JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 75, NO. 17, 2020

KCNQ1 Antibodies for Immunotherapy of Long QT Syndrome Type 2

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ABSTRACT

BACKGROUND Patients with long QT syndrome (LQTS) are predisposed to life-threatening arrhythmias. A delay in cardiac repolarization is characteristic of the disease. Pharmacotherapy, implantable cardioverter-defibrillators, and left cardiac sympathetic denervation are part of the current treatment options, but no targeted therapy for LQTS exists to date. Previous studies indicate that induced autoimmunity against the voltage-gated KCNQ1 K^+ channels accelerates cardiac repolarization.

OBJECTIVES However, a causative relationship between KCNQ1 antibodies and the observed electrophysiological effects has never been demonstrated, and thus presents the aim of this study.

METHODS The authors purified KCNQ1 antibodies and performed whole-cell patch clamp experiments as well as single-channel recordings on Chinese hamster ovary cells overexpressing I_{Ks} channels. The effect of purified KCNQ1 antibodies on human cardiomyocytes derived from induced pluripotent stem cells was then studied.

RESULTS The study demonstrated that KCNQ1 antibodies underlie the previously observed increase in repolarizing I_{Ks} current. The antibodies shift the voltage dependence of activation and slow the deactivation of I_{Ks}. At the single-channel level, KCNQ1 antibodies increase the open time and probability of the channel. In models of LQTS type 2 (LQTS2) using human induced pluripotent stem cell-derived cardiomyocytes, KCNQ1 antibodies reverse the prolonged cardiac repolarization and abolish arrhythmic activities.

CONCLUSIONS Here, the authors provide the first direct evidence that KCNQ1 antibodies act as agonists on I_{Ks} channels. Moreover, KCNQ1 antibodies were able to restore alterations in cardiac repolarization and most importantly to suppress arrhythmias in LQTS2. KCNQ1 antibody therapy may thus present a novel promising therapeutic approach for LQTS2. (J Am Coll Cardiol 2020;75:2140–52) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The long QT syndrome (LQTS) is responsible for a significant proportion of sudden cardiac deaths (1). Genetic mutations leading to a loss of function of the cardiac voltage-gated KCNH2 (LQTS type 2 [LQTS2]) or KCNQ1 (LQTS type 1 [LQTS1]) K^+ channels are the most common causes (2). As a result, the corresponding repolarizing currents across the channels, I_{Kr} and I_{Ks}, respectively, are reduced, which prolongs the cardiac repolarization phase. On a surface electrocardiogram (ECG), this delay is reflected by a prolonged QT interval predisposing patients to life-threatening arrhythmias. Current treatment options for LQTS patients include beta-blockers, left cardiac sympathetic denervation, and the implantation of a cardioverter-defibrillator (3). Whereas the former therapies consist in dampening the adrenergic drive, the trigger of ventricular tachyarrhythmias (VT), the latter terminates

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Manuscript received December 2, 2019; revised manuscript received February 4, 2020, accepted February 25, 2020.

ISSN 0735-1097 https://doi.org/10.1016/j.jacc.2020.02.067

already-occurring VT. In the search for a tailored therapy for LQTS, the KCNQ1 channel has naturally drawn our attention, given its substantial role in cardiac repolarization. Consequently, a compound that activates KCNQ1 channels represents an optimal approach to restore normal repolarization. In a previous clinical study, autoantibodies targeting an extracellular pore domain of the KCNQ1 channel were detected in a subset of patients and were associated with a shorter QT interval (4). When immunizing rabbits against the same target peptide epitope of the KCNQ1 channel, a shortening of the QT interval and ventricular effective refractory period could be reproduced (5). On the cellular level, cardiomyocytes isolated from the immunized rabbits exhibited shorter action potential (AP) duration (APD), as well as increased repolarizing I_{Ks} current densities (5).

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Taken together, these findings indicate that the humoral immune response following KCNQ1 peptide immunization accelerates cardiac repolarization. However, although reasonable, a causative relationship between circulating KCNQ1 antibodies and the observed activation of I_{Ks} has not yet been established. Thus, in view of a therapeutic use for patients with a pathological delay in cardiac repolarization as in LQTS, the aim of the present study was to: 1) provide evidence for the KCNQ1 antibody-induced I_{Ks} activation; 2) explore the biophysical mechanisms by which KCNQ1 antibodies enhance KCNQ1 channel function; and 3) investigate the therapeutic potential of KCNQ1 antibodies in LQTS2 using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMC).

METHODS

KCNQ1 ANTIBODY PURIFICATION. New Zealand White rabbits were immunized against the extracellular peptide sequence of the KCNQ1 channel, as previously described, and anti-KCNQ1 IgG were affinity-purified (Davids Biotechnologie, Regensburg, Germany, and ProteoGenix, Schiltigheim, France) (5). Of note, the concentration (30 μg/ml) used for our in vitro experiments was based on the estimated average amount of circulating immunoglobulin G (IgG) found in immunized rabbits.

