Preclinical efficacy and safety of *KCNH2-G628S* gene therapy for postoperative atrial fibrillation

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ABSTRACT

Background: Postoperative atrial fibrillation (POAF) is the most common complication occurring after cardiac surgery. Multiple studies have shown significantly increased risks of stroke, myocardial infarction, and death associated with POAF. Current prophylaxis strategies are inadequate to eliminate this problem. We examined the preclinical efficacy and safety of *KCNH2-G628S* gene transfer to prevent POAF.

Methods: Domestic pigs received Ad*KCNH2-G628S* by epicardial atrial gene painting and atrial pacemaker implantation for continuous-burst pacing to induce atrial fibrillation. In an initial dose-ranging evaluation, 3 pigs received 5×10^{10} to 5×10^{11} virus particles. In the formal study, 16 pigs were randomized to 3 groups: 5×10^{11} virus particles of Ad*KCNH2-G628S* with 20% Pluronic P407 in saline, 20% Pluronic P407 in saline with no virus, and saline alone. Animals were followed with daily efficacy and safety evaluations through the period of peak adenovirus-mediated transgene expression. After 14 days, pacing was discontinued, and the animals were followed in sinus rhythm for an additional 14 days to assess any longer-term toxicity.

Results: In the primary efficacy analysis, the G628S animals exhibited a significant increase in the average time in sinus rhythm compared with the Pluronic control group ($59 \pm 7\%$ vs $14 \pm 6\%$; $P = .009$). There was no significant difference between the Pluronic and saline controls ($14 \pm 6\%$ vs $32 \pm 12\%$; $P = .16$). Safety assessment showed improved left ventricular function in the G628S animals; otherwise there were no significant differences among the groups in any safety measure.

Conclusions: These data indicate that *KCNH2-G628S* gene therapy can successfully and safely reduce the risk of AF. (J Thorac Cardiovasc Surg 2017;154:1644-51)

KCNH2-G628S gene transfer decreases the risk of atrial fibrillation.

Central Message

G628S gene transfer safely and effectively prevents postoperative atrial fibrillation, supporting further study in a Phase I clinical trial.

Perspective

Postoperative atrial fibrillation (POAF) is a common and significant complication of cardiac surgery. The risks of stroke, heart failure, and death are increased in patients who POAF. We found that *KCNH2-G628S* gene transfer reduced the AF burden without any evident toxicity. The timing of adenovirus-mediated gene expression is such that the method may be directly applicable to preventing POAF.

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Approximately 700,000 cardiac surgical procedures are performed each year in the United States.¹ Postoperative atrial fibrillation (POAF) continues to be the most common complication after cardiac surgery, affecting 30% of patients overall and >75% of patients with risk factors (eg, valve surgery, heart failure, older age, hypertension,

Scanning this QR code will take you to supplemental tables, appendix, and video for this article.

Abbreviations and Acronyms

- AF = atrial fibrillation
- APD = action potential duration
- ECG = electrocardiography
- MAP = monophasic action potential
- POAF = postoperative atrial fibrillation
- SR = sinus rhythm
- vp = viral particles

pulmonary or renal dysfunction).²⁻⁴ POAF most often occurs during the first few days after surgery, with almost all cases developing before postoperative day 14.² Despite this time-limited risk, however, studies have consistently shown that POAF is associated with increased risks of stroke, myocardial infarction, and death.²⁻⁸

Although clinical trials have shown some reductions in POAF with amiodarone, β -blockers, an antioxidant diet, drug-releasing hydrogels, sotalol, and biatrial pacing,^{3,5,9-11} the persistent occurrence rate of 30% indicates the need for better POAF prevention.⁷ We propose gene therapy with AdKCNH2-G628S for prevention of POAF. This adenoviral vector induces temporary transgene expression over a 2-to-3-week interval, which mirrors the time frame of greatest POAF risk.¹²⁻¹⁴ KCNH2-G628S (G628S) is a dominant negative mutation that eliminates the function of a potassium channel that plays a central role in cardiac myocyte repolarization.¹⁵ In a previous study, we demonstrated the efficacy of atrial painting of AdG628S with 200 mg/mL

Pluronic P407 and 5 mg/mL trypsin.¹⁶ The use of trypsin in the clinical environment is potentially problematic owing to the narrow therapeutic window; a concentration of 5 mg/mL is needed for transmural penetration of the gene transfer vector, and a concentration of 10 mg/mL affects atrial structural integrity.¹⁷ We have previously shown that eliminating trypsin from the painting solution allows for surface gene transfer, but no penetration of the vector beyond the epicardial layer.¹⁷ Uncertainty about the efficacy of epicardial-only G628S expression motivated the present study.

MATERIALS AND METHODS

The study materials and methods are described in detail in the online supplement. In brief, the study comprised 2 components: a preliminary dose-ranging assessment in 3 animals and a randomized, double-blind, placebo-controlled study in 16 animals (Figure 1). All animals underwent an initial surgery that included the gene painting procedure (Video 1) and implantation of an atrial burst pacemaker for induction of AF. Burst pacing was activated immediately, and the animals were followed daily for rhythm analysis and safety assessment. The animals in the preliminary study were sacrificed at 14 days after gene transfer to verify transgene expression. The animals in the randomized study had the atrial burst pacing discontinued at day 14 and were followed for an additional 14 days of extended safety assessment.

RESULTS

Preliminary Dose-Ranging Study of 3 Animals

To inform the prospective design of the formal efficacy study, we first evaluated an escalating virus dose in 3 animals: 1 each received 5×10^{10} viral particles (vp), 1×10^{11} vp, and 5×10^{11} vp of AdG628S in a 5-mL total volume of Pluronic P407/saline (without trypsin). Atrial

FIGURE 1. Study design for initial dose-ranging study (A) and randomized, blinded, controlled efficacy study (B). EPS, Electrophysiological studies.

VIDEO 1. Gene painting procedure performed by applying 5 mL of virus/Pluronic gel onto the atria using a foam applicator (swab). Video available at: [http://www.jtcvsonline.org/article/S0022-5223\(17\)31098-X/addons](http://www.jtcvsonline.org/article/S0022-5223(17)31098-X/addons).

pacemakers were implanted at the time of gene therapy, and the AF-inducing burst pacing protocol remained active for the entire study. Rhythm was assessed daily from the off-pacing segments of the 2-second on/off cycles of the burst pacing protocol (Figure E1). Over the 14-day study period, the animal receiving 5×10^{10} vp of AdG628S had 6 days with sinus rhythm, the animal receiving 1×10^{11} vp had 3 days with sinus rhythm, and the animal receiving 5×10^{11} vp had 9 days with sinus rhythm. Expression and functional analyses showed an increased duration of atrial monophasic action potential (MAP) and increased KCNH2 expression in all animals at the time of sacrifice compared with baseline (Figure E2).

