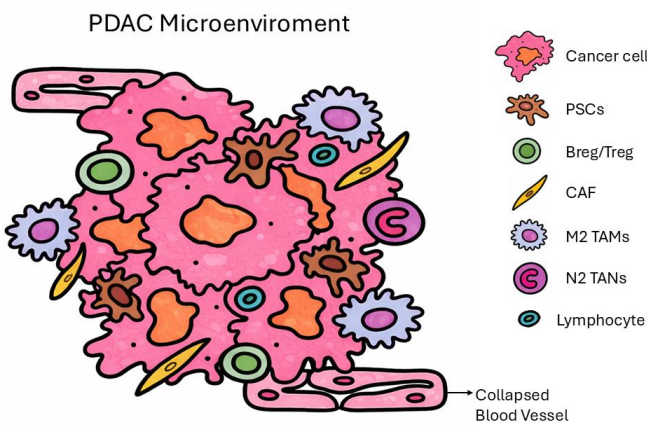


Background

Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival < 12%. Its hallmark is a dense desmoplastic stroma — a fibro-inflammatory matrix generated by pancreatic stellate cells (PSCs) — that collapses blood vessels and shields tumor cells from immune attack.

Healthy pancreas vs PDAC:

Normal ductal epithelium (HPDE) is organised and well-vascularised. In PDAC, cancer cells are surrounded by PSCs, CAFs, immunosuppressive M2 TAMs, N2 TANs, Breg/Treg lymphocytes, and collapsed vasculature.

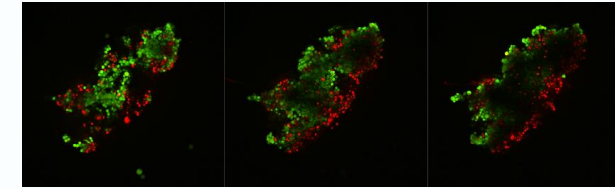


LICU (Low-Intensity Continuous Ultrasound) targets mechanosensitive channels overexpressed in PDAC

Materials & Methods (University of Florence)

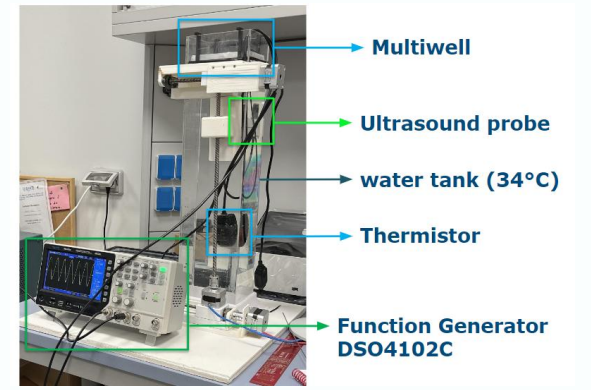
Cell Model: PANC-1 hanging-drop spheroids (1000 cells, 4 days). Controls: HEK-293, RLT-PSC stellate cells, HPDE (non-tumorigenic ductal epithelium).

Assays: LDH-Glo bioluminescent cytotoxicity assay and Calcein AM / PI confocal imaging (Zeiss LSM 880, 20×, 5 μm Z-slices).



LICU setup:

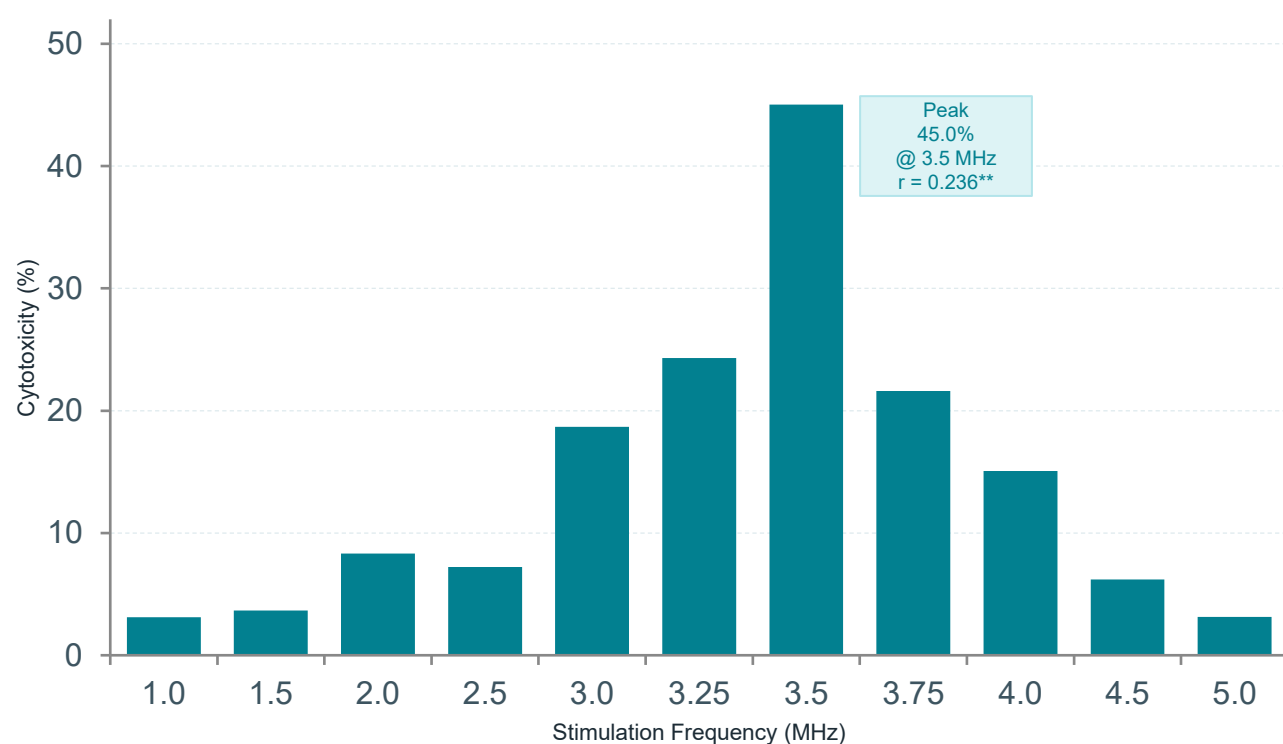
Cylindrical immersion transducers WS75-2 (1–3.5 MHz) & Olympus A37S (3.5–6 MHz). Input voltage 3.5 V, pressure characterised with HGL-0400 hydrophone. Duty cycles 100%.



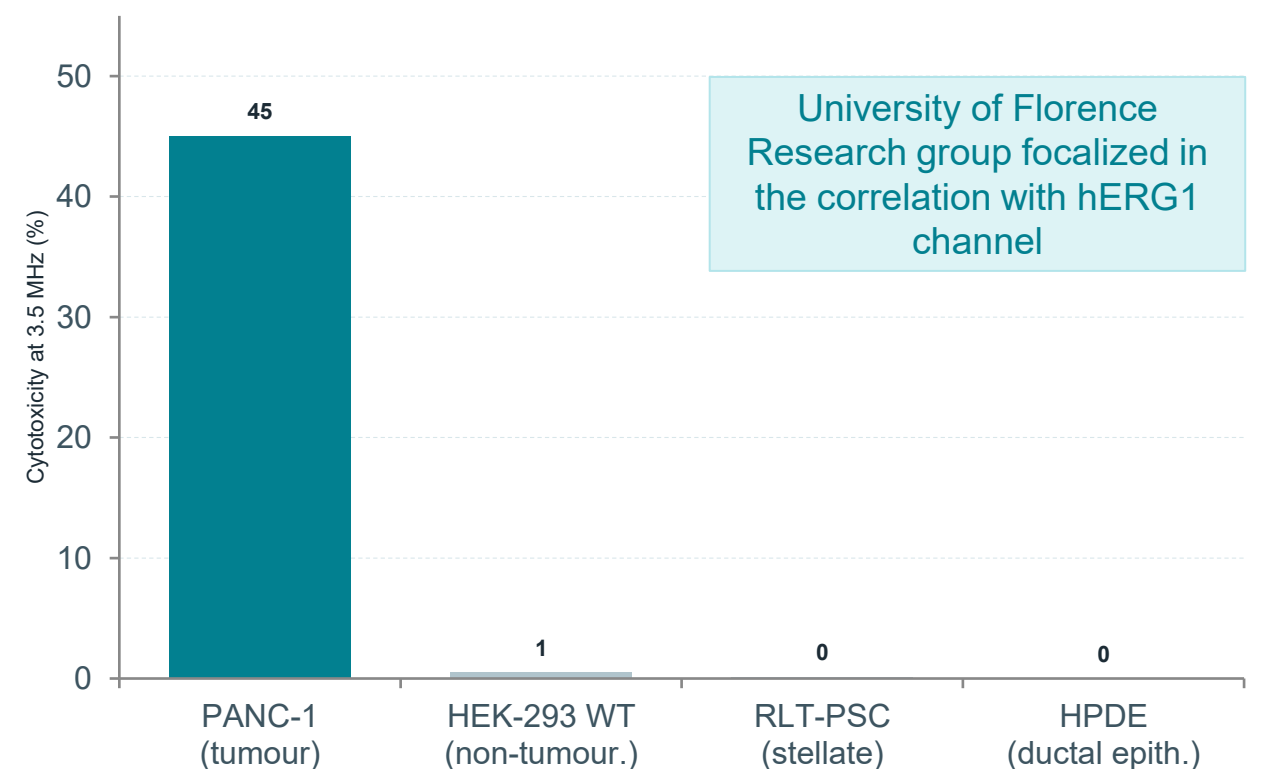
University of Florence LICU experimental setup