MAMMALIAN CELL CULTURE AND PATCH CLAMP RECORDINGS. Chinese hamster ovary (CHO) cells stably expressing the subunits KCNQ1/KCNE1 underlying human I_{Ks} were used. To study the interaction between KCNQ1 antibodies and the other major human cardiac ion channels, CHO ($Na_v1.5$, hERG) and human embryonic kidney (HEK) 293 cells ($Ca_v1.2$) stably expressing the respective channels were applied. More details on cell preparation methods are provided in the Supplemental Appendix. Macroscopic and single-channel currents were recorded at room temperature using an EPC-10 amplifier controlled by PATCHMASTER software version 2x90.5 (HEKA Elektronik, Lambrecht, Germany) on cells after 24 h of incubation with KCNQ1 antibodies at 2 different concentrations (30 μg/ml, 60 μg/ml) and compared with control conditions. A detailed Methods section regarding the patch clamp protocol is given in the Supplemental Appendix.

COMPUTER SIMULATIONS. We adopted the mathematical model proposed by Ten Tusscher-Noble-Noble-Panfilov to simulate the effects of KCNQ1 antibodies on the AP of human ventricular cardiomyocytes and on the QT in pseudo-ECGs (Supplemental Appendix).

hiPSC-CMC CELL CULTURE AND PATCH CLAMP RECORDINGS. Ventricular cardiomyocytes differentiated from hiPSCs (Pluricyte cardiomyocyte, Ncardia BV, Leiden, the Netherlands) were cultured according to the manufacturer's instructions. To induce a pharmacological LQTS2, hiPSC-CMCs were challenged with 25 nmol/l E-4031 (Alomone Labs, Jerusalem, Israel). In addition, hiPSC-CMCs derived from a patient with congenital LQTS2 (subsequently referred to as hiPSC-CMC-LQTS2) were used. Information on the donor patient's phenotype and his hiPSC reprogramming and validation can be found in the Supplemental Appendix. The amphotericin B–perforated patch method was used to record APs under current-clamp conditions ±24-h KCNQ1 antibodies. Details on the patch clamp protocol used are described in the Supplemental Appendix.

STATISTICAL ANALYSES. One-way analysis of variance was used to assess for differences between groups for normally distributed data, followed by Tukey's multiple comparisons test. For the comparison between 2 group means, 2-tailed Student's *t*-test was performed after the Shapiro-Wilk test asserted a normal distribution of the samples. The Mann-Whitney *U* test determined the statistical significance of non-normally distributed data. Analysis of categorical variables was carried out using chi-square contingency tables. A p value <0.05 was considered statistically significant. It should be noted that the p values presented in this report have not been adjusted for multiplicity, and therefore, inferences drawn from these statistical tests may not be reproducible. GraphPad Prism 7 software (GraphPad