Efficacy of AdKCNH2-G628S for AF

Based on the results of the dose-ranging study, we designed a randomized, controlled trial comparing AdG628S at a virus dose of 5×10^{11} vp in 5 mL of 20% Pluronic saline with 20% Pluronic/saline controls and saline-only controls. Group sizes were initially 5 pigs each for the AdG628S and Pluronic/saline groups and 3 pigs for the saline-only group. Animals that died during the burst pacing portion of the study were replaced after completion of the initial 13-animal cohort via an extended randomization table, so that all animals in the study were prospectively randomized.

We assessed the percentage of time in sinus rhythm (SR%) on daily telemetry recordings averaged over the 14-day burst pacing period (Figure 2). Animals in the G628S group had significantly increased SR% compared with the Pluronic control group ($59 \pm 7\%$ vs $14 \pm 6\%$; $P = .009$). There was no significant difference between the Pluronic and saline controls ($14 \pm 6\%$ vs $32 \pm 12\%$; $P = .44$). Even though the saline control group was not powered to determine differences from the G628S group and that comparison was not part of the predetermined analysis, the difference between those 2 groups nearly reached statistical significance ($P = .068$).

The control animals followed a general pattern of progressively increasing AF from the onset of burst pacing, similar to control animals in our previously published studies.^{16,18-20} The AdG628S animals showed competing

FIGURE 2. SR% per day after gene painting. Compared with Pluronic controls, animals exposed to G628S had a significant decrease in AF even with the continued stress of aggressive burst atrial pacing ($P = .0006$). Saline and Pluronic controls did not differ significantly.

influences of burst pacing-driven AF and antiarrhythmic efficacy, with an initial increase in SR% from the influence of the G628S transgene followed by a loss of SR% as transgene expression presumably waned and burst pacing continued to induce AF. The onset of antiarrhythmic efficacy in the AdG628S animals was consistent with the previously observed timing of adenovirus-mediated transgene expression in cardiac myocytes.¹² Compared with our previous study with AdG628S, Pluronic P407, and trypsin, the duration of efficacy in the present study was attenuated,¹⁶ likely owing to the reduced gene transfer and thus reduced transgene expression in the absence of trypsin.¹⁷

Safety of AdKCNH2-G628S for AF

We compared the 3 groups in terms of various safety measures, including qualitative measures of overall clinical status, activity, appetite, thirst, and urine and stool generation and quantitative measures of average heart rate, respiratory rate, body temperature, and QT interval on electrocardiography (ECG) measured on a daily basis and body weight measured weekly (Figures 3 and 4). We also evaluated general laboratory test results (Table E1), echocardiographic measures of cardiac structure and function, and electrophysiological measures of repolarization (Table E2) recorded at baseline and at sacrifice, and we assessed cardiac dissection time (as a surrogate for pericardial adhesion formation), organ weights, and effusion volumes at sacrifice (Table E3). Other than significantly better left ventricular function at sacrifice in the G628S group (a reflection of the increased SR% in that group), we found no significant differences among the 3 groups for any of these measures.

Microscopic evaluation of all tissues collected at necropsy demonstrated findings related to the open chest

FIGURE 3. Clinical observations. Daily assessments of overall clinical status (A), activity (B), appetite (C), thirst (D), urine output (E), and stool output (F) were recorded using a 2- to 5-point numerical scale with guidance from the descriptors shown on the graphs. The investigator evaluating the animals was masked to study group identity of each animal. *Black square*, G628S group; *red circle*, Pluronic group; *blue triangle*, saline group. There were no statistically significant differences among the 3 groups.

surgery or the tachycardiomyopathy inherent to the model. There were no differences among the groups in the prevalence of these findings, and there were no findings suggesting any adverse effect of AdG628S treatment (Figure 5).

During the course of the study, 2 animals died, including a G628S animal with cardiac tamponade from atrial lead perforation and a Pluronic animal with severe heart failure that never recovered from the initial surgical procedure. Interestingly, the Pluronic animal had a low-level positive troponin at baseline study, suggesting the presence of subclinical myocarditis that might have contributed to its intolerance of the AF and heart failure. We terminated 2 animals (1 Pluronic animal and 1 saline animal) early owing

to intractable, severe heart failure that is a described component of the model.¹⁸ The in-life clinical observations, gross and microscopic pathology in these animals were assessed for any treatment-related cause. Based on these data, all deaths were determined to be procedural or model-related and not product-related.

DISCUSSION

This preclinical study was designed to evaluate AdKCNH2-G628S gene therapy for prevention of POAF. It should be considered in the context of our previous work showing that atrial gene painting with reporter genes caused epicardial-only gene transfer in the absence of trypsin and dense transmural gene transfer with 0.5% trypsin, and that

FIGURE 4. Clinical measurements. Daily measurements of ventricular rate on ECG (A), respiratory rate (B), body temperature (C), and QTc from ECG (D) were performed. The animals were weighed weekly (E). The shaded box indicates the interval after burst pacing was discontinued. There were no statistically significant differences among the groups for any measurement.

gene painting with AdG628S and trypsin reliably increased atrial action potential duration (APD) and prevented AF at peak gene expression, with dissipation of efficacy over 2 to 3 weeks.^{16,17} In the present study, we found effective AF prevention without any observable toxicity in the animals receiving AdG628S in the absence of trypsin.

Porcine Model of AF and Heart Failure

No available animal model reliably produces POAF. To test AF therapies in an aggressive preclinical environment, we developed and validated a porcine AF heart failure model.^{16,18-20} The independent use of this model by other

laboratories has verified its reliability.²¹ The basic protocol includes burst right atrial pacing to induce persistent AF and an uncontrolled ventricular rate to cause a cardiomyopathy. Animals exhibit progressively increasing nonsustained AF over the first few days before developing persistent AF at a median of 5 ± 1 days after the onset of burst pacing.¹⁸ The tachycardiomyopathy worsens progressively over time, with left ventricular ejection fraction decreasing to $53 \pm 4\%$ after 1 week, to $28 \pm 2\%$ after 3 weeks and to $22 \pm 3\%$ after 5 weeks.¹⁸⁻²⁰ Along with this decrease in cardiac function, progressive dilation occurs in all cardiac chambers, and histological findings of cellular hypertrophy,

FIGURE 5. Example microsections from hearts of animals receiving AdG628S (*left*) and Pluronic/saline (*right*). There were no differences among the groups in terms of pathological findings in the right atria (A and E), right ventricles (B and F), left atria (C and G), or left ventricles (D and H). Pathological findings of hypertrophy and myolysis in all chambers from all animals were consistent with structural remodeling from AF and heart failure. No treatment-specific pathological findings were observed.

myolysis, apoptosis, inflammation, and fibrosis progress over the same time frame. For testing the efficacy against POAF, we lacked the facilities and expertise to put the animals on cardiopulmonary bypass and perform coronary bypass or valve surgery. As such, our animals did not have “typical” POAF, but we did test efficacy in the setting of median sternotomy and hands-on manipulation of the heart (in addition to the persistent AF and structural remodeling components of the model), and found efficacy in an aggressive AF-inducing postoperative environment.