KEY RESULTS

1. Frequency-Dependent Cytotoxicity — PANC-1 (LDH, spheroids)



2. Selectivity — Zero Cytotoxicity in Non-Tumorigenic Controls



Materials & Methods (RWTH – ExMI lab)

Cell Model: PANC-1 two-dimensional cultures.

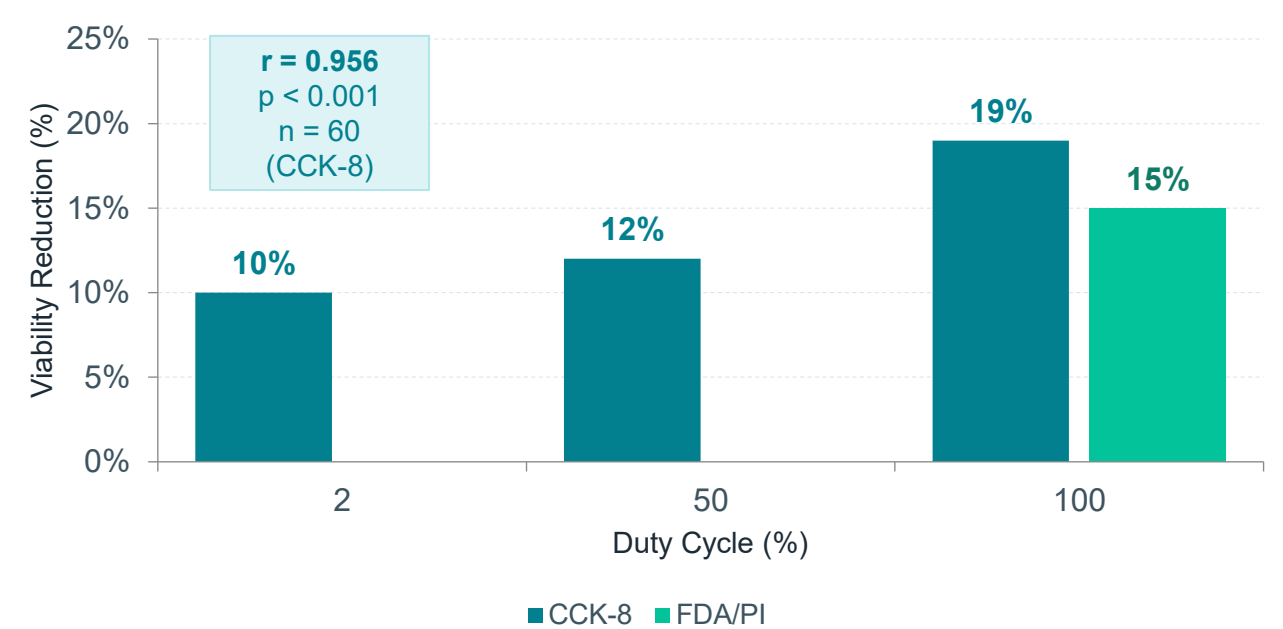
Assays: CCK-8 colorimetric viability.