ABBREVIATIONS AND ACRONYMS

AP = action potential

APD = action potential duration

CHO = Chinese hamster ovary

EAD = early afterdepolarization

ECG = electrocardiogram

hiPSC-CMC = human induced pluripotent stem cell-derived cardiomyocyte

IgG = immunoglobulin G

LQTS1 = long QT syndrome type 1

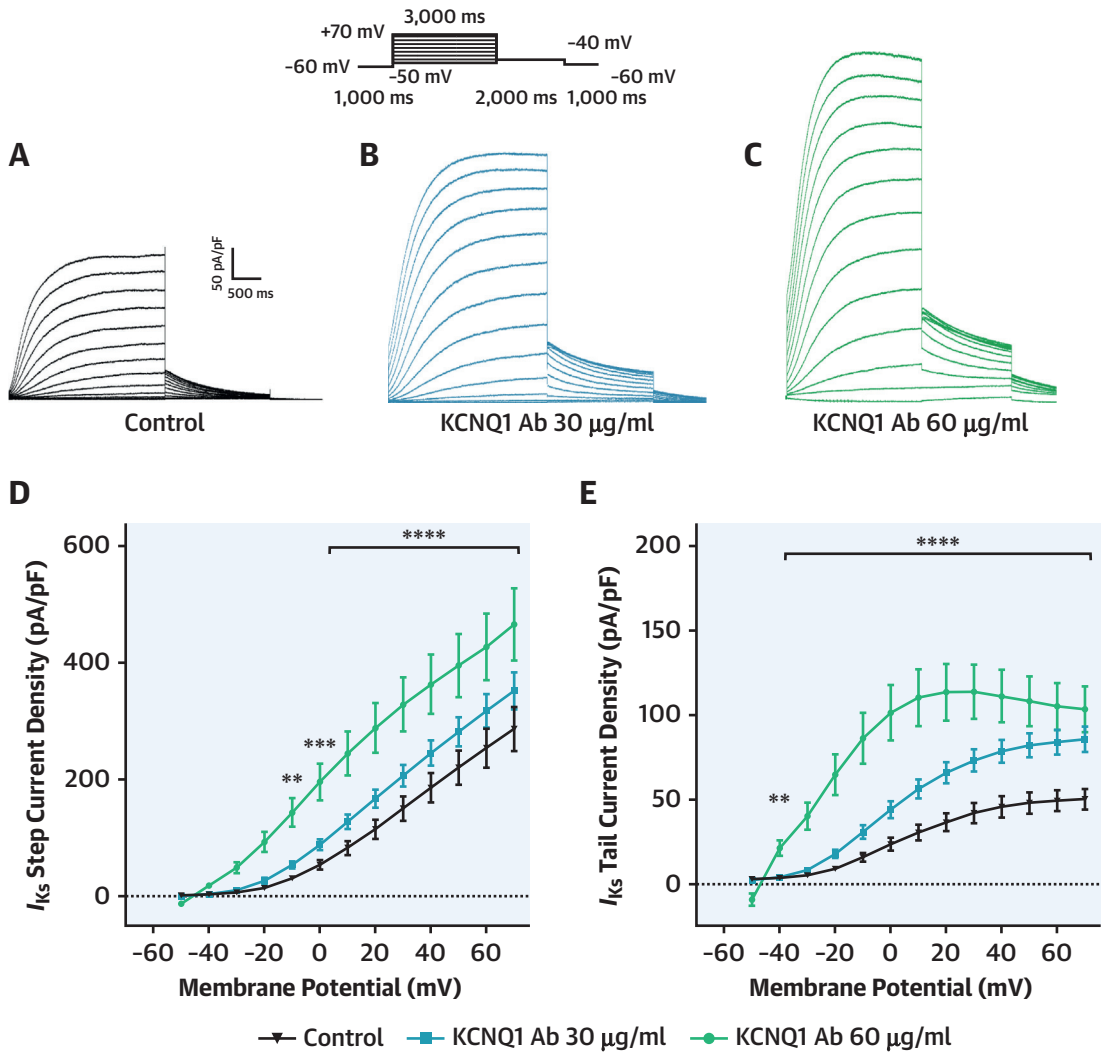
LQTS2 = long QT syndrome type 2

MDP = maximum diastolic potential

$V_{1/2}$ = half-maximal activation potential

VT = ventricular tachyarrhythmia

FIGURE 1 Effect of KCNQ1 Antibodies on I_{Ks}



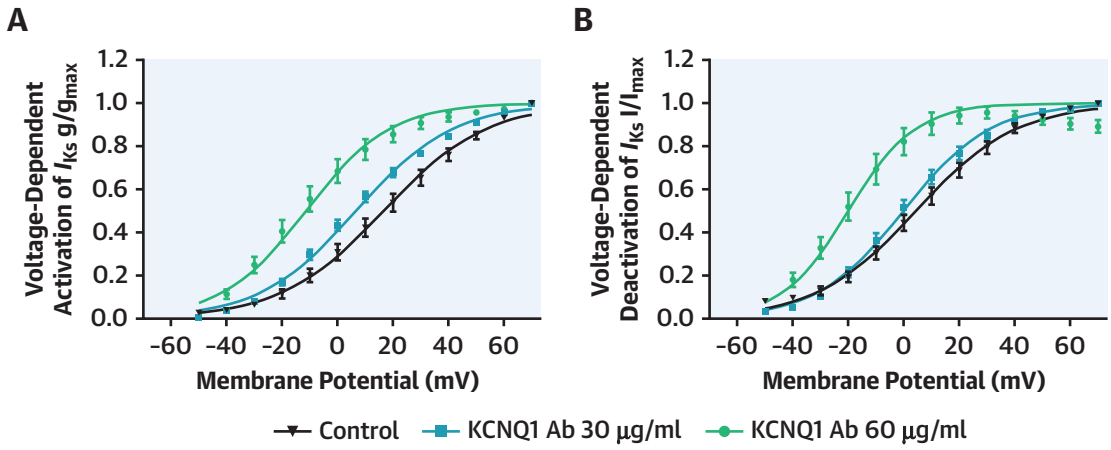
Representative I_{Ks} current traces recorded in Chinese hamster ovary cells under the control condition (**A**, cell capacitance 12.8 pF) and in the presence of 30 µg/ml (**B**, 14.7 pF) and 60 µg/ml (**C**, 17.4 pF) KCNQ1 antibodies. **(D and E)** Step and tail current densities as a function of the test potential. Indicated are mean ± SEM, comparing control cells (n = 19), and cells with 30 µg/ml (n = 23) and 60 µg/ml (n = 10) KCNQ1 antibodies. **p < 0.01; ***p < 0.001; ****p < 0.0001 comparing control versus KCNQ1 Ab 60 µg/ml. Ab = antibody.

Software, La Jolla, California) was used for statistical analyses.

