Paclitaxel and Rose Bengal Loaded Microbubbles for the Ultrasound Targeted Chemo-Sonodynamic Therapy of Pancreatic Cancer.

Jack Wright[1], Keiran Logan[1], Thomas McKaig[1], Sukanta Kamila[1], Chloe McClenaghan[1], Heather Nesbitt[1], Mark A. Taylor[2], Mark Love[3], Eleanor Stride,[4] Jia-Ling Ruan,[4] Anthony P. McHale[1], John F. Callan[1*]

1. Biomedical Sciences Research Institute, University of Ulster, Coleraine, Northern Ireland, UK; 2. Department of HPB Surgery, Mater Hospital, Belfast, Northern Ireland, U.K. BT14 6AB. 3. Imaging Centre, The Royal Victoria Hospital, Grosvenor Road, Belfast, Northern Ireland, U.K. BT12 6BA; 4. Institute of Biomedical Engineering, University of Oxford, Oxford, U.K. OX3 7DQ.

* Address to whom correspondence should be addressed: j.callan@ulster.ac.uk.

Abstract: Despite significant advances in cancer treatment over the past five decades, survival outcomes for pancreatic cancer have remained largely unchanged. The effectiveness of chemotherapy as a treatment for pancreatic cancer is limited by the dense, protective tumour stroma, which impedes drug delivery. Ultrasound-targeted microbubble destruction (UTMD) has emerged as a promising strategy for enhancing the delivery of chemotherapy agents to solid tumours. In this study, we report the development and evaluation of a novel microbubble (MB) formulation, ST-001, which incorporates paclitaxel chemotherapy and a Rose Bengal sonosensitiser for targeted chemo-sonodynamic therapy of pancreatic cancer. The principle of UTMD using ST-001 was demonstrated in a murine model of pancreatic cancer, where B-mode ultrasound imaging was used to visualize MB accumulation within the tumour and its subsequent clearance following the application of therapeutic ultrasound. Preclinical efficacy studies demonstrated a significant survival advantage in ST-001 treated mice, which survived more than twice as long as those treated with standard Taxol, despite receiving only 14% of the paclitaxel dose. Additionally, a preclinical toxicology study in healthy mice demonstrated an excellent safety profile for ST-

001, with no adverse effects observed in key hematological and blood biochemical markers, or in the histology of the spleen, liver, and kidneys.

Keywords: Pancreatic Cancer, MBs, Paclitaxel, Rose Bengal, Ultrasound, Targeted therapy.

Introduction: Survival rates for pancreatic cancer have remained largely unchanged over the past five decades, and it is projected to become the second leading cause of cancer-related deaths by 2030 [1]. Chemotherapy is employed at all stages of the disease, whether as a neoadjuvant, adjuvant, or palliative treatment. Gem-Abraxane (a combination of gemcitabine and Abraxane) is one of the most widely used chemotherapy regimens for pancreatic cancer [2]. Abraxane, which consists of nanoparticulate albumin-bound paclitaxel, was developed to address the delivery challenges associated with conventional paclitaxel treatment [3]. Due to paclitaxel's poor aqueous solubility, the polyoxyethylated castor oil solvent (Cremaphor) is typically used to solubilize paclitaxel for intravenous administration, a formulation marketed as Taxol [4]. However, this formulation is associated with infusion hypersensitivity reactions, requiring premedication with steroids and antihistamines [5]. The solvent-free Abraxane formulation was shown to offer more favourable pharmacologic properties, allowing for the delivery of a higher dose of paclitaxel compared to Taxol [6].

Inspired by this development, we hypothesized that, although Taxol is not typically used to treat pancreatic cancer, paclitaxel can be an effective treatment for the disease when delivered more efficiently, as exemplified by the success of Abraxane. We have previously demonstrated that ultrasound-targeted microbubble destruction (UTMD) is an effective strategy for delivering a variety of payloads to solid tumours [7]. UTMD utilizes lipid or polymer-stabilized, gas-filled microbubbles (MBs) loaded with drugs. After intravenous administration, low-intensity ultrasound is used to rupture the MBs at a target site, such as a tumour, facilitating localized drug release [8]. An additional advantage of this approach is

that the micro-jetting effect associated with MB inertial cavitation is known to enhance the microscale mass transfer of the drugs through otherwise impermeable tissue [9,10]. This is especially important for pancreatic cancer, as the dense fibrotic stroma is known to hinder the uptake of drugs delivered through conventional systemic administration [11].

In this manuscript, we report the formulation, characterization and preclinical efficacy / safety testing of a phospholipid-stabilized MB carrying paclitaxel and the sonodynamic therapy (SDT) sensitizer Rose Bengal (RB). The paclitaxel/RB-loaded MBs (ST-001) were prepared as a pre-MB suspension and activated immediately before use. The efficacy of UTMD mediated chemo-SDT using ST-001 was evaluated in two pancreatic cancer cell lines (Panc-01 and T110299) and in a murine model of the disease. The process of UTMD was demonstrated *in vivo* using B-mode ultrasound imaging, and the safety of the ST-001 formulation was confirmed in healthy, non-tumour-bearing mice.

