tumours (Fig. 4a, b). Comparing tumour volumes from week 5 (Fig. 4c) onwards, a statistically significant difference between the combined treatment group and the gemcitabine group versus control was seen. There was no statistical difference in primary tumour volume when comparing the gemcitabine group to the control group; however, statistically significant differences were observed between the combined treatment group versus both control ($p<0.05-0.001$) and gemcitabine-only groups ($p<0.05-0.001$).

**Combined Gemcitabine and Sonoporation Treatment Moderately Impacts Systemic Disease and Survival** Although whole-body bioluminescent imaging revealed a slower onset of metastatic development in combination-treated animals (from weeks 6 to 8; Fig. 3c) with lower bioluminescence observed at weeks 9 and 10, all animals eventually succumbed to metastatic disease. While disseminated metastasis of the liver and abdomen were noted in control and, to a lesser extent, gemcitabine-treated animals from weeks 7 to 10, combination-treated mice from weeks 6 to 8; Fig. 3c) with lower bioluminescence observed at weeks 9 and 10, all animals eventually succumbed to metastatic disease. While disseminated metastasis of the liver and abdomen were noted in control and, to a lesser extent, gemcitabine-treated animals from weeks 7 to 10, combination-treated mice demonstrated restricted metastatic phenotype mostly constrained to the liver (Fig. 3c). Histopathology indicated a very aggressive form of PA with nuclear polymorphism, atypical mitosis and an invasive growth pattern. However, the combination-treated group showed a less invasive growth pattern seen at the border between normal pancreatic and tumour tissue (Fig. 5). While both combination and gemcitabine groups demonstrated significant increases in survival versus controls (median survival times of 88, 84 and 80 days, respectively), significance could not be demonstrated between the treated cohorts ($p=0.0570$; Fig. 6).

**Discussion**

Here we describe the development of an orthotopic PA mouse model exhibiting very similar characteristics to its human counterpart. A custom-made ultrasound transducer was used to induce sonoporation using clinically approved microbubbles (SonoVue®) at an extremely localised area using identical acoustic settings shown to induce sonoporation in our previous work [19, 24]. To follow the disease progression, tumour volume was measured weekly using 3D ultrasound, and optical imaging was used to observe whole-body disease development. Two treatments with combined gemcitabine and sonoporation resulted in statistically significant lower primary tumour volume. This statistical difference was persistent for the further duration of the study. Furthermore, bioluminescence imaging indicated that there was a delay in the onset of metastasis in responding mice.

Sonoporation in combination with gemcitabine was employed to treat orthotopic PA xenografts, resulting in a significant reduction of tumour growth, inhibited metastatic development and a moderate increase in survival when compared to controls. These results indicate potential to enhance the localised drug delivery of chemotherapeutics, in consequence reducing the systemic toxic side effects and increasing local drug delivery. In this study, we have used very low acoustic conditions that comply with current clinical safety regulations with the inclusion of a contrast agent [28–30], allowing for easy translation from pre-clinical to clinic studies. Clinical increase of local neoadjuvant chemotherapeutic exposure by sonoporation should reduce the growth rate of the primary tumour and allow for the possibility of surgical resection and increased survival [3].

While gemcitabine therapy is the current standard of care in the therapy of pancreatic cancer, its clinical effect is modest, only extending survival by 1 month [41]. Similarly, in our pre-clinical study, gemcitabine improved survival over controls modestly, with both cohorts exhibiting disseminated metastatic disease. Additional sonoporation of the primary tumour following gemcitabine therapy limited primary tumour development and metastatic dissemination, resulting in some improvement to overall survival, compared with gemcitabine alone. As such, this may add a clinical benefit in limiting primary tumour growth, making resection and curable treatment possible.