KCNH2-G628S

KCNH2 is the alpha subunit of an ion channel that generates I_{Kr} , one of the principal cardiac repolarizing currents. The KCNH2-G628S mutation replaces a glycine with a serine at a position within the channel pore. The larger serine blocks the pore.²² An *in vitro* study showed no current if equal quantities of wild-type and G628S messenger RNA were injected, and only 12% of the expected current if a ratio of 5 wild-type KCNH2 to 1 G628S was injected.²² This dominant negative action is required for therapeutic efficacy because patients would presumably express normal endogenous KCNH2. We do not have the tools available to quantify the percentage of I_{Kr} channels in each myocyte that contained the transgene-derived mutant channel, but the stoichiometry of wild-type to mutant channels is undoubtedly a component in the overall time course of efficacy for our therapy.

Insight Into Mechanisms of Arrhythmia

The mechanisms of postoperative AF remain controversial. Previous studies have implicated various factors, including preexisting atrial fibrosis, expression levels of various ion channels, inflammation, metabolic or oxidative stress on myocytes, alterations in connexin expression, and adrenergic, purinergic, and/or cholinergic stimulation.²³⁻²⁹ These associations potentially could support either triggered or reentrant mechanisms for POAF. Manipulations to prolong APD could help distinguish among these mechanisms. Triggered activity by early after depolarization mechanisms would worsen with APD prolongation, because the increased repolarization time would provide more opportunity for early afterdepolarizations to occur. Delayed afterdepolarizations and automatic activity would not be directly affected by APD. I_{Kr} block is a well-established method of prolonging APD. The efficacy of I_{Kr} block, both in our study and in the sotalol clinical trials, suggests reentry as the underlying mechanism of POAF.

The contribution of pulmonary veins to AF was established by Hassaiguerre and colleagues and has since become an accepted paradigm for paroxysmal AF.³⁰ Decidedly lower efficacy has been demonstrated for pulmonary vein ablation in patients with long-standing persistent AF and/or structural heart disease, suggesting that the pulmonary vein contribution is less relevant in those settings.³¹ The pulmonary veins in the

pig are not accessible through a pericardial approach, so our intervention did not affect pulmonary vein function. Nonetheless, we saw efficacy with our intervention, suggesting that POAF is likely an intra-atrial and not a pulmonary venous process. That conclusion is supported by the findings of Ryu and colleagues, who reported decreased intra-atrial connexin43 expression in dogs with POAF,²⁷ by data from Zaman and colleagues showing reduced levels of right atrial sarcolipin, myosin heavy chain, and SERCA2a in patients with POAF,³² and data from Dizon and coworkers demonstrating that POAF occurs in recipients of double lung transplantation despite pulmonary vein isolation.³³

Additional insight into the mechanism of AF can be gleaned from the efficacy in our present study, in which trypsin was no longer included in the gene painting solution. We previously showed that transgene expression was limited to the epicardial layer when trypsin was not part of the adenoviral painting solution.¹⁷ That observation, combined with our present results, indicate that epicardial-only expression of G628S is sufficient to prevent AF. These data suggest that the epicardium is an obligatory component in the reentry circuits that sustain AF. Overall, our data indicate that POAF is a reentrant process that is not dependent on pulmonary vein activity, but is critically dependent on conduction through the epicardium.

Safety of G628S Gene Therapy

Our extensive safety analysis revealed no evidence of any adverse effects of therapy. This comprehensive safety analysis comprised 36 observation variables, including overall clinical status, specific measures of the animals' condition, and daily QTc and cardiac rhythm analysis. The sole between-group differences suggest a benefit of therapy, likely related to the reduced incidence of heart failure stemming from the overall lower average heart rate and increased SR in the active treatment group. None of our findings were suggestive of any potential harm from therapy. In particular, we saw no evidence of QT prolongation or ventricular arrhythmias, consistent with the atrial specificity of the delivery method. Our overall assessment of safety for this therapy should be considered in the context of the work of Kikuchi and colleagues and Amit and colleagues, where 18 pigs that received AdKCNH2-G628S by atrial painting with trypsin (a more effective delivery method than the current trypsin-free protocol) demonstrated no QT prolongation and no significant adverse events compared with the 21 control animals.^{16,17}

Study Limitations

Our data present compelling evidence supporting the efficacy and safety of G628S gene painting for prevention of POAF. In addition to the lack of cardiopulmonary bypass discussed above, other considerations for these data include the younger substrate and starting good health of the pigs

relative to the typical elderly cardiac surgery patient. Age, hypertension, heart failure, diabetes, and pulmonary disease are consistent risk factors for AF overall and for POAF in particular. Our model does include severe heart failure developing from the uncontrolled ventricular rate during either burst pacing or AF.¹⁸ Antiarrhythmic efficacy is maintained in the setting of heart failure, suggesting that the therapy may withstand these other comorbidities. In the end, only clinical testing will answer the questions of efficacy and safety in the clinical environment.

CONCLUSIONS

Overall, the findings of our preclinical study indicate that G628S is safe and effective in treating AF. The animals in the G628S group had a significantly reduced AF burden and no toxicity attributable to the test. These data support further study of G628S in a Phase I clinical trial to prevent POAF.

Conflict of Interest Statement

J.K.D. is listed as an inventor on gene therapy patents issued to Johns Hopkins University. All other authors have nothing to disclose with regard to commercial support.

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Key Words: atrial fibrillation, gene therapy, potassium channel, operative complications

APPENDIX E1. MATERIALS AND METHODS

Study Test Article Preparation

Adenovirus creation and quality testing. Adenovirus encoding the cytomegalovirus immediate/early promoter and enhancer, the KCNH2-G628S transgene (G628S), and the SV40 virus poly-A sequence was constructed using the AdEasy system as described by He and colleagues.⁴¹ The resulting virus was expanded by infecting HEK-293 cells and purified using Adenopur columns (Purosyn, Philadelphia, Pa). All viruses were aliquoted to minimize repeated freezing and thawing and then stored at -80°C until use.

The identity of the resulting virus construct was confirmed by sequencing the entire transgene using a series of nested primers. Transgene expression was confirmed by infecting COS-7 cells, harvesting protein after 3 days, and assessing for KCNH2 by Western blot analysis. Transgene function was confirmed by infecting primary cultures of porcine ventricular myocytes and measuring I_{K_r} current after 3 days in culture. The absence of wild-type adenovirus was verified by polymerase chain reaction using primers directed to the adenoviral E1 gene (absent in the recombinant virus and necessary for function in the wild-type virus). Virus particle titer was measured by ultraviolet spectroscopy, and virus infectious titer was measured by a plaque assay using standard methods. The column-purified virus was used for the 4 preliminary study animals, and it was sent to the Cornell University Virus Vector Core for production of the virus used in the formal study.

Pluronic-saline solution. Pluronic P407 (also known as poloxamer, Lutrol, or Kolliphor and either F127 or P407) was donated by BASF (Florham Park, NJ). Pharmaceutical-grade saline solution (0.9% sodium chloride in water) was purchased from Baxter (Deerfield, Ill). At 3 to 5 days before the study, stock solution of Pluronic P407 in saline was prepared by adding the requisite amount of Pluronic to saline in a glass beaker to achieve final concentrations of 300 mg/mL (30%) for the preliminary study and 250 mg/mL (25%) for the formal study. The beaker containing the Pluronic/saline was placed in a cold room (3°C to 5°C) on a stir plate and stirred until a homogeneous solution was achieved (generally 24 to 48 hours), at which time chilled saline was added to achieve the desired stock Pluronic concentration. The Pluronic/saline solution was sterile-filtered inside the cold room by passage through a chilled $0.2\text{-}\mu$ filter and then refrigerated until use.