2.0 Material and Methods

2.1 *Materials and Reagents*: The phospholipids 1,2-dibehenoyl-sn-glycero-3-phosphocholine (DBPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000] (DSPE-PEG(2000)) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). DSPE-RB was prepared as reported by McKaig *et al*. [7]. Cholesterol was purchased from Sigma Aldrich (St. Louis, MO, USA), perflouoropropane (PFP) from F2 Chemicals Ltd (Preston, UK) and paclitaxel was purchased from LC laboratories (China). Triacetin was purchased from Sigma Aldrich (St. Louis, MO, USA). The T110299 cell line, a gift from Prof. Jens Siveke, (Klinikum rechts der Isar, Technical University Munich, Munich, Germany), was isolated from primary pancreatic tumours in KPC mice (Ptfla-Cre; LSL-^{KrasG12D}; LSL-$^{Trp53fl/R172H}$)- hereafter referred to as KPC. Panc-01 cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). BALB/c nude mice were bred in house. CD-1 mice (8–10-week-old) were purchased from Envigo RMS (UK) Ltd. 3D modelling of tumours in animals was performed using the Peira TM900 tumour measuring

device. Roswell Park Memorial Institute (RPMI 1640) culture media, foetal bovine serum (FBS), penicillin and streptomycin were obtained from ThermoFisher Scientific (Waltham, MA, USA). 96-well plates were purchased from Sarstedt (Nümbrecht, Germany) and MTT reagent from Merck (Rahway, NJ, USA). UV absorbances were recorded using a Fluostar Omega plate reader (BMG Labtech, Ortenberg, Germany). Optical microscope images were obtained using a 40x-2000x Lab Trinocular Lab Microscope from AmScope (Irvine, CA, USA). Realtime continuous B-mode ultrasound imaging was performed using the Ultrasound Advanced Open Platform (ULA-OP) (MSDLAB of the University of Florence, Sesto Fiorentino, Italy) system in conjunction with a linear array probe (LA523, Esaote spa, Firenze, Italy). Therapeutic ultrasound was delivered using a Sonidel SP100 sonoporator (Sonidel Limited, Dublin Ireland). MBs were activated using a VialMix shaker (DuPont Pharmaceuticals, Billerica, Massachusetts, USA).

2.2 Preparation of ST-001 formulation: DBPC (2.9 eq), DSPE-PEG(2000) (1.0 eq), DSPE-RB (3.8 eq), and cholesterol (1.0 eq) were dissolved in a chloroform / methanol mixture (2:1 v/v). The solvent was removed by rotary evaporation and the resulting film dried *in vacuo* at 25^{o}C. The film was then hydrated with a PBS : propylene glycol : glycerol (8:1:1 v/v/v) mixture to reach a final lipid concentration of 4.62 mg/mL, which was stirred at 80^{o}C for 45 min. The solution was cooled to 25^{o}C, sonicated in a bath sonicator for 45 min and then aliquoted (1 mL) into 2 mL vials. The required amount of a paclitaxel in triacetin solution (75 mg / mL) was added to the vials to reach the required concentration. The headspace of each vial was exchanged with PFP by sparging for 10 sec and the vials stoppered and crimped. The vials were then stored at 5-8^{o}C and activated immediately before by agitating for 45 using a VialMix device. MB size distribution was determined by analysis of optical images using a bespoke MATLAB algorithm (2010B, MathWorks, Natick,MA, USA). MB concentrations were determined from optical images by manually counting the number of MBs per unit area and multiplying by the appropriate factor.

2.3 Determination of ST-001 paclitaxel loading and in-use stability of ST-001: To determine the amount of paclitaxel encapsulated within the ST-001 MBs, an appropriate volume of ST-001 MBs was transferred to a microcentrifuge tube and centrifuged at 2000 RPM for 9 minutes. The infranatant solution was removed and added to a separate microcentrifuge tube. 750 µL of isopropanol (IPA) was added to both the microcentrifuge tube containing the infranatant solution and the tube containing the supernatant MB suspension. Each sample was then vortexed for 30 seconds before being sonicated using a bath sonicator for 2 minutes. The resulting solution was transferred to a 5 mL volumetric flask and made up to the mark using IPA. This process was repeated in triplicate. The paclitaxel concentration was determined using HPLC on a Kinetex 5 µm EVO C18 100 Å column maintained at 25°C. The analysis was performed using an isocratic mobile phase (50:50 v/v) of water with 0.05% TFA (A) and acetonitrile with 0.05% TFA (B) at a flow rate of 1 mL/min for 10 min, with a 5 µL injection volume and a detection wavelength of 227 nm.

For the in-use stability study, 5 vials (vials 1-5) of ST-001 were activated consecutively and stored at room temperature. For vial 1, the mean MB concentration and mean particle diameter were determined using optical microscopy t=0h. The same process was repeated for vial 2 at t=2h, vial 3 at t = 4h and vial 5 at t =6h.

2.4 In vitro cytotoxicity of ST-001 in pancreatic cancer cell lines: KPC pancreatic cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5g/L glucose and supplemented with 10% foetal bovine serum (FBS), 100 U/mL penicillin, 100 mg/mL streptomycin, 1% L-Glutamine and 1% non-essential amino acids in a humidified 5% CO_2 atmosphere at 37°C. A single cell suspension was prepared by treatment with trypsin-EDTA and cells were dispensed into 500 µL Eppendorf tubes at a cell density of 16000 cells in 200 µL of medium. ST-001 was then added to obtain final concentrations ranging from2.43 – 38.80 µM of paclitaxel and 1.12 µM – 17.90 µM of DSPE-RB. Selected Eppendorf tubes were treated with ultrasound using a Sonidel SP100 sonoporator for 10 s (3.0 W cm^{-2}, 1 MHz, 30% duty cycle, and PRF = 100 Hz; PNP = 0.48 MPa; MI = 0.48) using ultrasound

gel to mediate contact and incubated at 37°C for 30 minutes. After incubation the Eppendorf tubes were insonated for an additional 30 s. Following treatment, 100 µL aliquots of the Eppendorf cell suspension were dispensed into each well on a 96 well plate yielding a final cell density of 4000 cells per well, 4 wells per Eppendorf. The plate was incubated at 37°C for 24h and cell viability then determined using a MTT assay. Untreated cells, cells treated with US alone and cells treated with ST-001 alone (i.e. no ultrasound) were used for comparative purposes. Data are expressed as % cell viability by comparison with untreated controls. Statistical analysis was undertaken for group comparisons using a one-way ANOVA (GraphPad Prism version 9.5.0.) between the following groups (i) untreated versus ultrasound only (ii) untreated versus ST-001 + ultrasound and (iii) ST-001 + ultrasound versus ST-001 alone.