RESULTS

KCNQ1 ANTIBODIES INCREASE I_{Ks} IN A CONCENTRATION-DEPENDENT MANNER. We measured the effect of KCNQ1 antibodies on activating I_{Ks} step currents under voltage-clamp conditions using 3-s depolarizing pulses applied in 10 mV incremental steps from −50 mV to +70 mV from a holding potential of −60 mV. After each test pulse, the membrane was repolarized to −40 mV to record the deactivating I_{Ks} tail currents. KCNQ1 antibodies significantly increased I_{Ks} (Figures 1A to 1C). At a concentration of 60 µg/ml, a 1.7-fold increase in mean I_{Ks} step current density at +70 mV (from 271.1 ± 35.1 pA/pF to 466.1 ± 61.7 pA/pF; p < 0.0001) could be observed, as well as a 2.3-fold increase in mean I_{Ks} tail current density after depolarization to +70 mV (from 45.9 ± 6.3 pA/pF to 103.6 ± 13.5 pA/pF; p < 0.0001). There were no statistical differences in cell capacitance

FIGURE 2 Effect of KCNQ1 Antibodies on the Biophysical Properties of I_{Ks} Channels



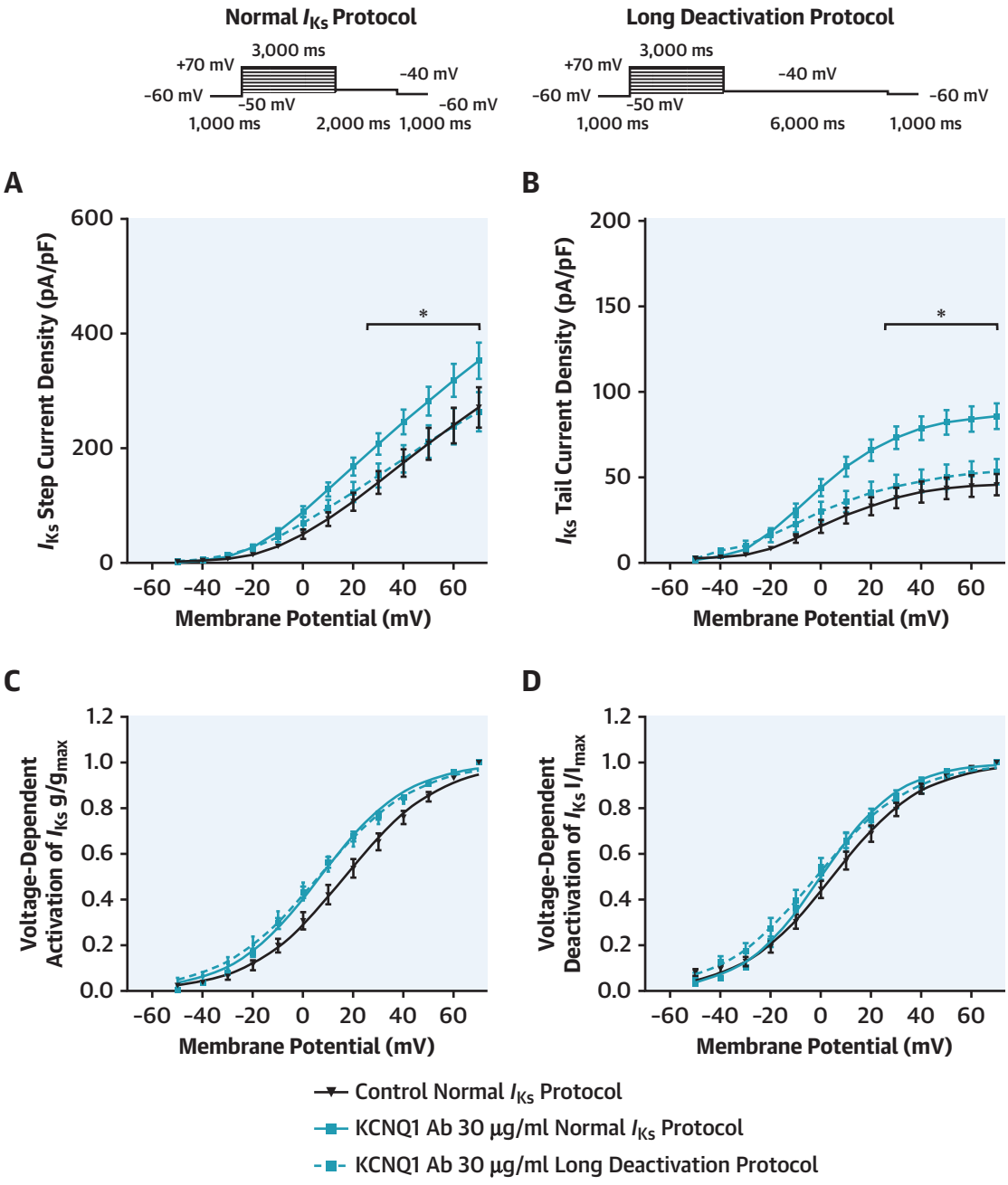
Indicated are mean ± SEM. **(A)** Voltage-dependent activation of I_{Ks}. Control n = 19 ($V_{1/2}$ = 16.7 ± 0.9 mV, slope factor k = 18.5 ± 0.8 mV), KCNQ1 Ab 30 µg/ml n = 23 ($V_{1/2}$ = 6.7 ± 0.5 mV, k = 17.4 ± 0.5 mV), KCNQ1 Ab 60 µg/ml n = 10 ($V_{1/2}$ = −11.4 ± 1.1 mV, k = 15.2 ± 1.0 mV). $p < 0.0001$ when comparing $V_{1/2}$ between control versus KCNQ1 Ab 30 µg/ml and 60 µg/ml. $p < 0.05$ when comparing the slope factor k of control cells versus KCNQ1 Ab 60 µg/ml. **(B)** Voltage-dependent deactivation of I_{Ks}. Control n = 19 ($V_{1/2}$ = 4.6 ± 0.8 mV, k = 18.1 ± 0.7 mV), KCNQ1 Ab 30 µg/ml n = 23 ($V_{1/2}$ = 0.1 ± 0.7 mV, k = 15.7 ± 0.6 mV), KCNQ1 Ab 60 µg/ml n = 10 ($V_{1/2}$ = −19.8 ± 1.3 mV, k = 12.0 ± 1.1 mV). $p < 0.0001$ when comparing $V_{1/2}$ between control versus KCNQ1 Ab 30 µg/ml and 60 µg/ml, respectively. $p < 0.05$ when comparing the slope factor k between control versus KCNQ1 Ab 30 µg/ml and 60 µg/ml, respectively. Ab = antibody.

