Nonviral, Ultrasound Mediated Gene Delivery to the Heart of Mice and Non-Human Primates

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Background:

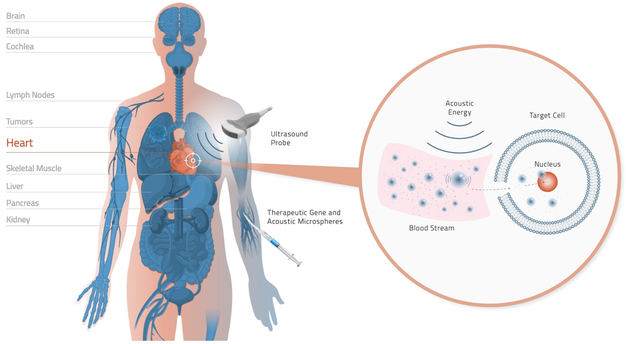
While gene therapy holds promise as a revolutionary approach for treating cardiovascular diseases, several hurdles remain that need to be overcome. Transcutaneous ultrasound mediated gene delivery (UMGD) has emerged as a pivotal tool in delivering noninvasive *in vivo* gene therapy to the heart with precision and efficacy. SonoThera is developing a novel ultrasound-guided nonviral gene therapy platform based on UMGD that allows selective targeting of specific organs and tissues within the body in a safe, redosable, durable, and titratable manner.

Methods:

To evaluate the potential of UMGD for safe and efficient transgene delivery to the heart, we developed a technology platform utilizing novel acoustic profiles, next-generation genetic payloads, and FDA-approved ultrasound components. The delivery process involves intravenous co-administration of nucleic acid payloads (e.g. DNA or RNA) and ultrasound contrast agents (a.k.a microbubbles), coupled with targeted application of externally applied ultrasound energy to direct the DNA into specific tissues via sonoporation. In vivo bioluminescence imaging of firefly luciferase reporter gene expression in the heart in wild type mice was evaluated using an IVIS imaging system at multiple timepoints. Mouse heart tissues were analyzed for vector delivery. NHP heart tissues were analyzed for vector delivery and expression. Multiple clinical and molecular safety assessments were evaluated in mice and NHP using established methods.

SonoThera delivery platform

1. Ultrasound enhancing agents (a.k.a. microbubbles) co-infused intravenously with nucleic acid payload (e.g. DNA/RNA)
2. Ultrasound energy is externally applied to target organ
3. Expression of the therapeutic payload occurs in target cells



UMGD Offers Targeted, Redosable, Durable, and Safe Transgene Delivery to the Heart

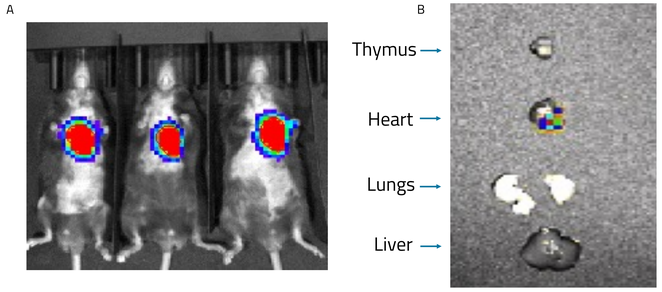


Figure 1: Expression of Luciferase in C57 mice 18 days following UMGD. A) Bioluminescent signal shows luciferase protein expression following UMGD. B) Ex vivo tissue bioluminescent signal of luciferase protein expression shows targeted signal in heart tissue but not in adjacent tissues (Thymus, Lungs, and Liver).

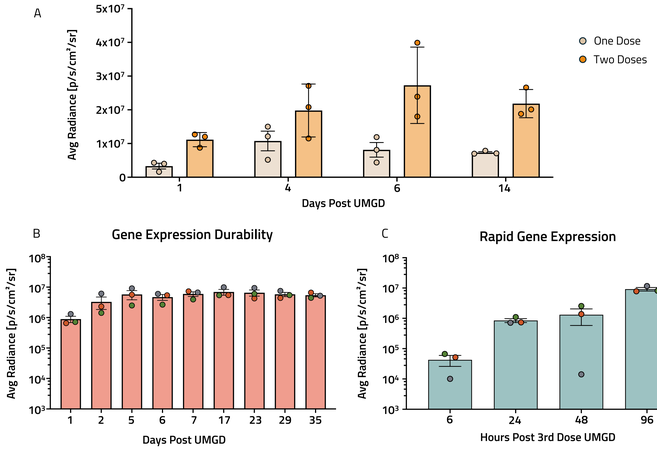
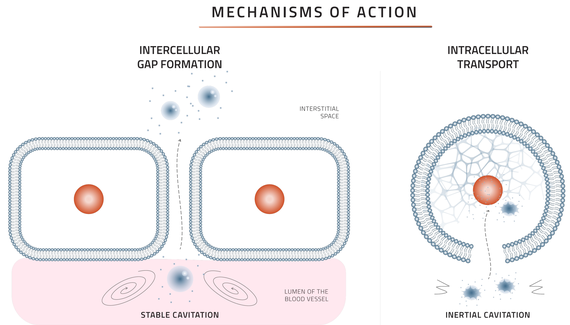