Test article preparation. For the preliminary dose-ranging study of 4 animals, the stock virus particle concentration was 8.3×10^{11} vp/mL and the stock Pluronic concentration was 300 mg/mL. In the randomized controlled trial, the stock virus concentration was 2.3×10^{12} vp/mL and the stock Pluronic concentration was 250 mg/mL.

On the day of the experiment, the virus and Pluronic solutions were removed from storage and kept on ice until immediately before use. At that time, the virus was added to the Pluronic solution using sterile technique. Ice-cold saline solution was added to bring the final solution volume to 5 mL and the final Pluronic concentration to 200 mg/mL (20%). The solution was mixed thoroughly and placed on a 37°C heating block for just enough time to achieve a firm, gel-like consistency, which was verified by turning the tube upside down and confirming the absence of solution movement.

Overview of the Experimental Protocol

All animals received humane care in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee. Yorkshire pigs (25 to 35 kg) were used in this study. The overall study protocol is shown in [Figure 1](#). Specific items in the overall protocol are described below.

For the dose-ranging study, 1 pig each received 5×10^{10} , 1×10^{11} , and 5×10^{11} vp of AdG628S. During an initial procedure, the animals underwent an invasive electrophysiological study, atrial gene painting of

the test article, and permanent atrial burst pacemaker insertion to induce persistent AF. Pacing was activated immediately after the surgery and was continued uninterrupted until animal sacrifice. The animals were checked daily for heart rhythm as described below. Animals were sacrificed at 14 days after the painting procedure, at which time they underwent a repeat electrophysiological study. The heart was extracted, and samples of the atria were snap-frozen for Western blot analysis of transgene expression.

In the formal efficacy study, pigs were randomized to 3 groups: 5×10^{11} vp of Ad-G628S in 20% Pluronic, 20% Pluronic with no virus, and saline alone. In accordance with the prospective design, the virus and Pluronic groups started with 5 animals each and the saline group had 3 animals, and all animals that died within the 14-day burst pacing AF portion of the study were replaced after completion of the initial cohort of 13 animals using a prospectively generated, extended randomization scheme. Four animals died or were terminated early for humane reasons (1 in the G628S group, 2 in the Pluronic group, and 1 in the saline group). One animal in the Pluronic group was terminated for intractable heart failure on postoperative day 14 after completion of efficacy data collection, so that animal was not replaced. The final numbers in the formal study included 6 in the G628S group, 6 in the Pluronic-alone group, and 4 in the saline-alone group. All animals, including all who died prematurely, were included in the safety analyses.

In the randomized efficacy study, each animal underwent an initial procedure that included electrophysiological studies, echocardiography, blood analyses, atrial pacemaker implantation, and atrial painting of the test article. At the end of the initial procedure, the pacemaker was activated to deliver burst atrial pacing. The atrial burst pacing algorithm was continued uninterrupted for 14 days. Animals were followed daily with clinical observations, rhythm recordings, and QTc analysis.

Antiarrhythmic efficacy was tested for 14 days after surgery to mirror the risk period of POAF. After 14 days of burst pacing, the pacemaker was turned off, and the animals were followed for an additional 14 days to observe for any longer-term toxicity before repeat electrophysiological studies, echocardiography, blood analyses, and sacrifice.

Pacemaker Implantation and Gene Delivery

Before the procedure, the infection solution was prepared as described above in the section on study test article preparation. After sedation (1.5 mg/kg each of ketamine, xylazine, and telazol), intubation, anesthesia (isoflurane 1.75%), and sterile preparation, the right external jugular vein was accessed by cutdown, and the chest was opened by median sternotomy. The pericardium was incised. Echocardiographic and electrophysiological recordings were performed as described below. After the electrophysiological study, the gene painting procedure was performed by applying 5 mL of virus/Pluronic gel onto the atria using a foam applicator (25-1605-1PSF-RECT; Puritan Medical, Guilford, Me). The heart was manipulated to expose all epicardial atrial surfaces, and all parts of the atria accessible from within the pericardium were painted (including the anterior and lateral portions of each atrium from the venae cavae on the right to the left pulmonary veins on the left, and the posterior-inferior atria around the coronary sinus). Painting occurred in a pattern of left atrial painting for 60 seconds, right atrial painting for 60 seconds, repeat left atrial painting for 60 seconds, and then right atrial painting for 60 seconds. After painting, the heart was left exposed to air for 10 minutes to allow for virus penetration. The painting procedure is shown in [Video 1](#).

After completion of the painting procedure, an active-fixation pacing lead was placed through the right external jugular vein into the right atrium. Adequate pacing parameters (electrogram >1 mV, pacing threshold <1 V, no diaphragmatic pacing at 10 V output) were confirmed, the lead was connected to a Medtronic Itrel-3 stimulator (Medtronic, Dublin, Ireland), and the system was placed in a subcutaneous pocket in the neck. Once the incisions were closed, the pacemaker was programmed to burst pace the right atrium at 42-Hz frequency and 7.5 V output in 2-second

increments alternating with 2-second pauses between burst pacing (Figure E1, A). The burst pacing algorithm was activated at the end of the surgical procedure and it remained active without interruption for 14 days.

Echocardiography

Echocardiography was performed at gene transfer and the termination study by placing a sterile sleeve-encased probe directly on the heart after median sternotomy. Conventional 2-chamber and 4-chamber images were obtained to measure atrial and ventricular chamber dimensions and left atrial and ventricular ejection fractions using software available on the echo machine (Vivid 7; GE Healthcare, Chicago, Ill).

Electrophysiological Studies

During gene transfer and the termination study, APD and atrial conduction time were measured as follows. MAPs were acquired using a decapolar catheter (Biosense Webster, South Diamond Bar, Calif). Signals were recorded without high-pass filtering with lead 3 (-) and lead 1 (+), a configuration validated to give an MAP signal.^{E2} The MAP catheter was positioned at the center of 10 predefined epicardial atrial regions and in the basal and apical regions of the ventricles following a previously published protocol.^{E3} The basal ventricular regions were adjacent to atrial sites 4 and 9. An endocardial MAP was recorded from a region corresponding to epicardial site 4. Atrial conduction time was recorded with catheters positioned over the sinus node and left atrial appendage.

For all recordings, the cardiac chamber of interest was paced at cycle lengths of 400, 350, 300 and 250 ms for 10 beats, and the last 3 captured beats were used to measure the condition of interest (APD or conduction time). Electrograms were recorded and analyzed using the EP Workmate (St. Jude Medical, Saint Paul, Minn).

