Supporting Information

Tri-modal Therapy: Combining Hyperthermia with Repurposed Bexarotene and Ultrasound for Treating Liver Cancer

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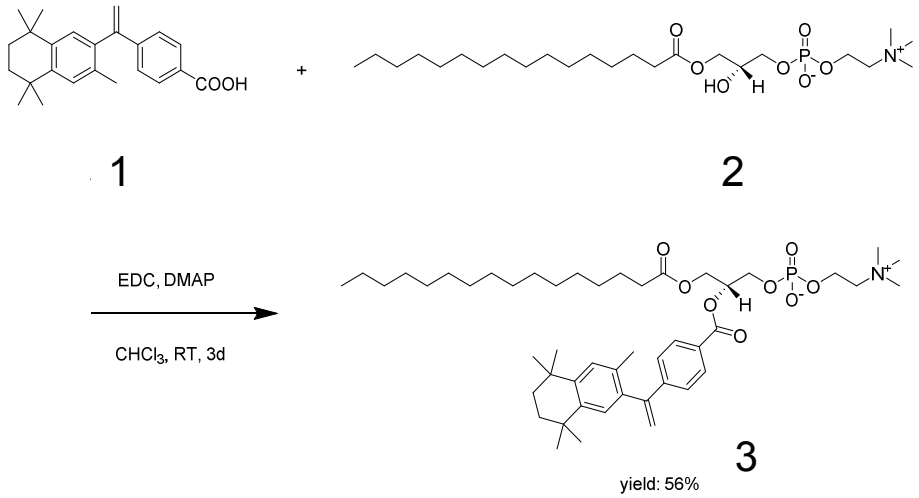
A

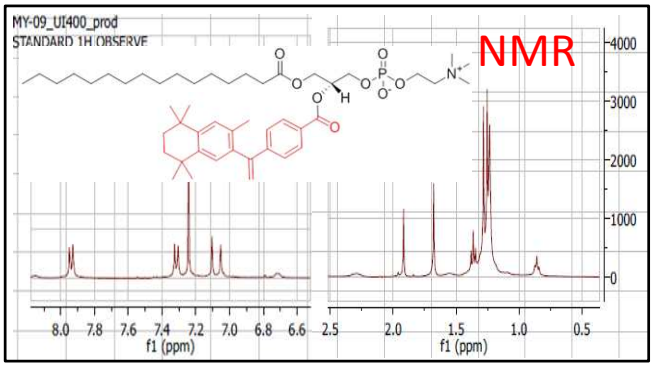


1 2

3

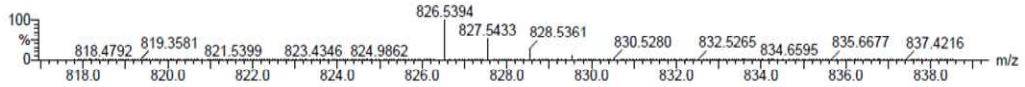
yield: 56%

B



C

HR-MS Spectroscopy



Minimum:				-1.5		
Maximum:		5.0	10.0	600.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
826.5394	826.5387	0.7	0.8	11.5	216.9	C48 H77 N O8 P

D

University of Illinois SCS Mass Spectrometry Laboratory

File: MY-20140926HR
Sample:

Date Run: 09-26-2014 (Time Run: 15:20:21)

Ionization mode: EI+
Instrument: VG 70-VSE(B)

Scan: 67-72

Base: m/z 361; 5.2%FS

Selected Isotopes : C H O_{0-3}

Error Limit : 5 ppm

Unsaturation Limits : -.5 to 30

Measured Mass	*% Base*	*Formula*	*Calculated Mass*	*Error*	*Unsaturation*
347.2012	20.8%	$C_{24}H_{27}O_2$	347.2011	0.3	11.5

E

Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
349.2169	349.2168	0.1	0.3	10.5	259.0	n/a	n/a	C24 H29 O2

Scheme S1. (A) Synthesis of pro-bexarotene (3). Bexarotene (1) 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (16:0 Lyso PC), (2) , EDC/DMAP (catalytic amount) in $CHCl_3$, 24 h RT. (yield: 56%); (B) ^{1}H-NMR($CDCl_3$, 400MHz): δ 7.93 (d, J = 8.0 Hz, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.10 (s, 1H), 7.06 (s, 1H), 5.78 (s, 1H), 5.30 (s, 1H), 4.35 (q, J = 4.0 Hz, 2H), 3.89(s, 2H). 3.22 (s, 4H), 2.30 (s, 2H), 1.93 (s, 4H), 1.68 (s, 6H), 1.53 (s, 3H), 1.36 (m, 6H), 1.69 (m, 12H), 1.23 (m, 24H), 0.85 (m, 3H); (C) HRMS m/z: 826.5394 $(MH)^+$, calcd for $C_{48}H_{76}NO_8P$: 826.5387; (D) HRMS analysis after incubation with enzyme (lipase) m/z: 347.2012 (M-H) for bexarotene, calcd for $C_{24}H_{27}O_2$), calculated 347.2011 MH+ for bexarotene; (E) HRMS analysis after incubation with phosphate buffer at pH 4.6 m/z: 349.2169 (MH+ for bexarotene calcd for $C_{24}H_{29}O_2$), calculated 349.2168 MH+ for bexarotene.