Figure 2: Expression of Luciferase in C57 mice following UMGD of A) 1 treatment shows peak average radiance of 1E7 at 4 days while 2 treatments shows an elevated peak average radiance of 2.7E7 at 6 days post final UMGD. B) Following UMGD treatment, luciferase expression shows durability through 35 days. C) Luciferase expression is detected as early as 6 hours following UMGD and continues to increase through 4 days post UMGD.



Gene Construct Detected in Mouse and NHP Heart Cells

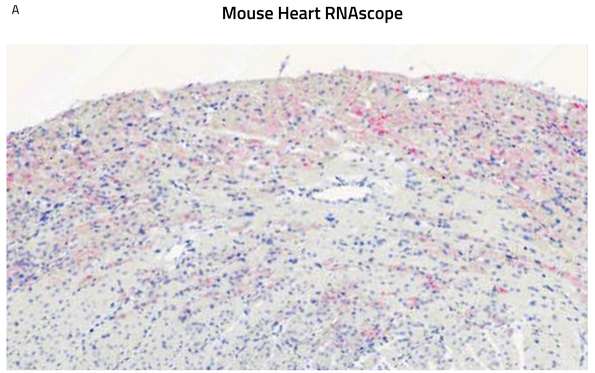


Figure 3: RNAscope analysis of mouse and NHP heart tissue following UMGD. A) 24 hours post UMGD show high level of DNA signal (red) in UMGD-treated mouse heart. B) Representative RNAscope image of naïve and UMGD-treated NHP heart at 5 days post UMGD with black arrows indicating cells with transgenic RNA signal (red).

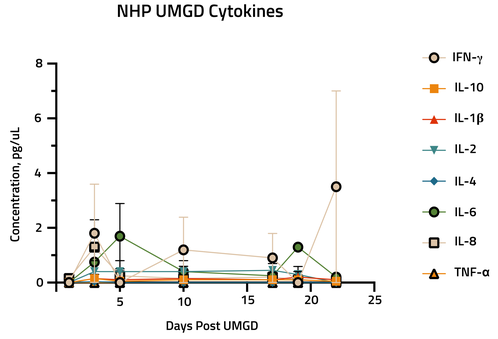


Figure 4: Inflammatory cytokine levels after administration of UMGD were all within normal range (n=2), Error Bars: SEM.

Efficient Gene Delivery in Mouse and NHP Heart

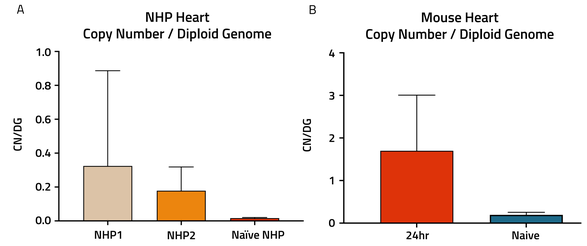


Figure 5: Transgenic DNA copy number per diploid genome (CN/DG) in non-human primate and mouse heart tissue following UMGD. A) CN/DG in hearts of two NHPs following UMGD comparing two different acoustic profiles. B) CN/DG in mouse heart at 24 hours post UMGD.

Results:

Efficacious delivery of non-viral DNA payloads using SonoThera's proprietary UMGD platform led to rapid, robust, durable, redosable, transgene expression in the hearts of mice and NHP with excellent tolerability and safety. Gene expression durability was followed for 1 month after single treatment in mice. Following repeated treatments, a significant increase of gene expression was observed compared to a single treatment. Vector delivery was detected in mouse heart tissues. Vector delivery and expression was detected in NHP tissues. Ex-vivo bioluminescence imaging of excised heart tissue after UMGD showed targeted delivery to the heart without off-target delivery to adjacent tissues.

Summary:

- Established ultrasound-mediated gene delivery platform for targeted, redosable, and durable payload expression in mouse and non-human primate heart.
- UMGD is safe and well tolerated in mice and non-human primates.
- Confirmed UMGD has efficient cell transfection using IVIS, ddPCR, and RNAscope