between groups (Supplemental Figure 1). The slightly dampened I_{Ks} current densities of cells treated with KCNQ1 antibodies recorded at higher depolarizing potentials most likely result from reduced driving force for K^+ efflux due to extracellular K^+ accumulation. As shown on the representative I_{Ks} current traces in **Figures 1B and 1C**, a prominent instantaneous current component could be discerned in the presence of KCNQ1 antibodies (6). At the test potential of −50 mV, an inward current could be recorded in CHO cells treated with the 60 µg/ml KCNQ1 antibodies, suggesting an altered selectivity for ions other than K^+, a phenomenon not observed when 30 µg/ml KCNQ1 antibodies were applied (**Figures 1D and 1E**). Both the considerable instantaneous current component and the inward current at a potential above the reversal potential for K^+ suggest an accumulation of channels in the open state due to KCNQ1 antibodies.

KCNQ1 ANTIBODIES SHIFT THE VOLTAGE-DEPENDENCE OF ACTIVATION AND SLOW DEACTIVATION OF I_{Ks}. KCNQ1 antibodies (30 µg/ml) shifted the half-maximal activation potential ($V_{1/2}$) by −10 mV compared with control cells ($p < 0.0001$) without manifest effect on the slope factor k (**Figure 2A**). With 60 µg/ml KCNQ1 antibodies, I_{Ks} currents were activated at more negative potentials ($V_{1/2}$ = −11.4 ± 1.1 mV compared with control cells 16.7 ± 0.9 mV; $p < 0.0001$) and the voltage sensitivity was increased (k = 15.2 ± 1.0 mV with 60 µg/ml KCNQ1 antibodies vs. 18.5 ± 0.8 mV for control cells; $p < 0.05$). Similarly, a concentration of 60 µg/ml KCNQ1 antibodies shifted the voltage dependence of deactivation to more negative potentials by 24.4 mV ($p < 0.0001$) (**Figure 2B**). These results suggest that 1 mechanism of the I_{Ks} increase by KCNQ1 antibodies is the modulation of voltage sensitivity of the channel.