The above procedure was repeated for the Panc-01 cell line with the following modifications. Panc-01 cells were maintained in (DMEM) containing 4.5 g/L glucose supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin and 10% FBS. Cells were seeded at a density of 20000 cells per Eppendorf, with a final cell density of 5000 cells per well on the 96 well plate.

2.5 B-mode ultrasound imaging of ST-001 UTMD in vivo: An athymic nude mouse bearing an ectopic KPC tumour generated using the procedure as outlined in 2.6 was used in this study. The animal was anesthetised, and a B-mode ultrasound imaging probe positioned at the tumour using US gel to mediate contact. The therapeutic Sonidel probe was also positioned on the tumour at a 90° angle to the imaging probe using US gel to mediate contact. Continuous B-mode imaging was commenced to determine baseline contrast levels. ST-001 (100 µL) was then administered via tail vein injection and therapeutic ultrasound treatment (Sonidel; 3.5 W cm^{-2}, 1 MHz, 30% duty cycle, and PRF = 100 Hz; PNP = 0.48 MPa; MI = 0.48) started 6 s later for a period of 70 s. The mean contrast levels in the tumour over the course of the experiment was determined by analysing regional pixel intensity using computer assisted image analysis. Briefly, individual TIFF images were

extracted from video recordings at 1-second intervals. Using ImageJ software (NIH, Bethesda, MD), specific regions of interest (ROIs) were manually traced on each image and applied consistently across sequential frames. Grayscale pixel intensity measurements were obtained within these defined regions and used to determine mean pixel intensity.

2.6 Efficacy of ST-001 mediated UTMD in a murine model of pancreatic cancer: All animals were treated humanely and in accordance with licenced protocols under the Animals (Scientific Procedures) Act 1986. KPC cells (5×10^5) in 100 µL PBS were subcutaneously implanted into the rear dorsum of 8 – 12 week old male athymic nude mice,. Tumours started to form approximately 5 days after cell implantation. Once the tumours became palpable, dimensions were measured using Vernier callipers. On days 0-7, tumour measurements were also recorded using a Peira tumour measuring device. The geometric mean diameter was used to determine tumour volume using the equation tumour volume = $4\pi R^3/3$. 10 days post implantation the tumours reached an average of 70.28 ± 5.40 mm^3 and the animals were randomly distributed into 3 treatment groups. Mice were anaesthetised through inhalation of isoflurane delivered via nose cone. Group 1 received a tail vein injection (100 µL) of ST-001 containing 4.10 $x10^9$ MB/mL (1.37 x 10^{10} MB/kg), paclitaxel (1.43 mg/kg) and DSPE-RB (5.83 mg/kg) plus US. Treatments were administered on days 0,1, 2, 6, 7, 8,13,14,15, 20, 21 and 22. Group 2 received a tail vein injection of Taxol (10 mg/kg) on days 0, 5, 8 and 12 while Group 3 received no treatment. Taxol was prepared by dissolving paclitaxel in a mixture of ethanol and Cremophor EL (1:1 v/v) at a concentration of 6 mg/mL. This paclitaxel concentrate was subsequently diluted in sterile saline to achieve a dose of 10 mg/kg. In Group 1, US was applied to the tumour during and after injection of ST-001 (for a total of 3.5 min) with a second 3.5 min US exposure 30 min following injection (US was delivered using a Sonidel SP100 sonoporator using the parameters outlined in 2.5.) Tumour volume and mouse weight were measured at regular intervals throughout the study. Survival was determined for each animal based on the time taken for the tumour to reach the maximum dimensions as permitted by the licence. When

animals were removed from the study for any other reason, this was indicated on the Kaplan Meier curve with an asterisk. All animals recovered normally from anaesthesia and exhibited no observable treatment related ill effects. Treated groups were compared with the untreated control group or each other using an unpaired t-test (GraphPad Prism version 9.5.0.).

2.7 Assessing the safety of ST-001 in healthy non-tumour bearing mice: CD-1 mice (8–10-week-old) were divided into 5 groups of 20, with each group consisting of 10 male and 10 female animals. Group 1 was untreated. Group 2 received a tail vein injection of ST-001 and blood was harvested 24 h post administration with the animals then terminated to harvest spleen, liver and kidneys. Group 3 received a tail vein injection of Taxol (15 mg/kg) and bloods / organs harvested as per group 2. Groups 4 and 5 received the same treatments as groups 2 and 3 but bloods / organs were harvested 7 days post treatment. Due to the differing body weight of male and female animals, male animals received a slightly lower dose (4.78 x 10^{10} MBs per kg; [paclitaxel] = 4.37 mg/kg and [DSPE-RB] = 4.48 mg/kg) than females (4.97 x 10^{10} MBs per kgMB/kg; [paclitaxel] 4.54 mg/kg and [DSPE-RB] = 4.64 mg/kg). Bloods were harvested in lithium heparin containing tubes and blood smear films were generated for each sample. The spleen, liver and kidneys from each animal were immediately harvested and immersed in formalin fixative. All samples were blinded and provided to Veterinary Pathology Group Ltd, UK (VPG Ltd.) for further analysis.

