



produced by separate  
challenge *in vivo*. This  
due to space constraints  
benefits of magnetic  
addressed by using  
magnetic fields in  
periments described  
upon drug delivery  
subsequently  
murine pancreas

## 2. Material

This section describes the experimental setup. The first part describes the magnetic field and the non-invasive measurement. The second part describes the periodic loading and the solution.

[illegible]

1-yl) 8,8'-(((2-(5-(2-oxooctanoyl)pentanamido)ethyl)azanobis(8-oxooctanoate) (5)

2-oxooctanoic acid (0.5 g, 1.3 mmol) and TEA (0.5 g, 4.6 mmol) were dissolved in DMF was added disuccinimidyl carbonate (0.5 g, 1.3 mmol) and the reaction mixture stirred at room temperature under nitrogen atmosphere. After completion of the reaction (1 h), DMSO (200 mL) was added to the reaction mixture. The mixture was filtered and washed 3 times with DMSO (20 mL x 3). The crude product was purified by column chromatography on basic (TEA) silica gel (methanol: DMSO = 1:1) to give **5** (0.83 g, 71% yield) as low melting solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 7.94 (brs, 2H, NH X 2), 7.67 (brs, 2H, NH X 2), 6.34 (brs, 1H, NH), 4.29 (s, 1H, CH), 4.12 (brs, 1H, NH), 2.88–2.72 (m, 6H, CH<sub>2</sub> X 3), 2.45–2.34 (m, 6H, CH<sub>2</sub> X 3), 2.20–2.06 (m, 22H, CH<sub>2</sub> X 11). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 1.60–1.21 (m, 22H, CH<sub>2</sub> X 11), 162.7 (C=O), 163.1 (C=O), 162.7 (C=O), 61.4 (CH), 53.9 (CH<sub>2</sub>), 53.9 (NCH<sub>2</sub>), 39.9 (CH<sub>2</sub>), 39.8 (CH<sub>2</sub>), 39.6 (CH<sub>2</sub>).



SO

F

... was stirred at 22 °C for 24 h under a ... completion of the reaction, Rose Bengal ... according to [22]), (0.43 g, 0.45 mmol) ... TEA (0.5 mL) were added to the reaction ... stir for 24 h. Once the reaction was complete, ... mL) was added to the solution and stirred for ... precipitate thus obtained was triturated with ... ethyl acetate (100 mL), acetone-water mixture ... and finally with ethyl acetate-hexane mixture ... respectively to afford a pink powder of compound **8** ... <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>), Fig. S1: δ 7.95 (brs, 2H, NH<sub>2</sub>),

6H, NCH<sub>2</sub> X 3),  
2.17–2.06 (m, 10H  
(DMSO-*d*<sub>6</sub>), (Fig. 8  
162.7 (C=O), 15  
(CH), 131.0 (C),  
69.3 (CH), 61.5  
(CH<sub>2</sub>), 40.2 (C  
31.0 (CH<sub>2</sub>), 28  
(Fig. S3): ca  
1925.90 (M

### 2.3. *In vitro* and *gemcitabine*

The h  
was mai  
100 U/ml  
serum  
PaCa-  
Medi  
100  
serv  
we  
pl  
c

from  
ure  
bles  
s then  
ensity at  
ample for  
ation, the  
posure were  
ber. A 2 MHz  
quency from the  
and storage onto  
calculated for each  
quantify cavitation  
) by determining the  
es ( $f_0^*(n + 0.5)$ ), with  
indicative of nonlinear

### control device

described by Barnsley et al. and as-  
ly, the magnetic body consisted of  
magnet material whose geometry was  
magnetic field of 0.2 T at a distance of  
edge. An integrated ultrasonic element  
10 mm provided a pressure field that  
the magnetic field peak, with sufficient am-  
cavitation of MBs used in this study. An alu-  
the MAD (hereafter referred to as “aMAD”) was  
US-only control for *in vitro* and *in vivo* experi-

the gap between the US element and the delivery  
the present work, a coupling cone (Fig. 2) was cast  
wax and secured with US gel. The cone material was