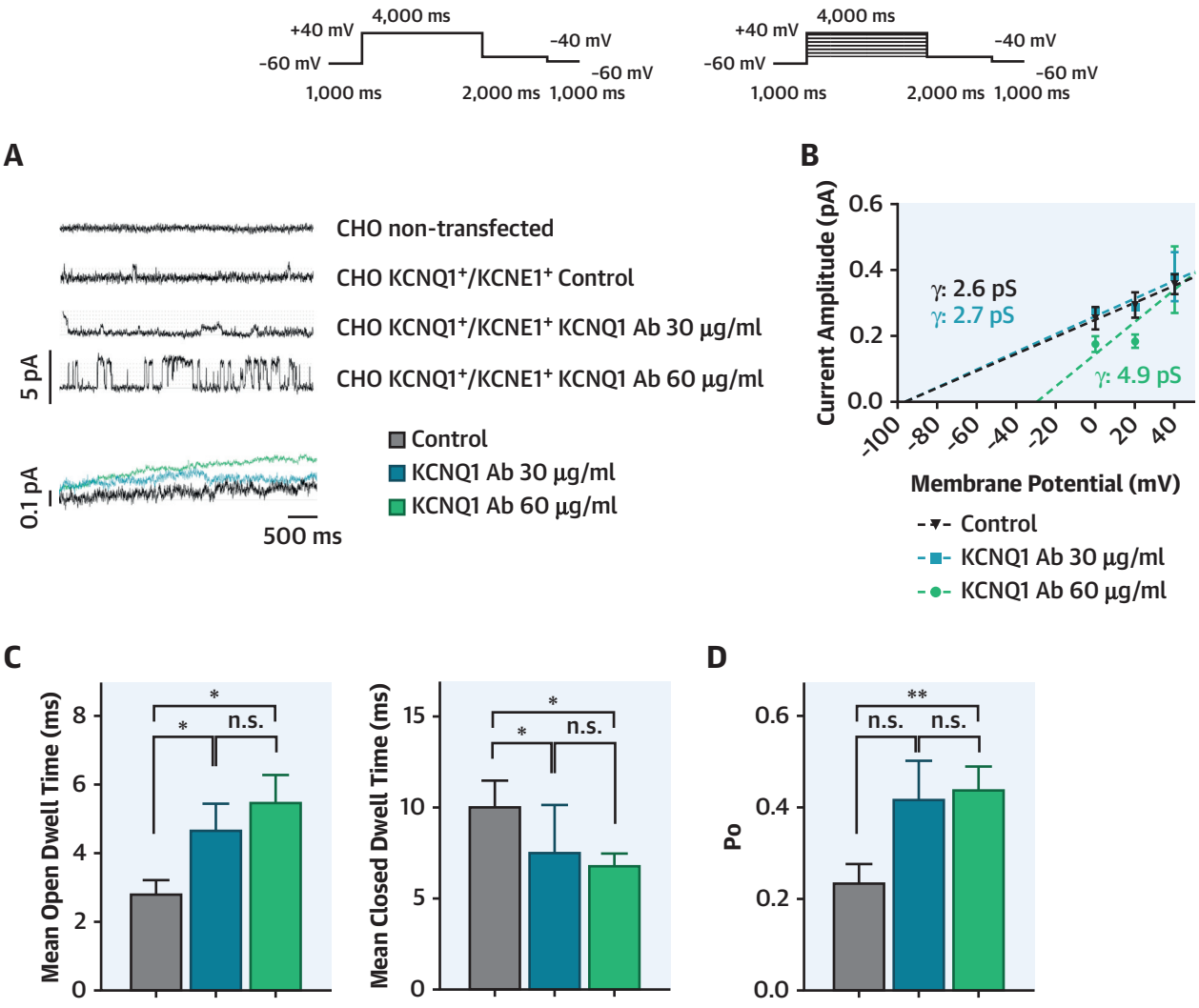
The time constants of I_{Ks} activation were best described by a biexponential function (Supplemental Figure 2A). Both fast (τ_{fast}) and slow (τ_{slow}) components of activation differed in the presence of KCNQ1 antibodies. A leftward shift could be distinguished in the voltage dependence of time constants, consistent with the $V_{1/2}$ shift of activation curves. When the voltage-dependent time constants were shifted by 10 mV to match peak time constants, both fast and slow components of activation become superimposed, thus indicating that KCNQ1 antibodies do not affect the I_{Ks} channel kinetics per se (Supplemental Figure 2B). The low I_{Ks} tail current amplitude made a reliable fit to estimate the deactivation time constants difficult (Supplemental Figure 2C). As the kinetics of deactivation could not be reliably discriminated, we estimated the channel availability at the end of the −40 mV pulse as the

FIGURE 3 Effect of KCNQ1 Antibodies on I_{Ks} Using a Protocol With a Prolonged Deactivation Phase



Represented are mean ± SEM. *p < 0.05 when comparing the normal versus long deactivation protocol. **(A and B)** Step and tail current densities as a function of the test potential. **(C)** Voltage-dependent activation of I_{Ks}. Control ($V_{1/2}$ = 16.7 ± 0.9 mV, slope factor k = 18.5 ± 0.8 mV), KCNQ1 Ab 30 μg/ml ($V_{1/2}$ = 6.7 ± 0.5 mV, k = 17.4 ± 0.5 mV) versus KCNQ1 Ab 30 μg/ml long deactivation protocol ($V_{1/2}$ = 6.3 ± 0.9 mV, k = 19.2 ± 0.8 mV). **(D)** Voltage-dependent deactivation of I_{Ks}. Control ($V_{1/2}$ = 4.6 ± 0.8 mV, k = 18.1 ± 0.7 mV), KCNQ1 Ab 30 μg/ml ($V_{1/2}$ = 0.1 ± 0.7 mV, k = 15.7 ± 0.6 mV) versus KCNQ1 Ab 30 μg/ml long deactivation protocol ($V_{1/2}$ = −1.8 ± 1.1 mV, k = 18.9 ± 1.0 mV). The long deactivation protocol did not affect the leftward $V_{1/2}$ shift occurring in the presence of 30 μg/ml KCNQ1 antibodies (p < 0.01 when comparing control versus KCNQ1 Ab with either pulse protocol). The slope factors were not significantly different. Ab = antibody.

