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## RESEARCH LETTER

# Ultrasound-Guided Optogenetic Gene Delivery for Shock-Free Ventricular Rhythm Restoration

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Implantable cardioverter defibrillators are the standard therapy for patients at risk for ventricular tachycardia (VT) and are associated with lower all-cause mortality in selected patients.<sup>1</sup> However, high-voltage electroshocks delivered by these devices can cause decreased quality-of-life and depression disorders, in addition to myocardial tissue damage.<sup>2</sup> Development of novel therapeutic approaches overcoming these adverse effects is therefore of high importance.

Optogenetics is a novel technology that utilizes light to enable fast, reversible, and precise control of the membrane potential of cardiomyocytes after forced expression of light-gated ion channels in these cells.<sup>3</sup> This technique has been applied to optically terminate VT in rodent models, thereby providing a potential novel therapeutic approach for acute, pain-free rhythm restoration. Still, genetic modification of whole hearts by systemic viral vector delivery, as performed in these studies, is considered a major challenge in the clinical translation of cardiac optogenetics, whereas local transduction has proven to be safe, feasible, and effective in cardiac gene-therapeutic trials<sup>4</sup>.

However, it remains unknown whether and how optogenetic VT termination can be realized through such localized myocardial transduction, thereby leaving an important aspect of its translatability unaddressed so far. Interestingly, evidence suggests that the cardiac apex may play an important role in arrhythmia sustainability because it can attract scroll waves (ie, reentrant activity) due to its geometric curvature combined with high levels of anisotropy.<sup>5</sup> Local and temporal depolarization of the cardiac apex during VT, as can be realized by optogenetics, might, therefore,

result in scroll-wave filament destabilization and subsequent VT termination.

To evaluate whether local transgene delivery and subsequent local illumination of the apex allows for effective optogenetic VT termination, adeno-associated virus (AAV) 9.45-pseudotyped AAV 2 vectors containing a transgene consisting of an enhanced mouse  $\alpha$ -myosin heavy chain promoter, the coding sequence of citrine-tagged red-activatable channelrhodopsin, the woodchuck hepatitis virus posttranscriptional regulatory element and the simian virus 40 polyadenylation signal, were apically administered to the adult rat heart via ultrasound-guided, stereotactic transthoracic injections (4×25  $\mu$ L, total genome-copies  $10^{11}$ ; Figure [A]). All animal experiments were approved by Animal Experiments Committee of the Leiden University Medical Center, the Netherlands (AVD1160020172929) and conformed to the Guide for the Care and Use of Laboratory Animals as stated by the US National Institutes of Health. Three to four weeks later, hearts (n=5) were excised and cannulated for Langendorff perfusion, followed by apical attachment of a custom-made light-emitting diode device consisting of four 565-nm light-emitting diode chips. Immunohistological staining of serial coronal sections along the sagittal axis of these hearts showed that transduction was mostly confined to the apical and septal region (Figure [B]). Optical ventricular pacing by 565-nm light-pulses (3 mW/mm<sup>2</sup>), targeting 2 mm<sup>2</sup> of the apex, was feasible in all hearts tested (Figure [C]), but not when other regions of the ventricles were illuminated. Following induction of sustained VTs (>10 s), apical illumination for up to 3× (500 ms duration, 500 ms interval) with

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one light-emitting diode-chip (surface-area: 2 mm<sup>2</sup>) resulted in termination of 52% of the VTs (SEM 10%). However, when all 4 light-emitting diode chips were activated (surface-area: 8 mm<sup>2</sup>), optogenetic VT termination increased to 96% (SEM 4%), whereas none of the VTs were terminated during the no-light control experiments (Figure [D] and [E];  $P=0.003$  using the Kruskal-Wallis test).

Collectively, the data presented here suggest that the clinical exploration of optogenetic VT termination might be realized by local rather than global myocardial transgene delivery, which can for example be realized by percutaneous catheters<sup>4</sup>. In particular, the apex might be a target area for such local transduction since local depolarization of this region resulted in VT termination with high efficiency, which may provide a new incentive for the development of more refined, and also pain-free, antiarrhythmic therapeutic strategies. Future studies should, among others, focus on optimizing the local delivery method, but also assess the role of pathological substrates and distribution of transgene expression in determining optogenetic arrhythmia termination efficacy.