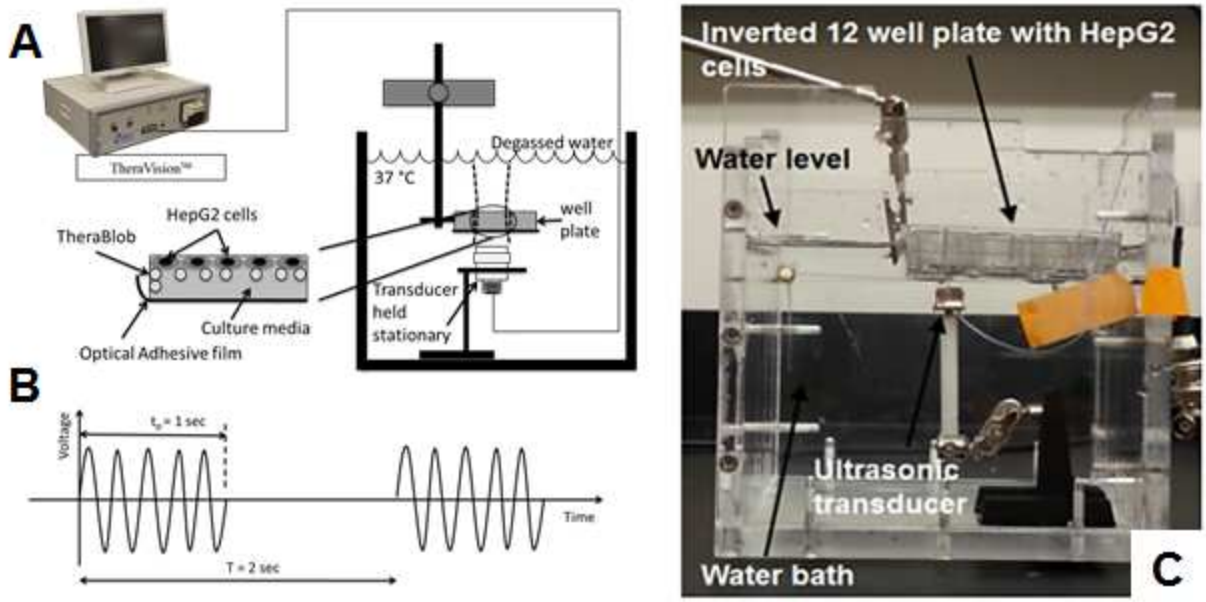


Figure S1. Experimental set up for *in vitro* US studies. (A) Schematics of the experimental l setup, and (B) pulse sequence used for the ultrasound exposure at 2.4 MHz and pulse rate of 0.5 Hz and (C) A photographical representation of the setup.

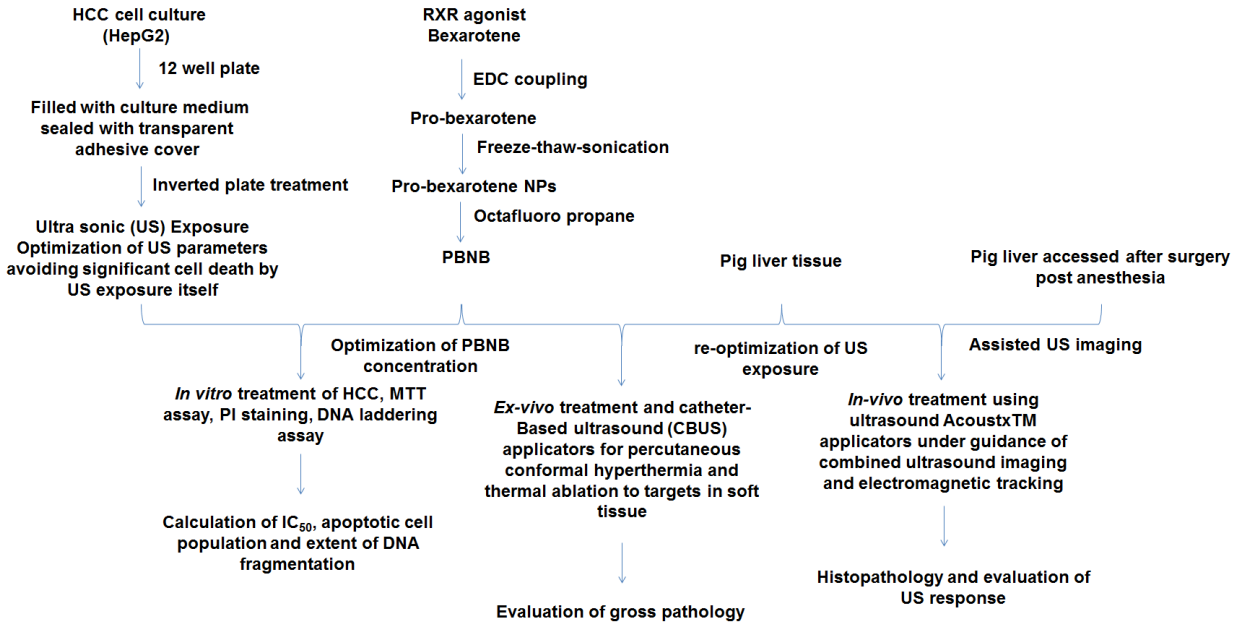


Chart S1. Representative stepwise plan for optimization of US power, exposure and concentration of PBNB used in present work.