Rhythm and QTc Analysis

Daily ECG recordings were performed using a 3-channel ECG system. Recording occurred in a quiet environment, and every effort was made to ensure that recording conditions were reproducible from one recording period to the next. The 2-second on/2-second off burst pacing algorithm was continuously active through the daily telemetry recordings to maintain the aggressive burst pacing stimulation. Intrinsic rhythm was observed during the 2-second off-pacing segments (Figure E1, B). We recorded 120 seconds of telemetry and then analyzed rhythm in the pacing-free intervals. We calculated and reported the %SR as the number of SR nonpacing segments divided by the total nonpacing segments $\times 100\%$. Effective burst pacing was confirmed daily by assessing telemetry strips for loss of P wave and increase in both the rate and irregularity of the QRS complexes during burst pacing.

The same telemetry strips were used to calculate QT interval, which was corrected for heart rate using Bazett's formula. QTc was measured from 10 beats during nonpaced segments (first 14 days of the study) or 5 beats during sinus rhythm (last 14 days of the study).

Clinical Observations

Overall animal status, activity level, food and water intake, urine and stool output, heart rate, respiration rate and body temperature were recorded daily at the time of ECG recording. Animals were weighed each week. Overall status and activity level were qualitatively graded on a scale of 1 to 5. Food and water intake and urine and stool output were qualitatively assessed as present or absent. The investigator performing these qualitative measures was masked to study group identity for each animal.

The overall animal status indicator was used to capture any health issues with the animal that might not have been picked up in the prospectively designed questionnaire of animal health indicators. The 5-point qualitative scale for overall animal status used the following descriptors to assist the

investigator in grading each animal on each day: 1 = normal animal appearance and behavior, 3 = moderately severe impairment in activity and/or behavior, 5 = moribund.

The animal activity indicator assessed spontaneous animal activity on a 5-point scale with the following descriptors to assist the investigator in grading the animals: 1 = spontaneously active without stimulation, 3 = some spontaneous movement but mostly quiescent, 5 = movement only observed with stimulation.

Western Blot Analysis

To quantify protein content of tissue extracts, Western blot analysis was performed on proteins extracted from the frozen tissue samples. The concentrations of proteins were determined by the BCA method (Pierce Chemical, Rockford, Ill). Proteins were fractionated by electrophoresis on 4% to 12% Bis-Tris gels and transferred to nitrocellulose membranes. After blocking with 5% nonfat dry milk membrane, membranes were blotted with anti-KCNH2 (mouse monoclonal, sc-377388; Santa Cruz Biotechnology, Dallas, Tex) or anti-calsequestrin (rabbit polyclonal, sc-28275; Santa Cruz Biotechnology) and secondary antibodies directed against the primaries and conjugated with horseradish peroxidase (goat anti-mouse IgG, sc-2005 and goat anti-rabbit IgG, sc-2004; Santa Cruz Biotechnology). Bands were detected with Western Blotting Luminol Reagent (sc-2048; Santa Cruz Biotechnology) and quantified using a gel imaging system (Imager 600; Amersham Biosciences, Piscataway, NJ).

Pathological Observations

At sacrifice, the time required for dissection to the heart (including removal of adhesions sufficient to perform MAP recordings) was recorded, and major organs were visually inspected, removed, and weighed. The following tissues were harvested for histopathology: heart (basal left and right ventricle in physical contact with the atria, mid-wall left and right ventricle, apical left and right ventricle, mid-ventricular septum, mid-atrial septum, and 3 widely spaced samples in each left and right atrial free wall); posterior pericardium; mid-diaphragm; ascending aorta, thymus, lung, liver, spleen, kidney, jejunum, ovary (no testes, because all males were castrated), quadriceps muscle, eye, spinal cord, brain, blood, and any gross lesions identified. All tissues except eyes and gonads were preserved in 10% neutral buffered formalin. Eyes and gonads were preserved in Bouin's fixative. Fixed tissues were shipped to Lovelace Respiratory Research Institute, where they were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Slides were examined by a board-certified veterinary pathologist (J.A.H.), and microscopic findings were recorded in the Provantis preclinical database (Instem LSS Ltd, Staffordshire, United Kingdom). Gradable histomorphological findings were assigned a severity score of 0 to 4.

Statistical Analysis

The investigator making the daily clinical observations and recording and interpreting the rhythm strips was masked to animal study group identity. The study was prospectively designed to compare the G628S and Pluronic-alone groups (5 animals per group). In consultation with the Food and Drug Administration, 3 saline-only animals were added before initiation of the study to identify any changes caused by the Pluronic. The study design had 95% power to detect a 66% reduction in AF for the active treatment group relative to the Pluronic-alone group (the treatment difference observed by Amit and colleagues^{E4}), which was the primary efficacy endpoint, and >80% power to detect a 10- ms between-group difference in QTc (based on average QTc of 399 and variance of 5 in a previous study) and an absolute 1-point difference in the "overall clinical status" rating (assuming an average clinical status rating of 2 and variance of 0.5), which were the primary safety endpoints.

Randomization was performed using the Excel random number generator function. Data are reported as mean $\pm$ SD. For rhythm analysis, the %SR over time was analyzed by Poisson regression with a generalized estimating equations model to compensate for repeated measurements. For nonrhythm analysis, the Student *t* test was used for comparisons between 2 groups and one-way analysis of variance was used for 3-way comparisons. Tukey post hoc testing was used for individual pair comparisons within the 3 groups. For the safety analyses, correction for multiple measures was not performed, to increase the sensitivity for adverse effects of AdKCNH2-G628S painting at the risk of increasing type II error. In all tests, a *P* value $<.05$ was considered statistically significant.

E-References

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A. atrial burst pacing protocol

Standard Programming:

- Pacing frequency = 42 Hz
- Pacing amplitude = 7.5 V
- Pacing pulse width = 0.5 ms
- Cycling = 2 seconds on/2 seconds off

Example of rhythm strip used for analysis:

- 3-lead telemetry is recorded daily. At least 2 minutes of interpretable rhythm is recorded.
- effective capture is confirmed by noting the loss of P waves and the irregular ventricular response during burst pacing
- non-pacing segments are manually assessed for sinus rhythm and marked on the recording
- if a sinus P wave is noted prior to restart of burst pacing, the segment is marked sinus (S)
- 60 non-pacing segments are analyzed per telemetry strip and the percentage of sinus segments is recorded

B. comparison of %SR for multiple recordings per day.

FIGURE E1. Atrial burst pacing protocol for induction of AF and rhythm assessment methods. A, The Itrel stimulator was programmed to pace at 42-Hz frequency in a pattern of 2-second on/off repeating cycles. Effective capture was confirmed on daily recordings by noting the rapid, irregular rhythm occurring during burst pacing segments. Pacing was not stopped for rhythm analysis. Rhythm was considered sinus for any pacing segment if conversion from AF to SR with a clear sinus P wave was observed before the end of the 2-second segment. SR% was recorded as the percentage of 2-second off-pacing segments that had SR for each animal on each day. B, Validation of rhythm analysis. In a previous study of 4 animals with atrial burst pacing and no antiarrhythmic therapy, we obtained 8 telemetry recordings of 2 minutes each over 3 days, one early after onset of burst pacing when the rhythm was predominately sinus, one at 2 to 3 days after onset of burst pacing when the rhythm was mixed, and one at 7 days after onset of burst pacing when the rhythm was predominately AF. The graph compares %SR from the first telemetry recording of the day to that of multiple recordings per day. The x-axis shows rhythm analysis from the first recording of the day, grouped into complete SR, partial SR, and complete AF. The y-axis shows final %SR based on the indicated number of recordings spaced evenly throughout the day. These data show that no significant amount of AF is missed when 100% SR is initially noted; no significant amount of SR is missed when 100% AF is initially noted, and the mid-range group has similar levels of SR regardless of the number of recordings. SR, Sinus rhythm.