Treatment could only be instigated once the tumour could be accurately located and correlated to a physical location on the mouse, only possible 3 weeks following implantation. It was important to be able to unequivocally locate the tumour in three dimensions to accurately position the therapeutic probe. As a result, it is not inconceivable that within 3 weeks micrometastasis may have already seeded, undetectable with either imaging modality, prior to initiation of therapy.

**Fig. 5.** Survival data of MIA PaCa-2 luc model treated with gemcitabine, gemcitabine microbubbles and ultrasound or control. A slight increase in survival time can be seen between the groups but was not statistically significant.
Owing to the complexity of the experimental design, including surgery, simultaneous therapy and imaging with two modalities in two separate facilities necessitated an extensively skilled workforce, limiting the total number of study mice, to improve experimental consistency and ethical regulations. While resulting in low power to survival analysis, significant inhibition of primary tumour growth is encouraging to note; our primary objective was demonstrated whilst complying with all ethical aspects of the 3Rs (replacement, refinement and reduction of animal numbers). Subsequent studies to evaluate the efficacy of sonoporation enhancement of chemotherapeutic treatment should use patient-derived xenografts using this protocol. Although individual mice will be treated at different times, similar to a clinical trial, imaging of the dense vasculature encapsulating the primary tumour (Fig. 4a) combined with optical imaging will aid in the standardisation of therapy initiation. Implanting and treating mice in this format will also reduce stress and enable greater numbers of animals to be treated. Further development of imaging in such pre-clinical studies should include near-infrared optical imaging agents [42, 43] to improve primary tumour delineation. Additionally, multispectral optoacoustic tomography (MSOT) or photoacoustic imaging may permit 3D tumour localisation at an earlier stage.

The encapsulating vascularisation, though maintaining tumour nutrition seems beneficial for the treatment with sonoporation as the microbubbles have better access to the tumour mass, and clinical contrast-enhanced ultrasonography demonstrate that microbubbles are not adversely affected by high intratumoral pressures [44]. Developments that may further increase the efficacy of sonoporation would be to introduce a drug into these microbubbles, creating anti-bubbles [45] and then inducing sonoporation. In this case, the toxic chemotherapeutic would only be delivered to the cancer cells. This would ensure maximum efficacy and reduce the side effects of the systemic chemotherapeutics.

As sonoporation has been shown to improve drug delivery to localised areas, other drug treatments in combination with sonoporation should be explored. Several new drugs that have indicated high effectiveness in treating pancreatic cancer cells are being investigated in vitro and in pre-clinical trials [46–48]; evaluating the efficiency of these drugs in combination with the benefits of sonoporation needs to be further studied.

Following these results, we have commenced a phase I study to evaluate the toxicity and/or efficacy of gemcitabine combined with inducing sonoporation in the clinic on pancreatic cancer tumours (2011/1601/REK).

Fig. 6. Histology images showing the invasive border between normal and tumour tissue in the control group and the less invasive border can be seen in the sonoporation-treated group as indicated by the asterisks. As the samples were taken at different time points, a very aggressive form of pancreatic cancer was observed in all cases.
Conclusion
In this study, we have developed a custom-made ultrasound transducer to induce sonoporation in a very localised region required for pre-clinical studies and a bioluminescent model of pancreatic adenocarcinoma. We demonstrated that by inducing sonoporation at the location of an orthotopic pancreatic xenograft primary tumour in combination with chemotherapeutic gemcitabine, we are able to significantly inhibit tumour growth compared with control or gemcitabine-treated mice, even in cohorts with a low sample number. Our results indicate that it is possible to enhance the efficacy of current chemotherapeutics with the simple addition of low-cost ultrasound contrast agents and a sound source. To further validate the clinical relevance of this study, repeated studies of primary patient-derived xenografts, possibly with the addition of near-infrared contrast reagents and MSOT imaging, should be considered. On the basis of these pre-clinical results, the combination of gemcitabine and sonoporation is currently undergoing phase I clinical trial at our centre.

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Conflict of Interest. The authors have no conflict of interest.

References