The *in vitro* experiments were conducted with a monolayer of cell in culture media where the goal was to enhance drug by mechanical vibration created by ultrasound wave propagation. Since the propagating media was liquid no thermal energy was delivered. On the other hand the propagating media in the *in vivo* experiments was tissue which is a high attenuating media where the mechanical energy is converted into thermal energy to produce ablative effects. Therefore, different acoustic powers were set for the *in vitro* and *in vivo* experiments. Higher frequency was used for the *in vivo* configuration to avoid cavitation during ablation. This is one of the several advantages compared with other ultrasound ablative technologies.

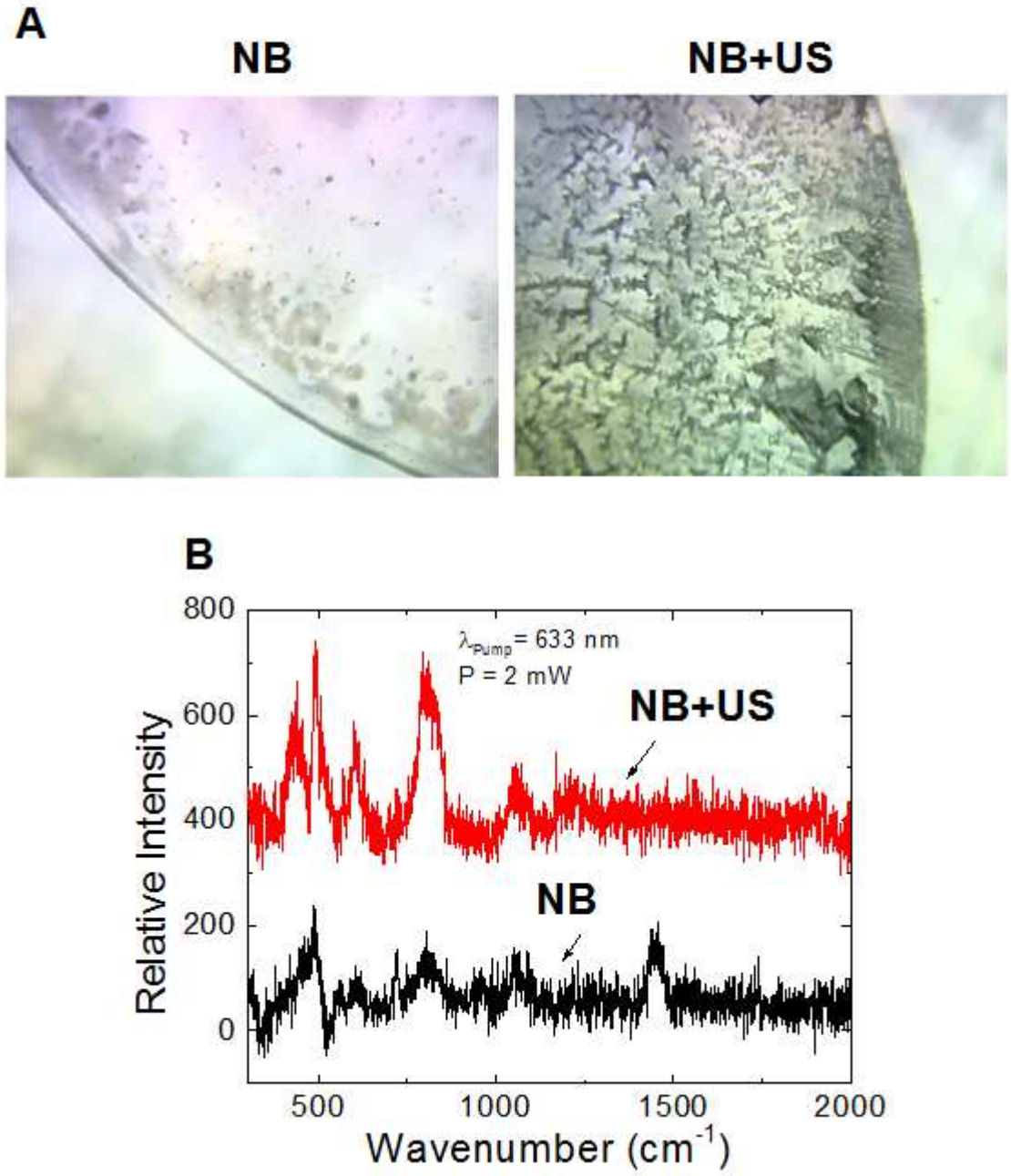


Figure S2. Raman spectroscopic details of NB samples before and after US exposure. (A) Optical micrographs of NB (left) and after ultrasonic exposure (right). (B) Characteristic Raman spectroscopic signature peaks of NB before and after the treatment of US.