A. KCNH2 expression by Western blot on tissues harvested 14 days after atrial gene painting

Protein expression (Western blot normalized to calsequestrin expression)

	LA	RA	
5x10 ¹⁰ vp	0.36	0.16	
1x10 ¹¹ vp	0.57	0.19	
5x10 ¹¹ vp	0.55	0.50	
[+] control		0.86	AdG628S pig from Amit study
[-] control		0.20	untreated AF pig

B. Functional effects of KCNH2 transgene expression

		average MAPD	
		right atrium	left atrium
5 x 10 ¹⁰ vp	day 0	147	124
	day 14	225	199
1 x 10 ¹¹ vp	day 0	134	125
	day 14	157	137
5 x 10 ¹¹ vp	day 0	131	100
	day 14	153	187

FIGURE E2. Transgene expression and function. A, KCNH2 expression at 14 days after atrial gene painting with AdG628S in the 3 dose-ranging animals. B, Results of electrophysiology assessment in the 4 dose-ranging animals showing some variability in MAP duration (MAPD), likely owing to the competing effects of transgene expression, AF, and heart failure. Overall, animals showed an increased MAPD relative to baseline, which is consistent with the functional effects of KCNH2-G628S. LA, Left atrium; RA, right atrium.

TABLE E1. Serum chemistry, cardiac enzyme, hematology, and coagulation laboratory results at baseline and sacrifice