FIGURE 4 Effect of KCNQ1 Antibodies on Single-Channel I_{Ks}



(A) Representative single-channel current traces from nontransfected Chinese hamster ovary (CHO) cells (tip resistance 18 MΩ) and CHO KCNQ1^{+}/KCNE1^{+} cells with 30 µg/ml KCNQ1 antibodies (20 MΩ, 2 active channels within the patch), 60 µg/ml (29 MΩ, 9 active channels within the patch) and without antibodies (27 MΩ, 6 active channels within the patch). Ensemble-averaged currents of successive recordings of representative cells under the control condition (31 sweeps) versus treatment with 30 µg/ml (40 sweeps) and 60 µg/ml KCNQ1 antibodies (46 sweeps). **(B)** The current amplitudes were plotted against pulse potentials between 0 mV and +40 mV in 20 mV incremental steps. The amplitude-voltage relationship resulted in an estimated slope conductance of 2.6 pS for control cells (n = 7), 2.7 pS and 4.9 pS for cells treated with KCNQ1 antibodies 30 µg/ml (n = 7) and 60 µg/ml (n = 10), respectively. **(C)** The **bars** represent the mean ± SEM open and closed dwell times (recorded at +40 mV) for control cells (n = 6) and cells treated with 30 µg/ml (n = 5) and 60 µg/ml KCNQ1 antibodies (n = 7). **(D)** Represented is the mean ± SEM open probability (recorded at +40 mV) in control cells (n = 6) compared with cells with 30 µg/ml (n = 5) and 60 µg/ml KCNQ1 antibodies (n = 7). *p < 0.05; **p < 0.01. Ab = antibody; Po = open probability.

ratio (amplitude at the end of the tail current)/(amplitude at the beginning of the tail current) (Supplemental Figure 3). The fractions were markedly increased in cells treated with 60 µg/ml KCNQ1 antibodies compared with control cells. Because the tail currents did not deactivate completely during the 2-s return potential of −40 mV, we modified the pulse protocol by prolonging the repolarizing pulse to 6 s (long deactivation protocol) to better estimate to what extent the slowing of deactivation is contributing to the increase of the I_{Ks} amplitude. When enough time was allowed for the I_{Ks} channels to deactivate completely, the ratio of nondeactivated I_{Ks} channels decreased accordingly (Supplemental Figure 3).

Interestingly, with the long deactivation protocol, the I_{Ks} current density increase measured with KCNQ1 antibodies (30 μg/ml) converges toward the control condition (**Figures 3A and 3B**), but the voltage-dependent activation and deactivation remained shifted (**Figures 3C and 3D**). The net effect is that KCNQ1 antibodies have a dual effect on I_{Ks} channels: they modulate the voltage sensitivity of the channel and markedly slow the deactivation. This latter process could lead to accumulation of activated channels and thus further contribute to the increased activation during the next test pulse. Our experiments with the higher IgG concentration (60 μg/ml) further support these findings (Supplemental Figure 4): whereas a prolongation of the deactivation phase allows the I_{Ks} current densities of KCNQ1 antibody-treated (30 μg/ml) cells to return to normal (**Figure 3A and 3B**), in the presence of 60 μg/ml KCNQ1 antibodies, not enough time is allowed for the channels to deactivate completely between pulses. Accordingly, the proportion of nondeactivated I_{Ks} channels with 60 μg/ml after 6 s of repolarization to −40 mV diminished, but without reaching values observed under control conditions (Supplemental Figure 3). Longer repolarizing pulses were avoided to minimize extracellular K^+ accumulation (7).