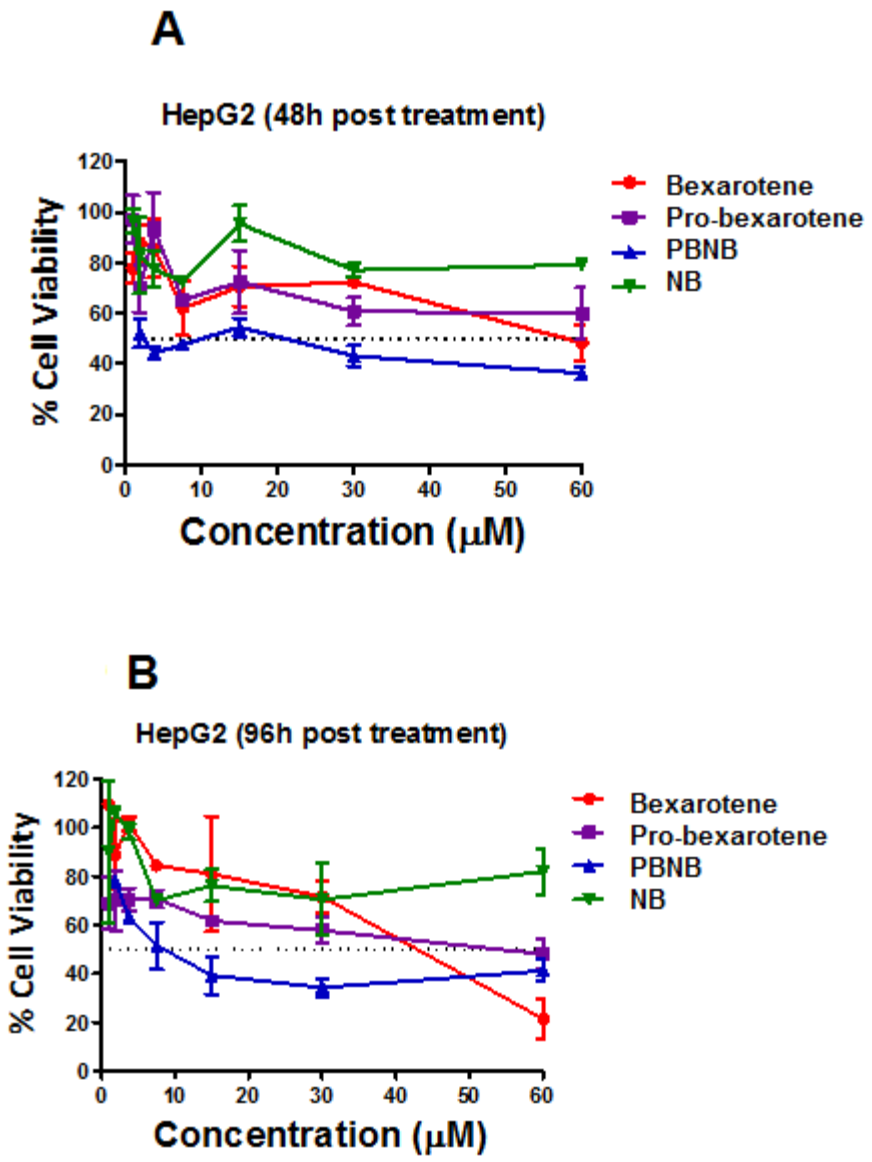


Figure S3. *In vitro* MTT assay experiments for establishing HCC growth regression in HepG2 cells after 72h of incubation. MTT assay on HepG2 cells treated with formulations to establish effective concentrations after 72h of treatment at (A) 48h and (B) 96h time points.