Pharmacology: GsMTx4 (PIEZO1 inhibitor, reduces membrane tension-gated channel opening).

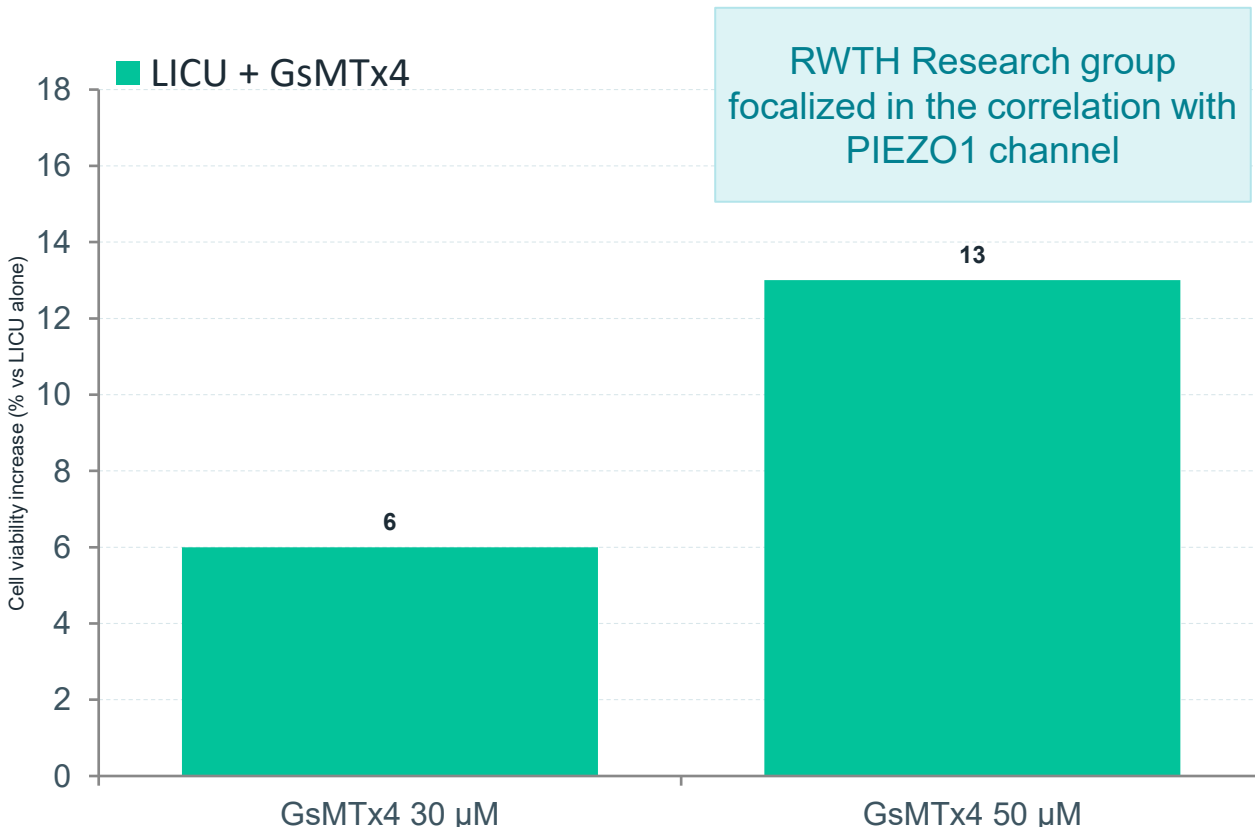
LICU setup:

Cylindrical immersion transducers Olympus focalized (1MHz). pressure characterised with HPN series hydrophone Onda, 130kPa. Duty cycles 2%, 50%, 100%.