For blood analysis, conventional haematology was performed together with biochemical analysis for the following analytes: ALT (alanine transaminase); AST (aspartate transaminase);, Haemoglobin (Hb), Hematocrit (Hc), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Platelets (Plat), Monocytes (Mono), Red Blood Cell (RBC) and White Blood Cell (WBC). For histological analyses, tissues were routinely processed to glass slides and stained with haematoxylin and eosin (HE). The standard veterinary pathology 4-point scale defined by Mann *et al* was used to grade the tissues [12]. Steatosis was graded as defined by Kleiner et al 2005 [13]. For the purposes of this study, portal and lobular inflammation were graded

separately as they are likely to indicate a separate pathogenesis. All treated groups were compared with the untreated control group using the Mann Whitney test in GraphPad Prism version 9.5.0.

3.0 Results and Discussion: A schematic of a cross-section of the ST-001 MB, prepared as outlined in Section 2.2, is presented in Figure 1a. Due to the hydrophobic nature of paclitaxel/triacetin and the amphiphilic properties of the phospholipids, self-assembly around the hydrophobic PFP gas leads to the incorporation of paclitaxel/triacetin within the hydrophobic acyl chain region of the MB shell, while the hydrophilic Rose Bengal is attached to the shell surface. Optical microscopy confirmed the formation of spherical particles (Figure 1b) with a mean diameter of between 1 and 2 µm (Figure 1c). The paclitaxel content within the ST-001 MBs was quantified as 82.0% via HPLC, following separation of the MBs from the infranatant by low-speed centrifugation (Figure 2a). This value represents a worst-case estimate, as some MBs are inevitably lost (burst) due to shear forces during the centrifugation process. An in-use stability study demonstrated that the ST-001 formulation remained stable for at least 6 h at 25°C post-activation, with no significant changes in particle concentration (Figure 2b) or diameter (Figure 2c).

Assessing the effectiveness of drug delivery systems like ST-001 using static cellular suspensions or 2D cell monolayers on well plates does not fully account for the beneficial targeting effect provided by the UTMD process. However, these experiments do offer insight into how paclitaxel chemotherapy and Rose Bengal SDT combine to induce the cytotoxic effects of ST-001. Figure 3 presents a plot of cell viability versus ST-001 concentration for the KPC and Panc-01 cell lines. Treatment with ultrasound alone had no statistically significant impact on the viability of either cell line. In the KPC cell line, ST-001 treatment alone resulted in only a slight reduction in cell viability up to a paclitaxel concentration of 19.40 µM but reduced to 50.5% at 38.80 µM. When ultrasound was applied to KPC cells treated with ST-001, significant reductions in cell viability were observed at all but the lowest

concentration. Specifically, at 19.40 µM, ultrasound application reduced viability from 87.2% to 27.1%, demonstrating a powerful combined paclitaxel/SDT effect. A similar trend was observed in Panc-01 cells, where significant reductions in viability were seen in cells treated with ST-001 + US compared to ST-001 alone. In KPC cells, the IC_{50} was determined to be 16.80 µM for the ST001 + US treatment, while in Panc-01 cells, it was 2.43 µM. KPC derived cell lines have previously been shown to be relatively resistant to paclitaxel treatment, with reported IC_{50} values ranging from 40 to 90 µM [14], whereas Panc-01 cells are more sensitive, with an IC_{50} for nab-paclitaxel of approximately 1.9 µM [15]. However, direct comparisons with published IC_{50} values should be made cautiously due to differences in experimental conditions. Still, the IC_{50} value for ST-001 + US in KPC cells is promising, given the known resistance of this cell line to paclitaxel treatment. While the effect of MBs alone (i.e., blank MBs without RB or paclitaxel) was not evaluated in this study, previous work in two 3D spheroid models of prostate cancer found no significant effect on cell viability when treated with blank MBs + ultrasound compared to an untreated control [7].

Prior to *in vivo* efficacy testing, it was important to demonstrate effective UTMD using the ST-001 formulation in a live animal model. To this end, a KPC ectopic tumour was established on the dorsum of an athymic nude mouse. Continuous B-mode ultrasound imaging was used to monitor ST-001 MBs within the tumour following tail vein injection, and a therapeutic ultrasound pulse was applied to induce MB rupture in the tumour region. A video demonstrating this process is included in the supporting information, showing the tumour outline clearly visible on B-mode imaging. As expected, contrast in the tumour area was minimal prior to ST-001 administration. Following injection, a dense bright signal appeared in the vascular net supplying the tumour, indicating ingress of the microbubbles within 30 seconds. Application of the therapeutic ultrasound resulted in the rapid disappearance of contrast, indicating successful inertial cavitation of the MBs. Representative still images from each stage are shown in Figure 4, alongside a plot of tumour contrast values over time. This analysis revealed a 2.5-fold increase in contrast

within one minute of administration, returning to baseline immediately after therapeutic ultrasound application. Collectively, these findings provide clear *in vivo* evidence of effective UTMD using the ST-001 formulation.

Building on the established responsiveness of the ST-001 formulation to therapeutic ultrasound, the next objective was to evaluate its UTMD-mediated efficacy *in vivo*. Ectopic KPC tumours were established in athymic nude mice, and animals were treated as outlined in Section 2.7. A plot of average tumour growth over time for each group is shown in Figure 5a. By Day 7 post-treatment, animals treated with ST-001 exhibited a significant reduction in tumour growth compared to those treated with Taxol ($p \leq 0.01$) or left untreated ($p \leq 0.001$). In contrast, no significant difference in tumour growth was observed between the Taxol and untreated groups. To visually underscore the differences in treatment response, representative animals from each group were photographed on Day 7, and their tumour volumes were quantitatively modelled using the Peira tumour measurement system (Figure 5b), clearly demonstrating the pronounced tumour suppression achieved with ST-001 treatment. In addition, no significant change in body weight was observed for ST-001 animals suggesting the treatment was well tolerated (Figure 5c). The Kaplan–Meier survival plot (Figure 5d) further highlights the therapeutic benefit of ST-001. By day 10, only 50% of animals in the untreated and Taxol-treated groups remained alive, whereas all animals receiving ST-001 survived. The average survival time in the ST-001 group was 21.3 ± 4.1 days, compared to 9.0 ± 1.7 days and 10.5 ± 1.2 days for the untreated and Taxol groups, respectively (Figure 5e). Notably, the two longest-surviving animals in the ST-001 group were euthanized for unrelated ethical reasons, not due to tumour burden.