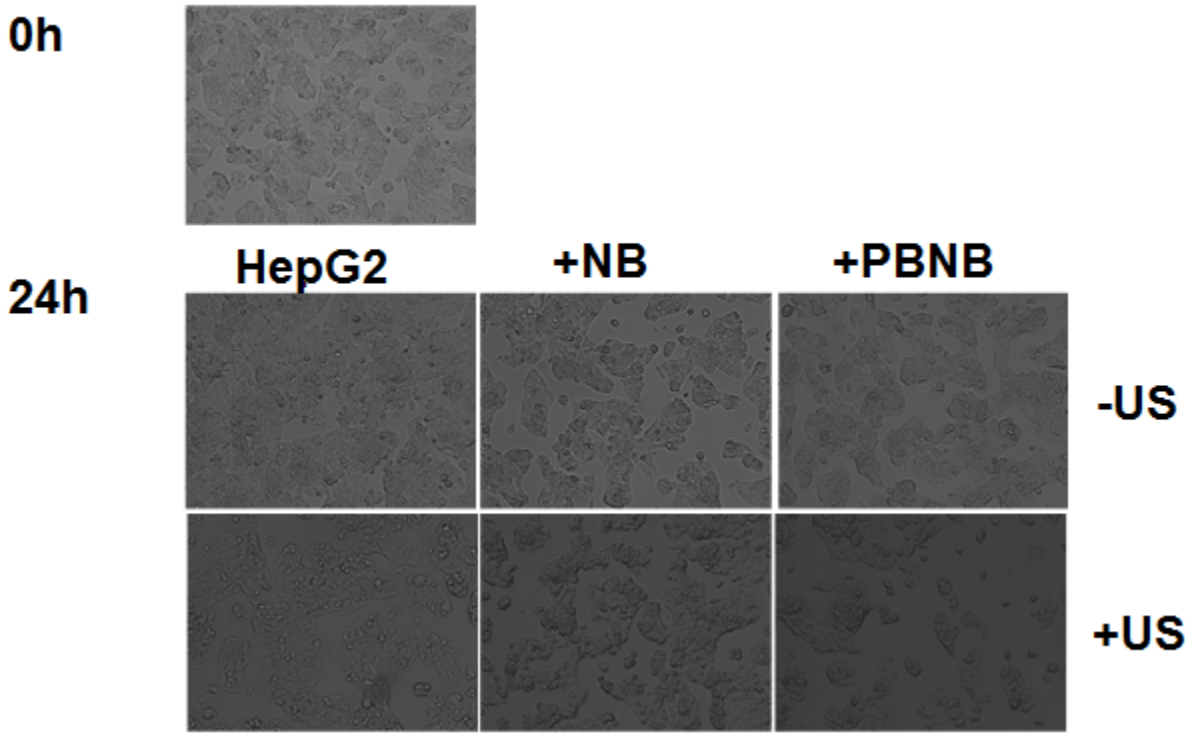


Figure S4. Bright field images of HepG2 cells with variation in cell density and morphology. The \effect of US on PBNB and NB treated HepG2 cells are shown after 24h of incubation.

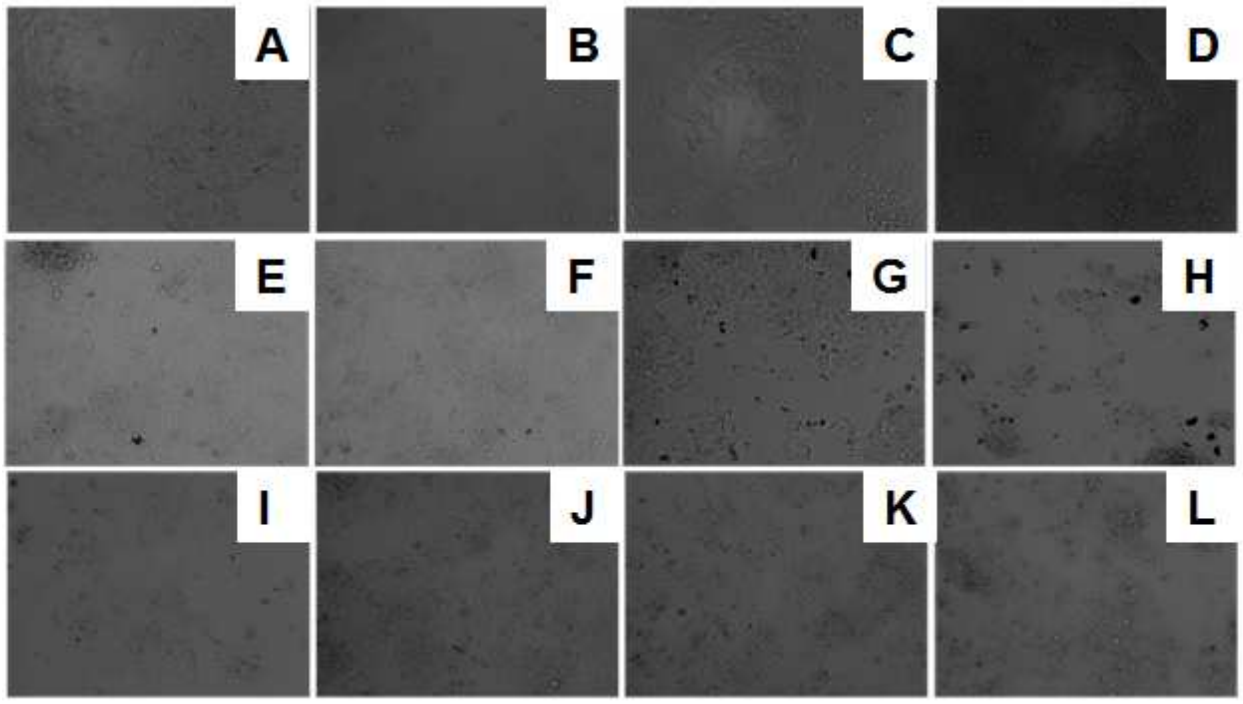


Figure S5. Bright field images of HepG2 cells before (A) and after US exposure (B). Images from 72h of treatments with 15 μM of (C) Bexarotene and (D) Pro-bexarotene without US exposure while 1.875, 3.75, 7.5 and 15 μM concentration of PBNB (E-H) and NBs (I-L) with US exposure.