Parameter	Baseline			Sacrifice		
	G628S	Pluronic	Saline	G628S	Pluronic	Saline
White blood cell count ($\times 10^3/\mu\text{L}$)	15.5 $\pm$ 1.0	15.8 $\pm$ 1.2	16.3 $\pm$ 0.7	11.7 $\pm$ 0.9	12.5 $\pm$ 1.3	13.3 $\pm$ 1.8
Red blood cell count ($\times 10^3/\mu\text{L}$)	5.7 $\pm$ 0.2	6.2 $\pm$ 0.4	5.3 $\pm$ 0.1	5.0 $\pm$ 0.2	5.7 $\pm$ 0.1	4.6 $\pm$ 0.6
Platelet count ($\times 10^3/\mu\text{L}$)	401 $\pm$ 50	265 $\pm$ 33	462 $\pm$ 84	342 $\pm$ 67	467 $\pm$ 30	357 $\pm$ 90
Hemoglobin (g/dL)	10.2 $\pm$ 0.3	10.8 $\pm$ 0.6	9.4 $\pm$ 0.2	8.4 $\pm$ 0.5	9.5 $\pm$ 0.4	7.9 $\pm$ 0.9
Hematocrit (%)	28.1 $\pm$ 0.7	30.2 $\pm$ 1.8	25.6 $\pm$ 0.4	23.9 $\pm$ 1.3	27.0 $\pm$ 1.2	22.4 $\pm$ 2.1
Mean cell volume (fL)	49 $\pm$ 0	48 $\pm$ 1	48 $\pm$ 1	47 $\pm$ 1	48 $\pm$ 1	50 $\pm$ 3
Mean cell hemoglobin (pg)	17.7 $\pm$ 0.1	17.2 $\pm$ 0.3	17.6 $\pm$ 0.2	16.7 $\pm$ 0.6	16.8 $\pm$ 0.4	17.1 $\pm$ 0.5
Mean cell hemoglobin concentration (g/dL)	36.1 $\pm$ 0.2	35.7 $\pm$ 0.2	36.6 $\pm$ 0.8	35.1 $\pm$ 0.6	35.4 $\pm$ 0.3	34.8 $\pm$ 1.0
Nucleated red blood cells (per 100 RBC)	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	2 $\pm$ 2
Reticulocyte count (%)	0.8 $\pm$ 0.4	0.7 $\pm$ 0.3	0.9 $\pm$ 0.8	3.9 $\pm$ 1.7	1.0 $\pm$ 0.4	4.1 $\pm$ 3.6
Absolute reticulocytes ($\times 10^3/\mu\text{L}$)	46 $\pm$ 24	44 $\pm$ 15	46 $\pm$ 39	185 $\pm$ 77	57 $\pm$ 21	125 $\pm$ 97
Neutrophil (%)	38.7 $\pm$ 5.3	48.2 $\pm$ 4.0	47.8 $\pm$ 8.0	35.6 $\pm$ 2.3	41.8 $\pm$ 2.1	48.5 $\pm$ 6.5
Lymphocyte (%)	55.0 $\pm$ 5.2	45.1 $\pm$ 3.8	48.0 $\pm$ 6.9	59.3 $\pm$ 1.9	52.0 $\pm$ 2.5	44.0 $\pm$.6
Monocyte (%)	4.0 $\pm$ 0.4	4.5 $\pm$ 0.6	2.8 $\pm$ 0.8	3.3 $\pm$ 0.6	3.1 $\pm$ 0.5	5.1 $\pm$ 0.9
Eosinophil (%)	1.6 $\pm$ 0.4	1.8 $\pm$ 0.2	0.9 $\pm$ 0.4	1.3 $\pm$ 0.2	2.3 $\pm$ 0.4	2.0 $\pm$ 0.7
Basophil (%)	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
Absolute neutrophils (per μL)	6176 $\pm$ 1010	7738 $\pm$ 1183	7740 $\pm$ 1246	4183 $\pm$ 464	5315 $\pm$ 763	6104 $\pm$ 271
Absolute lymphocytes (per μL)	8377 $\pm$ 668	6957 $\pm$ 507	7859 $\pm$ 1271	6896 $\pm$ 518	6439 $\pm$ 552	6182 $\pm$ 1496
Absolute monocytes (per μL)	610 $\pm$ 69	693 $\pm$ 75	459 $\pm$ 134	387 $\pm$ 83	377 $\pm$ 59	639 $\pm$ 70
Absolute eosinophils (per μL)	249 $\pm$ 57	295 $\pm$ 52	159 $\pm$ 80	152 $\pm$ 29	283 $\pm$ 57	301 $\pm$ 111
Absolute basophils (per μL)	44 $\pm$ 6	29 $\pm$ 10	75 $\pm$ 36	30 $\pm$ 7	40 $\pm$ 11	41 $\pm$ 17
Sodium (mmol/L)	139 $\pm$ 1	137 $\pm$ 1	138 $\pm$ 2	134 $\pm$ 4	137 $\pm$ 0	139 $\pm$ 1
Potassium (mmol/L)	6.0 $\pm$ 0.2	5.8 $\pm$ 0.4	5.8 $\pm$ 0.4	6.2 $\pm$ 0.1	6.6 $\pm$ 0.3	6.4 $\pm$ 0.3
Chloride (mmol/L)	100 $\pm$ 1	101 $\pm$ 1	100 $\pm$ 1	96 $\pm$ 4	100 $\pm$ 1	99 $\pm$ 2
Bicarbonate (mmol/L)	31 $\pm$ 0	27 $\pm$ 1	32 $\pm$ 1	28 $\pm$ 2	27 $\pm$ 1	31 $\pm$ 2
Calcium (mg/dL)	9.6 $\pm$ 0.2	9.3 $\pm$ 0.1	9.5 $\pm$ 0.3	8.9 $\pm$ 0.2	9.0 $\pm$ 0.2	9.2 $\pm$ 0.2
Phosphorus (mg/dL)	9.7 $\pm$ 0.4	9.3 $\pm$ 0.6	9.4 $\pm$ 0.4	8.9 $\pm$ 0.8	9.1 $\pm$ 0.1	9.1 $\pm$ 0.4
Magnesium (mg/dL)	2.0 $\pm$ 0.1	2.0 $\pm$ 0.1	1.9 $\pm$ 0.0	2.2 $\pm$ 0.1	2.1 $\pm$ 0.1	2.2 $\pm$ 0.1
Glucose (mg/dL)	79 $\pm$ 9	95 $\pm$ 22	74 $\pm$ 12	107 $\pm$ 21	84 $\pm$ 9	57 $\pm$ 9
Creatinine (mg/dL)	1.0 $\pm$ 0	0.9 $\pm$ 0.1	0.9 $\pm$ 0	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.0 $\pm$ 0
Blood urea nitrogen (mg/dL)	9 $\pm$ 1	8 $\pm$ 1	8 $\pm$ 1	9 $\pm$ 1	12 $\pm$ 1	18 $\pm$ 7
Alkaline phosphatase (IU/L)	164 $\pm$ 16	199 $\pm$ 22	143 $\pm$ 14	94 $\pm$ 15	134 $\pm$ 13	111 $\pm$ 5
Total bilirubin (mg/dL)	0.1 $\pm$ 0	0.1 $\pm$ 0	0.1 $\pm$ 0	0.3 $\pm$ 0.2	0.1 $\pm$ 0	0.5 $\pm$ 0.4
Direct bilirubin (mg/dL)	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.1 $\pm$ 0.1	0 $\pm$ 0	0.2 $\pm$ 0.2
Indirect bilirubin (mg/dL)	0.1 $\pm$ 0	0.1 $\pm$ 0	0.1 $\pm$ 0	0.2 $\pm$ 0.1	0.1 $\pm$ 0	0.2 $\pm$ 0.1
Alanine aminotransferase (IU/L)	42 $\pm$ 4	40 $\pm$ 3	36 $\pm$ 5	45 $\pm$ 9	30 $\pm$ 5	57 $\pm$ 18
Aspartate aminotransferase (IU/L)	31 $\pm$ 3	28 $\pm$ 2	27 $\pm$ 2	33 $\pm$ 6	34 $\pm$ 9	178 $\pm$ 157
Creatine phosphokinase (IU/L)	561 $\pm$ 83	445 $\pm$ 24	706 $\pm$ 226	531 $\pm$ 65	467 $\pm$ 55	1013 $\pm$ 384
Amylase (IU/L)	1996 $\pm$ 239	2170 $\pm$ 140	2179 $\pm$ 192	1462 $\pm$ 233	1915 $\pm$ 286	1825 $\pm$ 239
Albumin (g/dL)	2.9 $\pm$ 0.1	2.8 $\pm$ 0.1	2.7 $\pm$ 0.2	2.4 $\pm$ 0.1	2.5 $\pm$ 0.1	2.5 $\pm$ 0.2
Total protein (g/dL)	5.0 $\pm$ 0.1	5.1 $\pm$ 0.1	4.8 $\pm$ 0	5.1 $\pm$ 0.4	5.0 $\pm$ 0.1	5.2 $\pm$ 0.3
Globulin (g/dL)	2.1 $\pm$ 0.1	2.3 $\pm$ 0.1	2.1 $\pm$ 0.2	2.7 $\pm$ 0.2	2.5 $\pm$ 0.2	2.7 $\pm$ 0.5
Cholesterol (mg/dL)	82 $\pm$ 5	71 $\pm$ 6	79 $\pm$ 6	63 $\pm$ 3	61 $\pm$ 7	63 $\pm$ 11
Troponin I (ng/mL)	0 $\pm$ 0	0.007 $\pm$ 0.007	0 $\pm$ 0	0 $\pm$ 0	0.03 $\pm$ 0.03	0 $\pm$ 0
Prothrombin time (s)	14.3 $\pm$ 0.7	13.3 $\pm$ 0.8	12.5 $\pm$ 1.1	16.5 $\pm$ 1.0	14.5 $\pm$ 0.7	17.7 $\pm$ 4.1
Partial thromboplastin time (s)	10.4 $\pm$ 0.7	12.3 $\pm$ 0.8	37.7 $\pm$ 13.8	15.8 $\pm$ 3.0	14.0 $\pm$ 1.1	13.8 $\pm$ 2.4

All values are mean $\pm$ SD. There are no statistically significant differences among the 3 groups.

TABLE E2. Echocardiographic and electrophysiological measures obtained from animals at baseline and sacrifice