Several key factors contribute to the superior efficacy of ST-001, even when compared with a substantially higher dose of Taxol. Co-encapsulation of paclitaxel and Rose Bengal within the ST-001 lipid-stabilised MBs enables targeted delivery directly to the tumour site via ultrasound triggered cavitation. This approach not only enhances local drug concentration but also leverages the mechanical effects of cavitation to disrupt tumour

architecture and improve drug penetration [9,10]. This provides an especially important advantage in pancreatic cancer, where the dense desmoplastic stroma poses a major barrier to therapeutic delivery. Moreover, previous studies have shown that Rose Bengal-mediated (SDT) can enhance the effects of chemotherapy when delivered together via UTMD platforms, contributing to improved therapeutic outcomes [7]. Together, these mechanisms underpin the potent cytotoxic response observed in this aggressive and preclinical model of pancreatic cancer.

While no signs of acute toxicity were evident in ST-001–treated animals during the efficacy study, a dedicated safety evaluation was undertaken in healthy, non-tumour bearing mice to further investigate the potential impact of ST-001 on blood biochemistry and organ histology. Blood samples collected at 24 h and 7 days post-treatment were analyzed and compared with untreated controls. Across all white blood cell (WBC) and red blood cell (RBC) parameters assessed, no statistically significant differences were observed in ST-001 treated animals, indicating a lack of haematological toxicity. In contrast, mice treated with Taxol exhibited pronounced hematologic disturbances. At 24 h post-administration, WBC counts were significantly reduced ($p \leq 0.001$), with further analysis revealing marked decreases in both neutrophils ($p \leq 0.01$) and lymphocytes ($p \leq 0.001$). While neutrophil levels returned to baseline by day 7, lymphocyte counts remained significantly suppressed ($p \leq 0.001$), and overall WBC levels continued to show a statistically significant decline ($p \leq 0.001$), suggesting a sustained immunosuppressive effect. Additionally, Taxol treatment resulted in a significant reduction in RBC count at 24 h ($p \leq 0.001$), accompanied by decreased hematocrit (HCT) ($p \leq 0.001$) and elevated mean corpuscular hemoglobin concentration (MCHC) ($p \leq 0.001$). These red cell parameters, however, normalized by day 7, indicating that the effects were transient.

Histological analysis further supported the favourable safety profile of ST-001. Examination of the spleen, liver, and kidneys revealed no treatment related pathological changes. Mild findings such as glycogen-type vacuolation in the liver and mild portal

lymphoplasmacytic inflammation, along with minor inflammatory changes in the kidney, were observed sporadically across both treated and control groups. These were deemed incidental in nature, likely reflecting normal biological variation rather than a drug-related effect. Taken together, the absence of adverse haematological or histopathological effects in ST-001 treated animals, in contrast to the pronounced toxicity observed with Taxol, highlights the favourable safety profile of ST-001 and supports its continued development as a well-tolerated therapeutic candidate with minimal off-target effects.

4.0 Conclusions: To conclude, UTMD using the novel ST-001 MB formulation demonstrated significantly greater efficacy than conventional Taxol chemotherapy in an aggressive preclinical murine model of pancreatic cancer. By incorporating paclitaxel into the MB shell, this approach enables solvent-free paclitaxel delivery, which, when combined with the targeted release afforded by UTMD and the added therapeutic benefit of SDT, achieves enhanced anti-tumour effects at a fraction of the standard paclitaxel dose. Importantly, this improved efficacy is not accompanied by increased toxicity: ST-001 exhibited an excellent safety profile in healthy, non–tumour bearing mice, with no adverse haematological or histopathological effects observed. Collectively, these findings highlight the therapeutic potential of ST-001 as a safe and effective treatment strategy for pancreatic cancer.

Conflict of Interest

J.C, A.McH, E.S and M.A.T are co-founders of SonoTarg Ltd.

Acknowledgements

T.McK and S.K. acknowledge support from the UK's Engineering and Physical Sciences Research Council (EPSRC) for postdoctoral fellowships (Ref: EP/X033015/1).