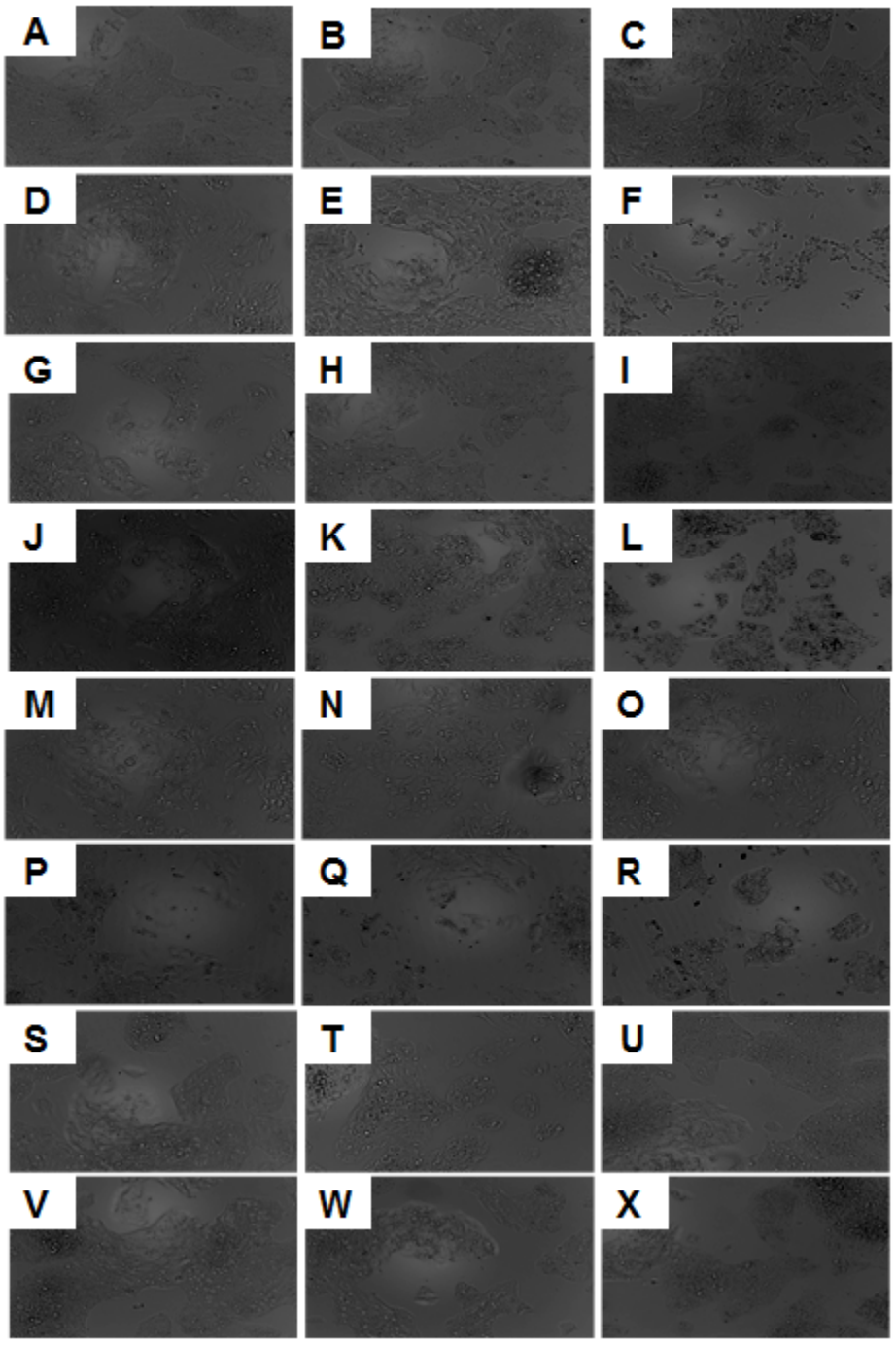


Figure S6. Bright field imaging of HepG2 cells treated with Bexarotene (A-F); Pro-bexarotene (G-L); PBNB (M-R) and NB (S-X) formulations at concentration of 1.875 (A, G, M and S), 3.75 (B, H, N and T), 7.5 (C, I, O and U), 15 (D, J, P and V), 30 (E, K, Q and W) and 60 μM (F, L, R and X) at 72 h time point.