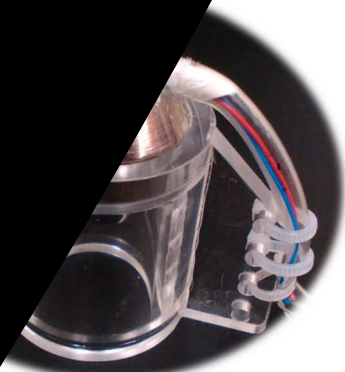


chosen  
1 MHz  
surem  
tion v  
both

2.1

in  
its  
) to  
own in  
control

lagMB-RB  
( $3 \mu\text{M}$ ) were  
3.5 min, after  
ed water. The  
and reserved for  
e completed in a



ested, with Perspex holder.

Fig. 3. *In vi*  
element tum  
water bath

Table 1  
*In vitro*

Group
Un
MB
Un
MB

...gel. In ...risk, the ...material ...transmis- ...from the ...Using these ...Subject weight ...first treatment. ...gemci- ...the results section

...times in the *in vivo* experi- ...value  $\pm$  one standard devia- ...as mean  $\pm$  standard error on ...tumour volume measurement. ...were established using an un- ...groups and a 1-way ANOVA followed ...comparing more than two groups using

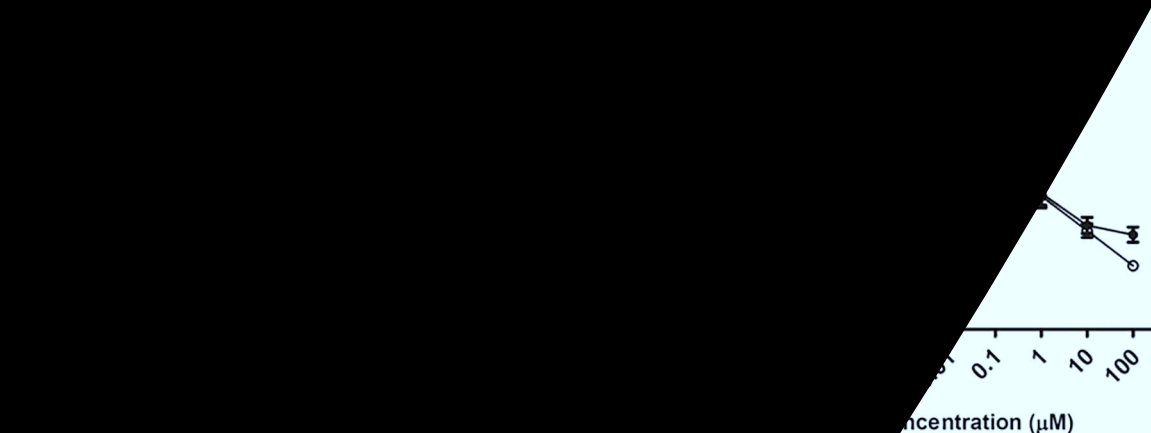
*B-gem (8) and its efficacy in pancreatic cancer*

...the SDT sensitiser RB and antimetabolite Gem to be ...MB surface, MBs were surface functionalised with ...podal ligand was designed to have a single biotin anchor

residues of **3**, yielding  
 tive esters. The acti  
 (6) and amine der  
 linkages respecti  
 of **8** was characte  
 electrospray ma  
 veals a base pea  
 addition,  $^1\text{H}$  N  
 ratio between  
 Gem (5.77 ppm)  
 present on B  
 biotin at 6.2

Following  
 next step  
 order to e  
 impair it  
 at a ran  
 determ  
 also tr  
 show  
 dose  
 gem  
 Mia  
 co  
 e

...in  
 The  
 Ma-  
 to Ma-  
 on of the  
 similar trend  
 recorded  
 and RB alone  
 cavitation ac-  
 be explained by  
 the surface func-  
 resolution in the de-  
 is further supported



(filled circles) (A) BxPC-3 and (B) Mia-PaCa-2 cell lines.