## ARTICLE INFORMATION

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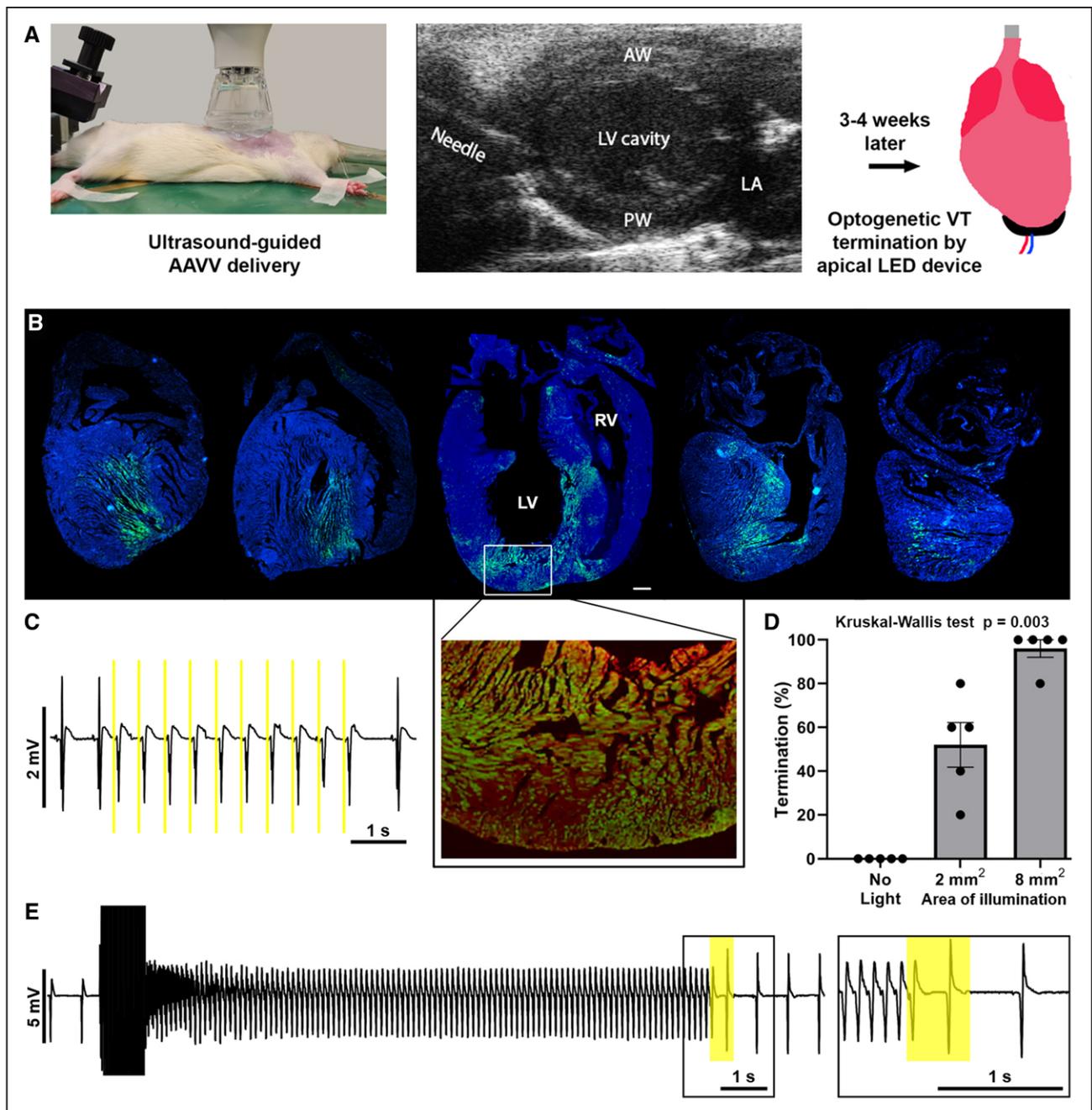
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### Disclosures

None.

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**Figure. Shock-free ventricular tachycardia (VT) termination by echocardiography-guided, light-emitting diode (LED) implant-based local optogenetic targeting.**

**A**, Experimental setup and study design, including parasternal long-axis view displaying apical needle insertion. **B**, Representative immunohistological staining of serial coronal sections along the sagittal axis from ventral (left) to dorsal (right). Green=citrine, Blue=nuclei, Red=cardiac troponin-I. Scale bar represents 1 mm. **C**, Optical ventricular pacing by 10-ms, 565-nm light-pulses directed at the apex. **D**, Quantification of optogenetic VT termination efficacy (n=5 animals with 5 independent VT termination attempts per animal). Error bars represent SEM.  $P=0.003$  using the Kruskal-Wallis test. **E**, Typical intracardiac ECG-trace demonstrating successful optogenetic termination by local illumination of the cardiac apex with a single 500 ms, 565 nm light-pulse. AW indicates anteroseptal wall; LA, left atrium; LV, left ventricular; PW, posterior wall; and RV, right ventricular.