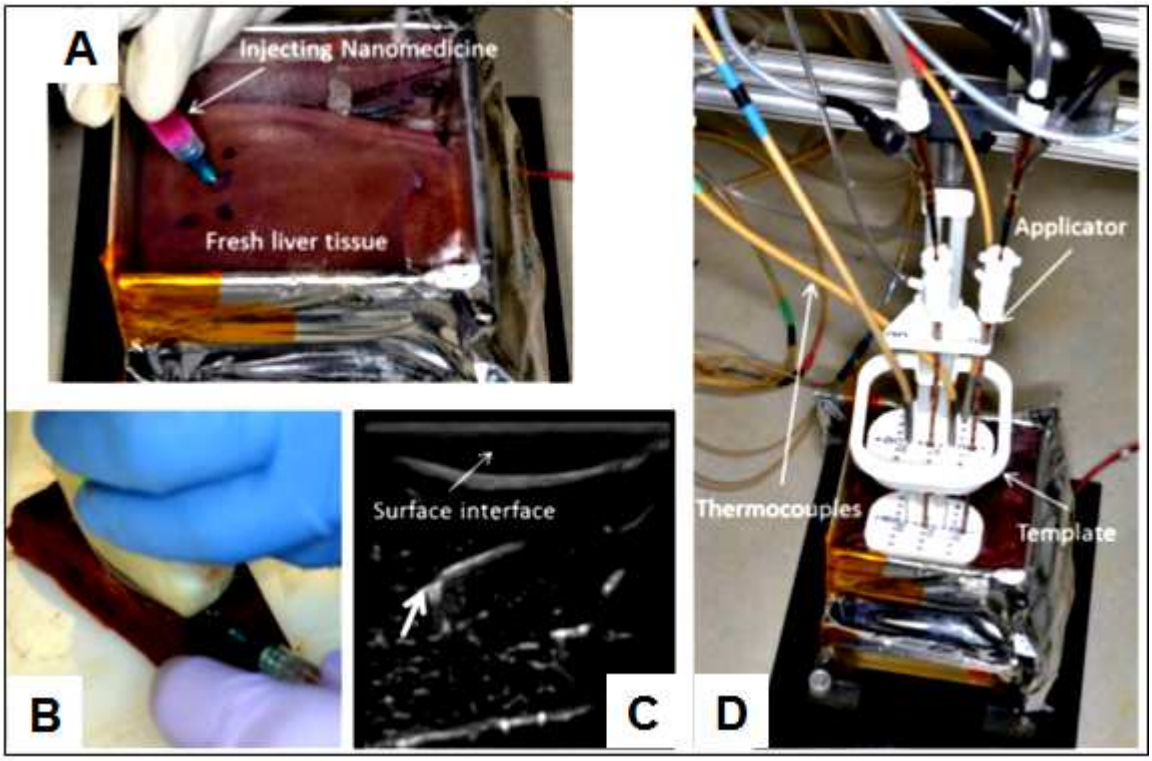


Figure S7. Experimental set-up for *ex vivo* pig liver tissue. (a) Freshly excised tissue placed in a thermo-electric box to keep the tissue temperature at 35-37 °C, (b) ultrasound imaging while injecting PBNB(RD), (c) ultrasound image guidance to inject nanomedicine into the tissue (the white arrow indicate the tip of the needle) and (d) the ultrasound ablation experimental setup. The applicator and the multi-sensor thermocouple needles were inserted using a custom template with pre-determined known sensor locations.

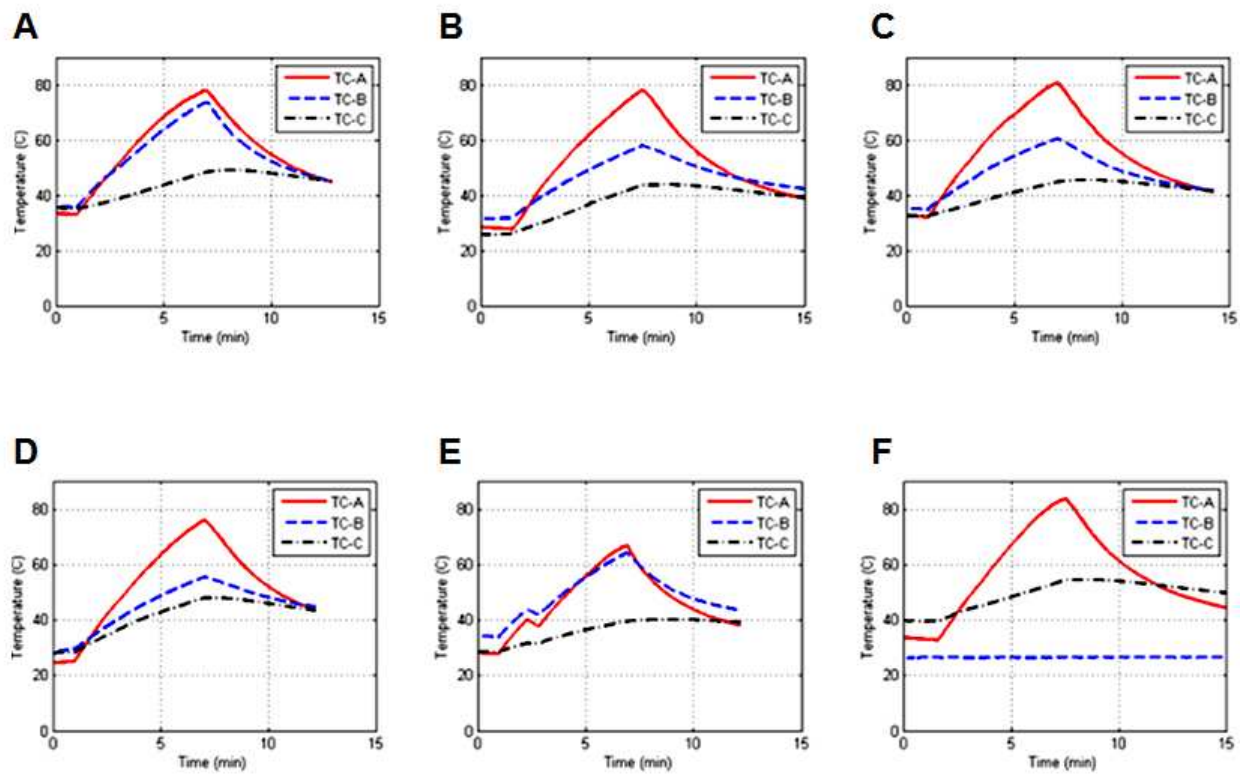


Figure S8. Temperature profile of the three thermocouples inserted into the tissue for the different configurations (a) Pro-bexarotene-NPs (RD)+ US, (b) NB (RD) + US, (c) Pro-bexarotene-NPs (RD) + US ablation, (d) PBNB(RD) + US ablation, (e) PBNB + US ablation, (f) US ablation only (TC-B was placed in air to record the room temperature).