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Figures and Diagrams

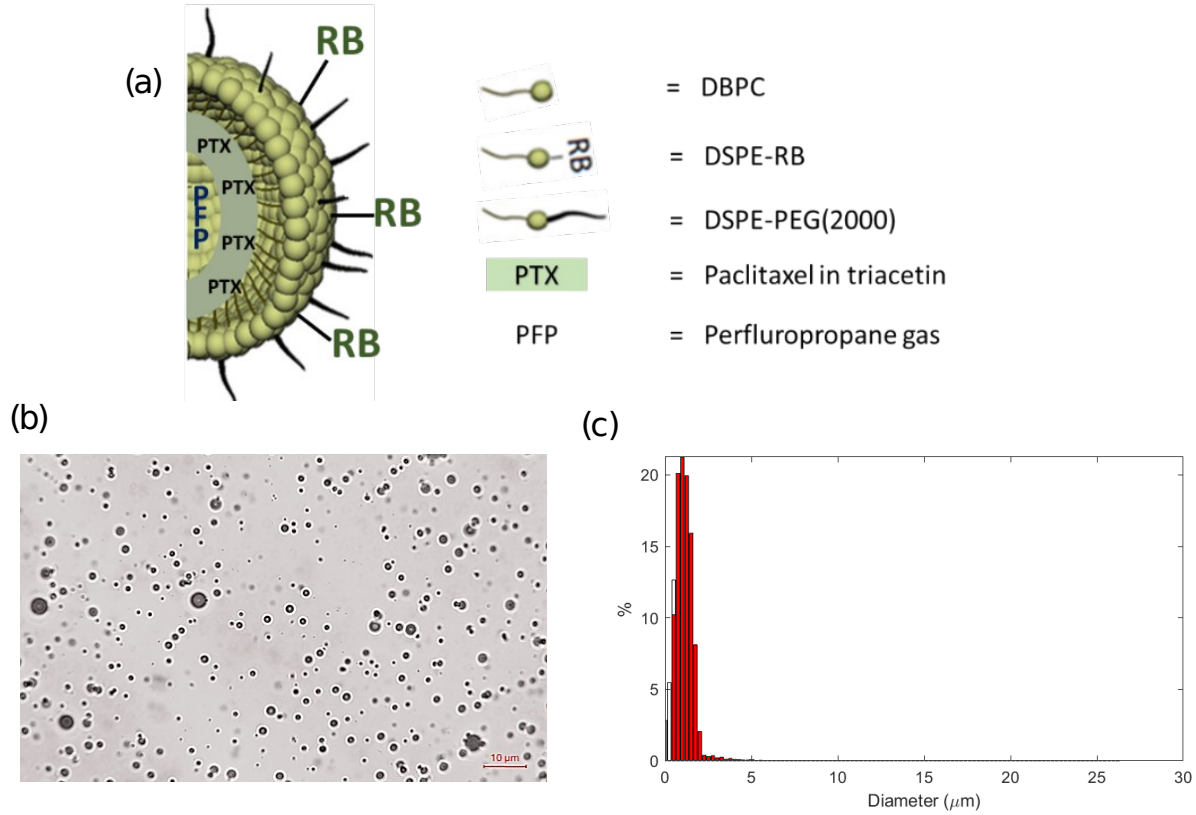

Figure 1 (a) Schematic representation of ST-001 formulation (b) optical micrograph and (c) size distribution data.

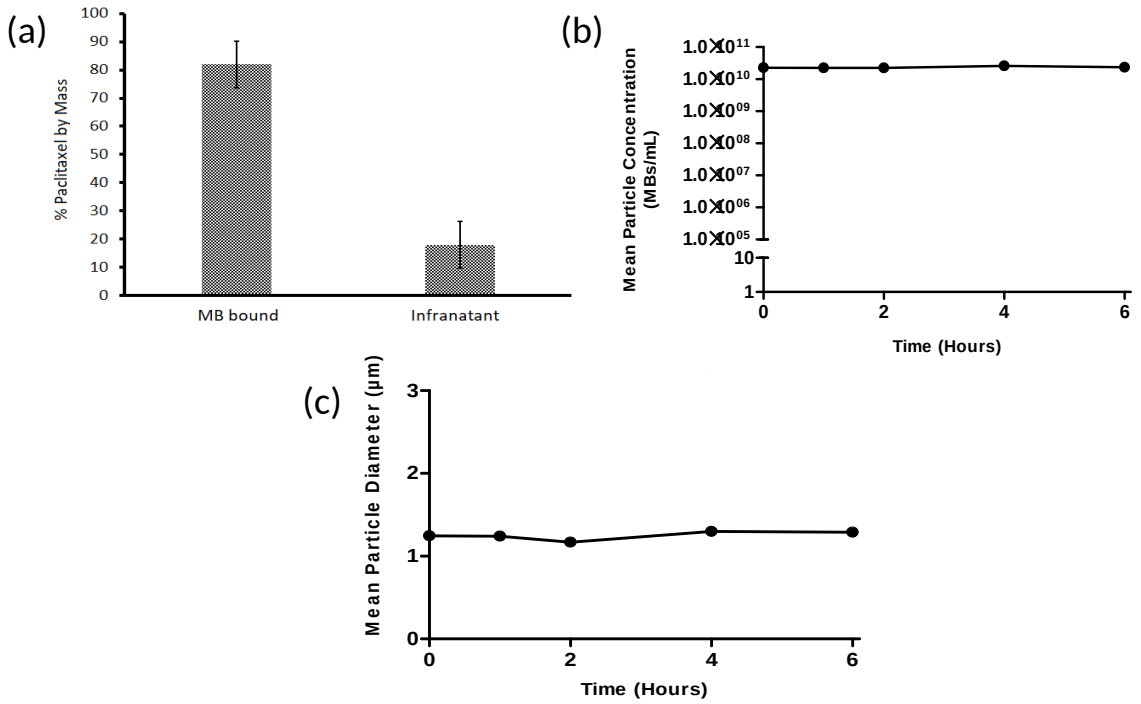

Figure 2 (a) Histogram showing the % of paclitaxel incorporated with the MBs or present in the infranatant solution following low speed centrifugation. (b) Plot of mean particle concentration and (c) mean particle diameter for ST-001 at various timepoints following activation. Error bars represent ± the standard error.