3. Cytotoxicity — 2D PANC-1 (RWTH Aachen)

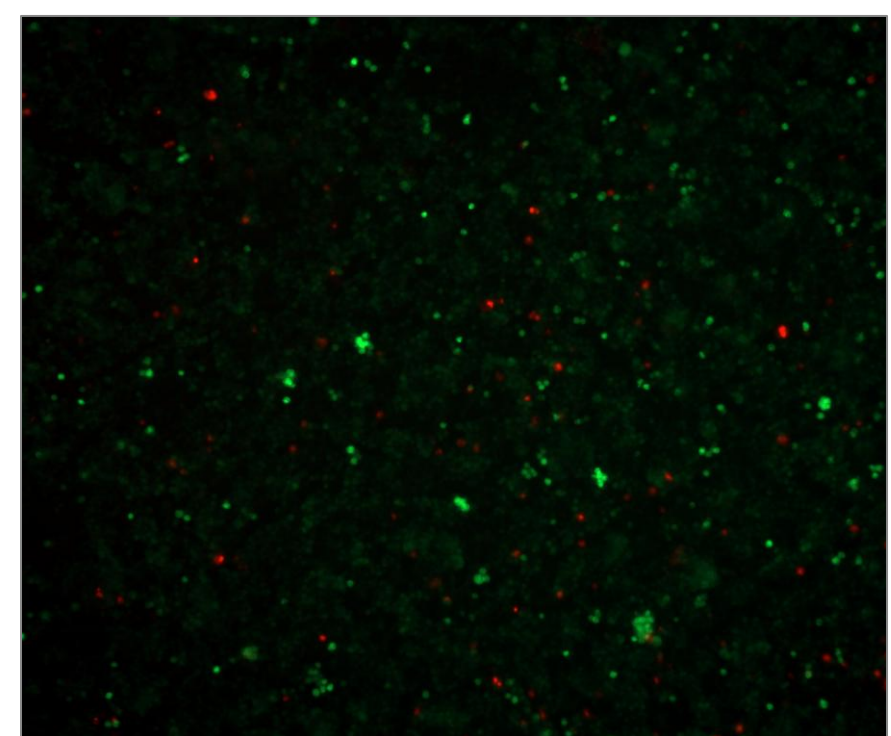


4. PIEZO1 Contribution: GsMTx4 Rescues Viability — 2D PANC-1 ★



★ Experiment on 2D PANC-1 monolayer cultures. GsMTx4 concentrations: 30 μM and 50 μM. GsMTx4-only control (≈8%) subtracted — net values shown. $p \leq 0.001$ (t-test).

5. Live/Dead Validation — FDA/PI Confocal Imaging (2D PANC-1)



■ Live (FDA) ■ Dead (PI)

FDA/PI staining of 2D PANC-1 monolayers after LICU (1 MHz, 130 kPa). Live cells (green) dominate; scattered dead cells (red) confirm LICU-induced cytotoxicity.

Conclusions

- LICU induces frequency-selective cytotoxicity in PANC-1 spheroids, peaking at 45.0% at 3.5 MHz ($r = 0.236$, $p \leq 0.01$)
- PIEZO1 acts as an independent co-contributor: GsMTx4 (30–50 μM) increases viability by +6–13% in 2D PANC-1 cells
- Results replicated at RWTH Aachen with focused transducer (130 kPa, ISPTA ≈ 121 mW/cm²): apparatus-independent biological response

References

- [1] Ricci M et al. (2025) Morphological analysis of US-treated PANC-1 spheroids. *Appl. Sci.* 15, 1707.
- [2] Ricci M et al. (2024) Sonoporation: a novel frontier for cancer treatment. *Appl. Sci.* 14, 515.
- [3] Song, Y., Chen, J., Zhang, C., Xin, L., Li, Q., Liu, Y., ... & Huang, P. (2022). Mechanosensitive channel Piezo1 induces cell apoptosis in pancreatic cancer by ultrasound with microbubbles. *Science*, 25(2).