Parameter	Pacing cycle length	Baseline			Sacrifice		
		G628S	Pluronic	Saline	G628S	Pluronic	Saline
Echocardiography							
LVEF (%)		58 ± 3	57 ± 2	60 ± 4	50 ± 3*	35 ± 2	39 ± 7
LVIDd (cm)		3.7 ± 0.2	3.9 ± 0.2	3.7 ± 0.2	4.3 ± 0.3	4.4 ± 0.4	4.2 ± 0.2
LAEF (%)		65 ± 3	63 ± 4	72 ± 4	56 ± 7	44 ± 7	54 ± 8
LADd (cm)		3.1 ± 0.1	3.1 ± .2	2.9 ± 0.2	3.9 ± 0	4.2 ± 0.4	4.0 ± 0.5
RADd (cm)		3.6 ± 0.1	3.9 ± 0.1	3.5 ± 0.2	4.4 ± 0.2	4.5 ± 0.3	4.6 ± 0.3
ECG							
P-Dur (ms)		65 ± 1	68 ± 4	65 ± 4	81 ± 9	97 ± 5	84 ± 8
PR (ms)		101 ± 5	101 ± 3	104 ± 8	114 ± 11	125 ± 6	121 ± 12
QRS (ms)		60 ± 2	58 ± 3	60 ± 4	65 ± 4	64 ± 3	65 ± 3
QTc (ms)		422 ± 5	415 ± 9	422 ± 3	386 ± 5†	401 ± 12	400 ± 20
RR (ms)		487 ± 23	541 ± 20	556 ± 73	528 ± 75	478 ± 18	638 ± 82
EPS-MAPD90							
RV base (in ms)	400	231 ± 6	230 ± 11	230 ± 10	234 ± 16	243 ± 16	245 ± 16
	350	216 ± 5	216 ± 8	217 ± 9	219 ± 11	226 ± 9	231 ± 12
	300	201 ± 5	197 ± 6	201 ± 8	200 ± 9	206 ± 7	213 ± 10
RV apex (in ms)	250	179 ± 3	176 ± 4	176 ± 9	179 ± 8	185 ± 7	190 ± 9
	400	232 ± 8	218 ± 5	206 ± 9	223 ± 19	229 ± 8	233 ± 9
	350	218 ± 6	207 ± 4	195 ± 12	210 ± 17	213 ± 6	222 ± 5
	300	197 ± 5	192 ± 2	181 ± 11	195 ± 14	192 ± 2	204 ± 3
LV apex (in ms)	250	176 ± 4	173 ± 2	171 ± 9	175 ± 15	179 ± 5	184 ± 1
	400	243 ± 9	233 ± 4	222 ± 13	234 ± 21	240 ± 9	252 ± 7
	350	227 ± 7	218 ± 5	215 ± 14	219 ± 18	222 ± 6	237 ± 4
	300	208 ± 6	204 ± 3	198 ± 9	201 ± 15	203 ± 4	221 ± 3
LV base (in ms)	250	186 ± 5	182 ± 2	174 ± 7	183 ± 11	181 ± 3	198 ± 3
	400	233 ± 9	220 ± 12	230 ± 6	219 ± 11	218 ± 6	238 ± 13
	350	220 ± 7	213 ± 8	214 ± 16	208 ± 9	211 ± 4	226 ± 10
	300	204 ± 6	200 ± 6	193 ± 17	195 ± 8	196 ± 2	210 ± 6
LA avg (in ms)	250	184 ± 5	180 ± 5	176 ± 12	179 ± 5	179 ± 3	192 ± 7
	400	95 ± 5	97 ± 2	108 ± 7	126 ± 14	129 ± 7	128 ± 7
	350	98 ± 5	99 ± 3	108 ± 6	129 ± 14	130 ± 8	136 ± 11
	300	98 ± 5	97 ± 3	106 ± 4	123 ± 9	127 ± 7	133 ± 10
LA dispersion (in ms)	250	96 ± 5	96 ± 3	101 ± 4	118 ± 10	125 ± 8	126 ± 9
	400	12 ± 2	11 ± 1	9 ± 1	17 ± 4	13 ± 2	13 ± 3
	350	12 ± 2	11 ± 1	8 ± 1	15 ± 2	13 ± 2	14 ± 4
	300	10 ± 2	9 ± 1	10 ± 1	16 ± 4	12 ± 2	11 ± 3
RA avg (in ms)	250	10 ± 2	9 ± 1	13 ± 3	14 ± 5	11 ± 3	16 ± 5
	400	112 ± 6	114 ± 4	127 ± 10	168 ± 8	161 ± 7	179 ± 18
	350	112 ± 5	115 ± 3	126 ± 8	168 ± 7	160 ± 5	173 ± 13
	300	111 ± 5	113 ± 3	119 ± 8	163 ± 9	156 ± 4	166 ± 16
RA dispersion (in ms)	250	108 ± 4	109 ± 3	112 ± 7	158 ± 7	148 ± 5	156 ± 6
	400	13 ± 3	10 ± 1	15 ± 1	21 ± 2	23 ± 5	14 ± 1
	350	12 ± 2	10 ± 1	15 ± 2	20 ± 3	23 ± 7	17 ± 1
	300	10 ± 2	10 ± 2	14 ± 2	15 ± 3	25 ± 9	23 ± 7
	250	10 ± 2	9 ± 2	13 ± 2	18 ± 3	19 ± 1	12 ± 4

(Continued)

TABLE E2. Continued

Parameter	Pacing cycle length	Baseline			Sacrifice		
		G628S	Pluronic	Saline	G628S	Pluronic	Saline
Endo RA (in ms)	400	115 ± 10	111 ± 9	121 ± 13	152 ± 12	172 ± 13	176 ± 11
	350	118 ± 11	115 ± 7	124 ± 14	151 ± 11	174 ± 11	175 ± 10
	300	114 ± 6	116 ± 5	122 ± 12	149 ± 10	182 ± 7	163 ± 15
Conduction time (in ms)	250	116 ± 6	117 ± 7	112 ± 8	139 ± 6	176 ± 0	141 ± 1
	400	65 ± 3	64 ± 3	69 ± 3	91 ± 6	97 ± 4	76 ± 2
	350	64 ± 3	64 ± 3	68 ± 3	94 ± 6	99 ± 5	76 ± 4
	300	65 ± 3	66 ± 3	69 ± 3	93 ± 8	101 ± 5	79 ± 2
	250	64 ± 3	67 ± 3	70 ± 4	97 ± 8	112 ± 6	99 ± 12

All values are mean ± SD. *LVEF*, Left ventricular ejection fraction; *LVIDd*, left ventricular internal diameter at end-diastole; *LAEF*, left atrial ejection fraction; *LADd*, left atrial diameter at end-diastole; *RADd*, right atrial diameter at end-diastole; *ECG*, electrocardiography; *P-Dur*, p wave duration; *RR*, average time interval between QRS waves; *EPS*, electrophysiological studies; *MAPD90*, monophasic action potential duration to 90% repolarization; *RV*, right ventricular; *LV*, left ventricular; *LA*, left atrial; *RA*, right atrial; *Endo RA*, endocardial right atrial site. *Analysis of variance, $P = .045$ for between-group differences, post hoc Tukey test significant for G628S versus Pluronic. No other significant differences among the groups. †Specifically, $P = .65$ for between-group differences in QTc at sacrifice.

TABLE E3. Measured dissection time, effusion volumes, and organ weights at sacrifice

Parameter	G628S	Pluronic	Saline
Time to open chest (min)	53 ± 10	59 ± 6	53 ± 5
Ascites volume (mL)	162 ± 72	484 ± 366	90 ± 26
Pleural/pericardial effusion volume (mL)	248 ± 133*	253 ± 88†	273 ± 207
Heart weight (g)	258 ± 24	242 ± 16	254 ± 25
Left lung weight (g)	140 ± 9	177 ± 30	140 ± 9
Right lung weight (g)	186 ± 11	203 ± 19	187 ± 11
Liver weight (g)	1095 ± 108	1100 ± 118	1098 ± 69
Spleen weight (g)	54 ± 4	45 ± 5	54 ± 5
Right kidney weight (g)	71 ± 4	69 ± 5	75 ± 4
Left kidney weight (g)	73 ± 2	70 ± 7	76 ± 2
Pancreas weight (g)	71 ± 8	51 ± 6	71 ± 12

All values are mean ± SD. *Volume includes blood in animal with perforation. Excluding that animal, the value is 128 ± 53, with no significant difference among the groups. †Value does not include 1 animal found dead in which pericardial effusion had solidified and could not be reliably measured.