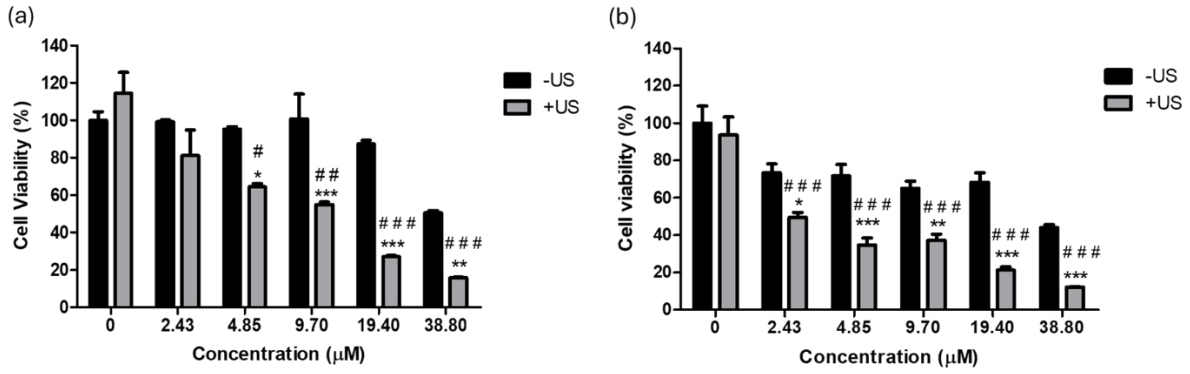

Figure 3 Plot of cell viability against concentration for (a) KPC cells and (b) Panc-01 cells following treatment with ST-001 ± US. The concentrations shown reflect the Paclitaxel concentration present within the formulation. The DSPE-RB concentration increased proportionally from 1.12 µM (at 2.43 µM [paclitaxel]) to 17.90 µM (at 38.80 µM [paclitaxel]). The MB concentration also increased proportionally from 0.17 x 10^8 MB/mL (at 2.43 µM [paclitaxel]) to 2.66 x 10^8 MB/mL (at 38.80 µM [paclitaxel]). * = $p \leq 0.05$; ** = $p \leq 0.1$ and *** = $p \leq 0.01$ for ST-001 + US versus ST-001. # = $p \leq 0.05$; ## = $p \leq 0.1$ and ### = $p \leq 0.01$ for ST-001+ US versus untreated. Unmarked groups exhibited no statistically significant differences. Error bars represent ± the standard error.

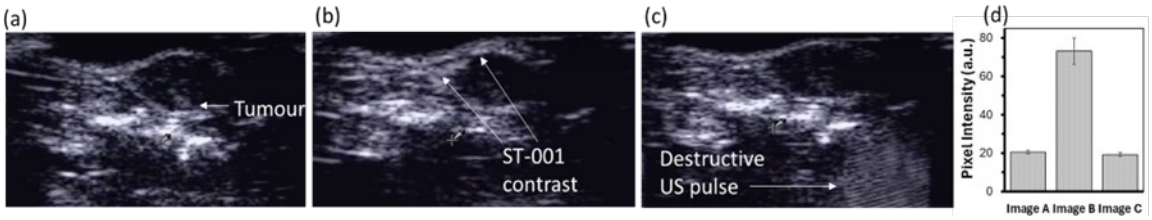

Figure 4 B-mode ultrasound images of murine pancreatic tumours (a) before and (b) 70 sec after tail vein administration of ST-001. The enhanced tumour MB contrast evident in (b) is then cleared in (c) following application of a therapeutic ultrasound pulse which ruptures the MBs, removing the resulting contrast. (d) Plot of mean pixel intensity determined using ImageJ for the images shown in a-c. Error bars represent ± the standard error