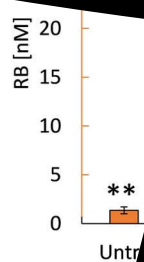
MB concen  
1.0  
5

Fig  
h

from  
ent ob-  
difficulty  
ased. The  
US) could  
ragments, in  
and was found  
( $p < .01$ ). In-  
similar degree, in  
[8].

production (orange,  $n = 3$ )  
after US exposure, with asso-  
cations from MBs (blue)  
linear oscillations. \*\* =  $p < .01$   
through a 1-way ANOVA with Tukey's  
sample was prepared with  $\pm 5 \times 10^7$   
41  $\mu\text{M}$  biotin-RB, and 1.25  $\mu\text{M}$  SOSG in  
PBS. US parameters were 1.17 MHz,  
peak negative pressure, 30% duty cycle,  
pulse repetition frequency for 3.5 min. (For  
interpretation of the references to colour in this  
figure legend, the reader is referred to the web ver-  
sion of this article.)





### 3.4. In vivo

To test the combination of MB with electrochemotherapy, the distribution of tumour volumes in Figure 4A was treated with MB with electrochemotherapy, while untreated tumours were used as a control. The results are shown in Figure 4B.

Figure 4B shows the results of the in vivo experiments. The tumour volumes were significantly reduced in the MB + electrochemotherapy group compared to the untreated group. This indicates that the combination of MB and electrochemotherapy is effective in reducing tumour volume.

The results of the in vivo experiments are encouraging, but there are several factors that need to be discussed. First,

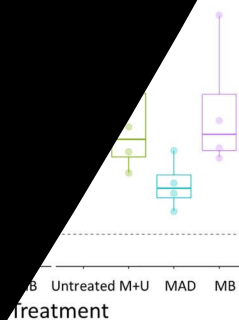


Figure 4B shows the results of the in vivo experiments. The tumour volumes were significantly reduced in the MB + electrochemotherapy group compared to the untreated group. This indicates that the combination of MB and electrochemotherapy is effective in reducing tumour volume.

however, likely that it determines the effect of ultrasound and MRE on therapeutic material in compensate entirely the variance in tumor size between the groups with Gem and Gem potentiated results, could be subjects receiving treatment of the MRE protocols to subjects.

#### 4. Concl

A no  
the ser  
thesis  
oxyge  
and  
incr  
pay  
re  
u

drug  
2–373,  
lan,  
O'Rourke,  
bles as de-  
of pancreatic  
016/j.jconrel.  
combined magnetic-  
netic and ultrasonic  
g/10.1002/admt.  
ochrane, C.C. Coussios,  
n carrying microbubbles  
J. Control. Release 203  
02.004.  
L. Rothenberg, M.R. Modiano,  
soff, R. Nelson, F.A. Dorr,  
survival and clinical benefit with  
advanced pancreas cancer: a  
83–2413, <https://doi.org/10.1126/>  
Thomas, B. Callan, M.A. Taylor,  
E. Stride, A.P. McHale, J.F. Callan,  
argeted chemo-sonodynamic therapy of  
79 (2018) 8–16, [https://doi.org/10.1016/j.](https://doi.org/10.1016/j)  
B. McCaughan, A.P. McHale, J.F. Callan,  
ates as therapeutics in sonodynamic therapy,  
12) 8332–8334, <https://doi.org/10.1039/>  
oughran, J. Casey, J.M. Seddon, M. Tang,  
d counting of microbubbles using optical microscopy,  
(2010) 2093–2096, [https://doi.org/10.1016/j.](https://doi.org/10.1016/j)  
shrivastava, E. Stride, Comparing strategies for magnetic  
microbubbles, ACS Appl. Mater. Interfaces 11 (2018)  
<https://doi.org/10.1021/acsami.8b18418>.  
mila, J. Owen, H. Nesbitt, B. Callan, M. Borden, N. Nomikou,  
M.A. Taylor, E. Stride, A.P. McHale, J.F. Callan, Combined sono-  
antimetabolite therapy for the improved treatment of pancreatic

[28] E. Stride, C. Forster,  
mediated gene del