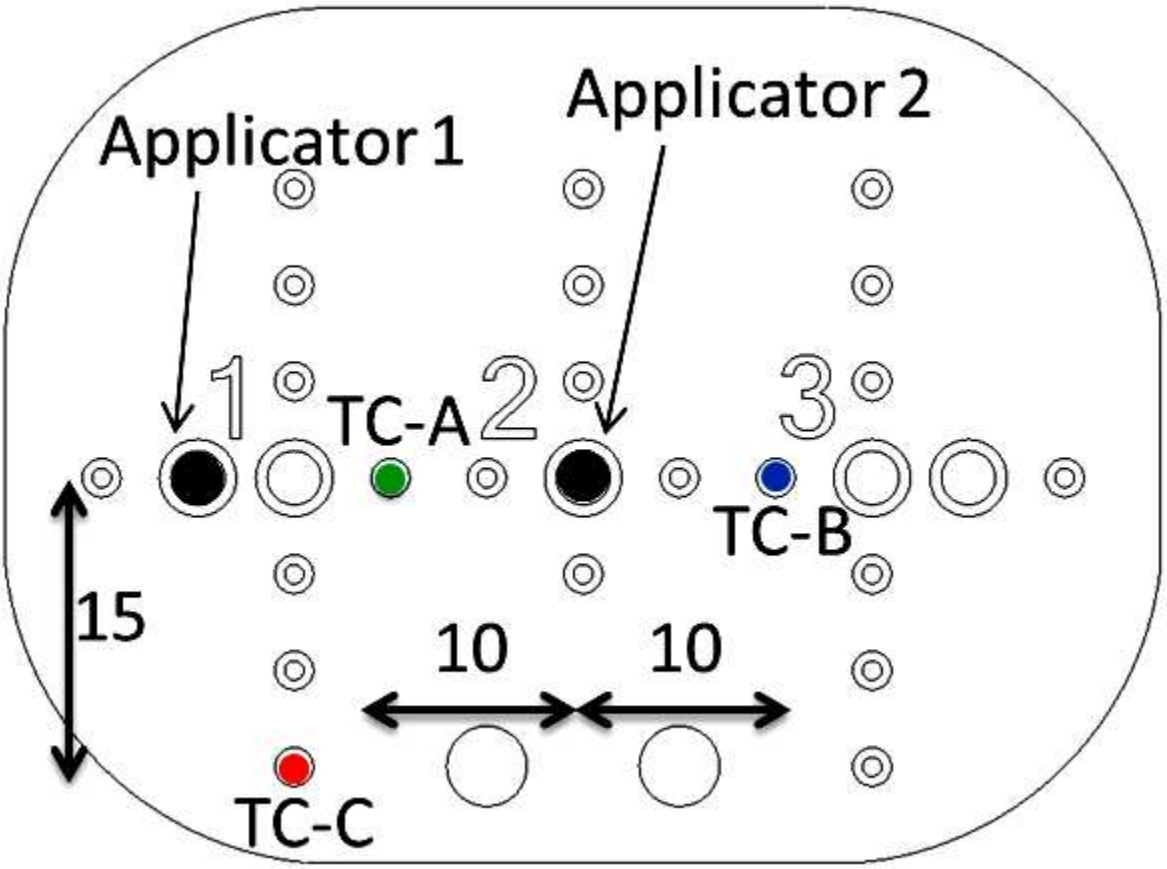


Figure S9. Location of the applicators and the flexible fine thermocouple needles in the treatment template.

Video Legends

Movie 1. DFT Simulation of bexarotene 782cm-1

Movie 2. DFT Simulation of bexarotene 1292cm-1

Movie 3. DFT Simulation of bexarotene 1316cm-1

Movie 4. DFT Simulation of bexarotene 1621cm-1

Movie 5. DFT Simulation of bexarotene 2829cm-1

Movie 6. DFT Simulation of pro-bexarotene 484cm-1

Movie 7. DFT Simulation of pro-bexarotene 713cm-1

Movie 8. DFT Simulation of pro-bexarotene 776cm-1

Movie 9. DFT Simulation of pro-bexarotene 872cm-1

Movie 10. DFT Simulation of pro-bexarotene 968cm-1

Movie 11. DFT Simulation of pro-bexarotene 993cm-1

Movie 12. DFT Simulation of pro-bexarotene 1061cm-1

Movie 13. DFT Simulation of pro-bexarotene 1174cm-1

Movie 14. DFT Simulation of pro-bexarotene 1434cm-1

Movie 15. DFT Simulation of pro-bexarotene 1453cm-1

Movie 16. DFT Simulation of pro-bexarotene 1570cm-1

Movie 17. DFT Simulation of pro-bexarotene 1587cm-1

Movie 18. DFT Simulation of pro-bexarotene 1650cm-1

Movie 19. DFT Simulation of pro-bexarotene 1894cm-1

Movie 20. Pro-bexarotene interaction with membrane NLMD simulation