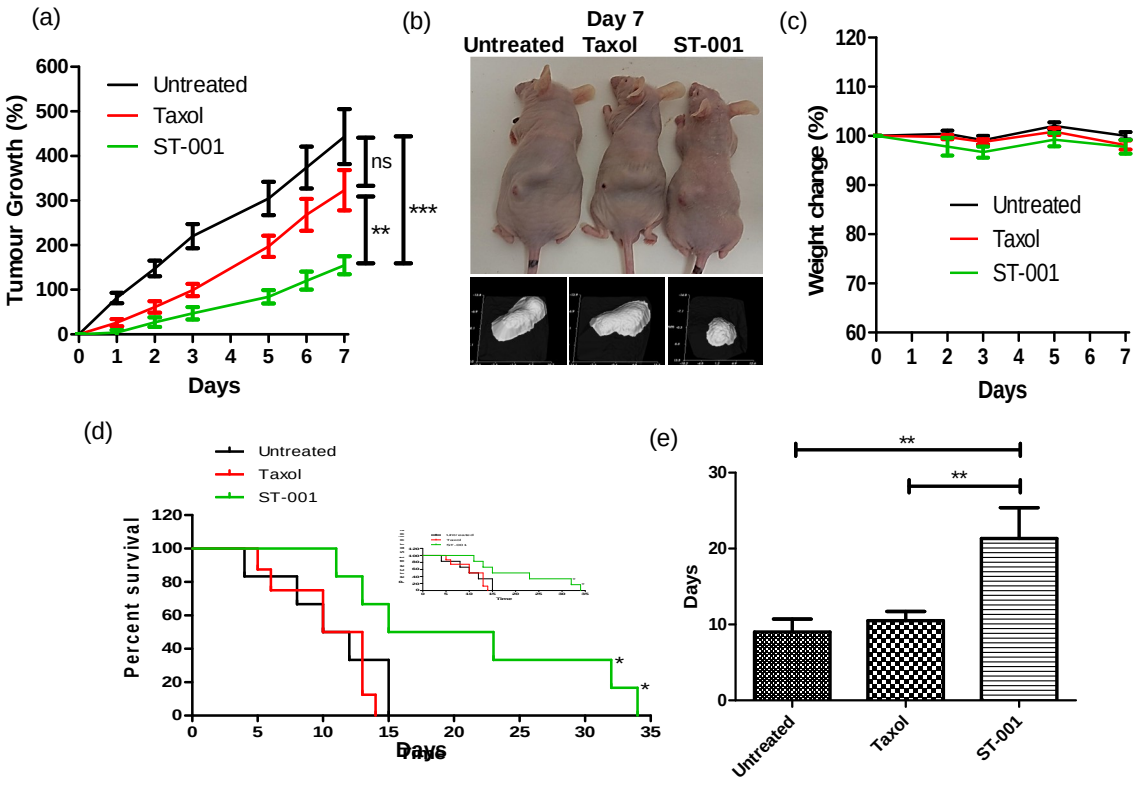

Figure 5 (a) Tumour growth delay plot for mice bearing ectopic KPC pancreatic tumours following treatment with ST-001 or a standard dose of Taxol. Untreated animals used for comparative purposes. (b) Photograph of tumours in animals referred to in (a) 7 days following treatment with the tumours modelled using the Peira tumour measuring device. Each of the animals shown had a tumour volume of 80 mm3 at the start of the experiment (c) Plot of average % weight change of animals in each group. (d) Kaplan Meier survival plot for animals treated as described in (a) with the average days the animals in each group remained alive shown in (e). Error bars represent ± the standard error. In (a) and (e) ** = $p<0.01$ and ***=$p<0.001$. In (d) * refers to animals that were removed from the study for reasons other than tumour size.

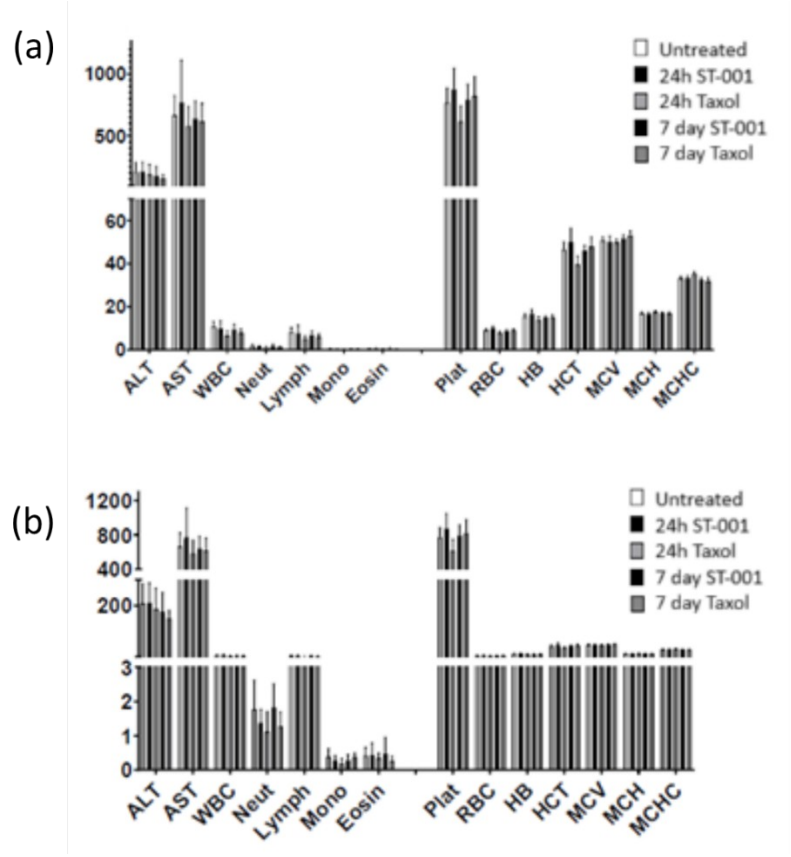

Figure 6 (a) Biochemical and hematological analysis of blood samples obtained from animals in the various treatment groups 24h and 7 days following treatment. Panel (b) shows the same analysis with expansion of the Y-axis between 0 and 1 to observe changes in neutrophils (N), monocytes (M) and eosinophils (E). Error bars represent + SD.

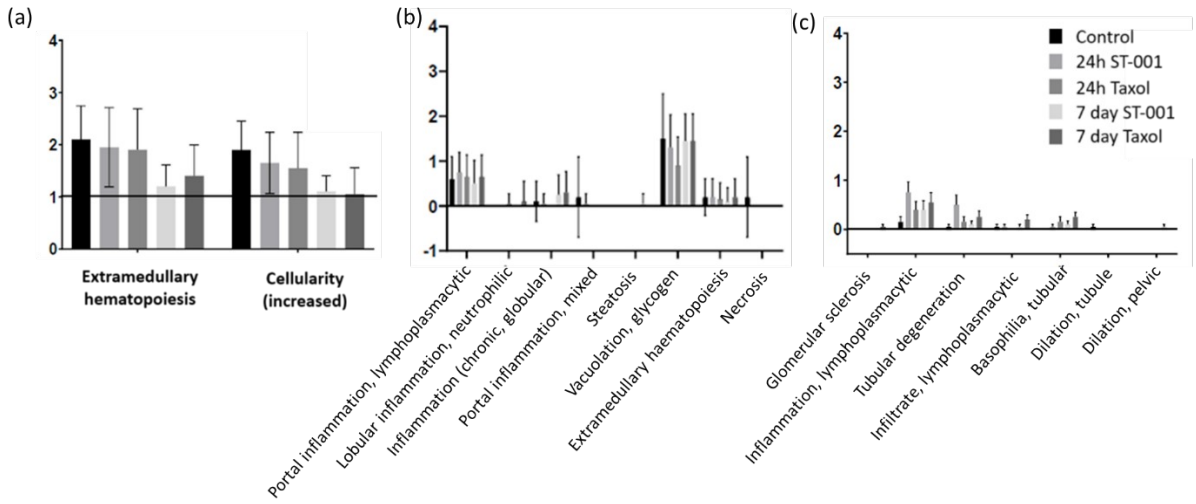

Figure 7 Histology analysis of (a) spleen, (b) liver and (c) kidney tissues harvested 24h and 7 days following treatment with ST-001 and Taxol. Error bars represent ± SD and n = 20.