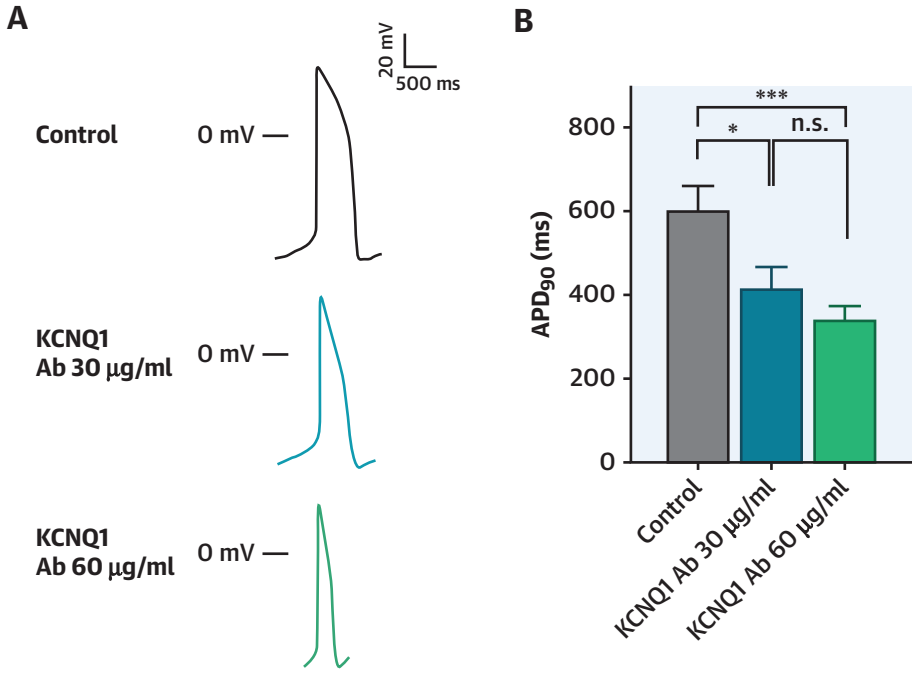
KCNQ1 ANTIBODIES INCREASE OPEN TIME AND PROBABILITY OF I_{Ks} CHANNELS. To investigate the molecular mechanisms of KCNQ1 antibodies on I_{Ks} gating, we performed single-channel recordings in CHO $KCNQ1^+/KCNE1^+$ cells comparing control conditions with cells exposed to KCNQ1 antibodies. Over the course of 50 sweeps at +40 mV for 4,000 ms, silent sweeps without channel activity prevailed in the recordings of control cells, supporting the previous observation that KCNQ1 channels open with a significant delay (8). However, when KCNQ1 antibodies were applied to the CHO cells, rapid flickering became apparent (**Figure 4A**). On average, the single-channel current at +40 mV was estimated at 0.36 ± 0.03 pA for the control cells, and 0.38 ± 0.07 pA and 0.34 ± 0.04 pA for CHO cells treated with 30 μg/ml and 60 μg/ml KCNQ1 antibodies, respectively. Non-transfected CHO cells lack any appreciable endogenous currents as shown in **Figure 4A**. From recordings at 3 different voltages, a linear fit yielded a value of 2.6 pS for the single-channel conductance under control conditions, which is in line with the published reports (8,9). The linear fit crosses the x-axis at the reversal potential expected for K^+-selective channels. Similar values were found in the presence of 30 μg/ml KCNQ1 antibodies, with a single-channel conductance of 2.7 pS. With 60 μg/ml KCNQ1 antibodies, the single-channel conductance was estimated at 4.9 pS (**Figure 4B**). Extrapolating to zero-current amplitude, the reversal potential was calculated at −30 mV, a value well above the equilibrium potential for K^+, indicating an altered ion selectivity in the presence of a high concentration of KCNQ1 antibodies. The mean open dwell time significantly rose from 2.9 ± 0.4 ms to 4.8 ± 0.7 ms and 5.6 ± 0.8 ms in the presence of 30 μg/ml and 60 μg/ml KCNQ1 antibodies, respectively ($p < 0.05$) (**Figure 4C**). At the same time, the mean closed dwell time decreased from 10.2 ± 1.4 ms to 7.6 ± 2.3 ms and 6.9 ± 0.6 ms in the presence of 30 μg/ml and 60 μg/ml KCNQ1 antibodies, respectively ($p < 0.05$) (**Figure 4C**). In addition, the open probability increased by 2-fold when cells were exposed to 60 μg/ml KCNQ1 antibodies ($p < 0.01$) (**Figure 4D**). Taken together, the single-channel kinetics are in accordance with the macroscopic I_{Ks} findings.

KCNQ1 ANTIBODIES DO NOT AFFECT KCNQ1 CHANNEL EXPRESSION. Because a change in KCNQ1 channel expression may influence the amplitude of I_{Ks}, we performed qPCR and Western blot experiments to quantify the channels comparing cells in the presence and absence of KCNQ1 antibodies. Incubation of CHO cells with KCNQ1 antibodies did not affect the relative expression of KCNQ1 mRNA nor the channel protein amount (Supplemental Figure 5).

KCNQ1 ANTIBODIES ARE SPECIFIC TO I_{Ks} CHANNELS. To address the question of off-target effects of KCNQ1 antibodies, we performed patch clamp experiments on mammalian cell lines stably expressing the other human cardiac voltage-gated ion channels that essentially shape the cardiac action potential, that is, CHO $Na_v1.5$, HEK 293 $Ca_v1.2$, and CHO hERG. We observed no changes in biophysical properties of the $Na_v1.5$, $Ca_v1.2$, and hERG channels in the presence of KCNQ1 antibodies (Supplemental Figure 6).

KCNQ1 ANTIBODIES SHORTEN APD AND THE QT INTERVAL IN AN IN SILICO MODEL OF HUMAN CARDIOMYOCYTE LQTS2. The effects of the shift of the steady-state activation relationship of I_{Ks} were evaluated in computer simulations using the Ten Tusscher-Noble-Noble-Panfilov human ventricular cell model. Supplemental Figure 7 shows the effects of shifting this relationship by −10 mV, corresponding to the shift observed in patch clamp experiments with 30 μg/ml KCNQ1 antibody. This shift shortened the AP in all simulated genotypes (wild-type, heterozygous, and homozygous LQTS2) and in all 3 ventricular cell types (Supplemental Figure 7A). Importantly, in the

FIGURE 5 Effect of KCNQ1 Antibodies on hiPSC-CMC

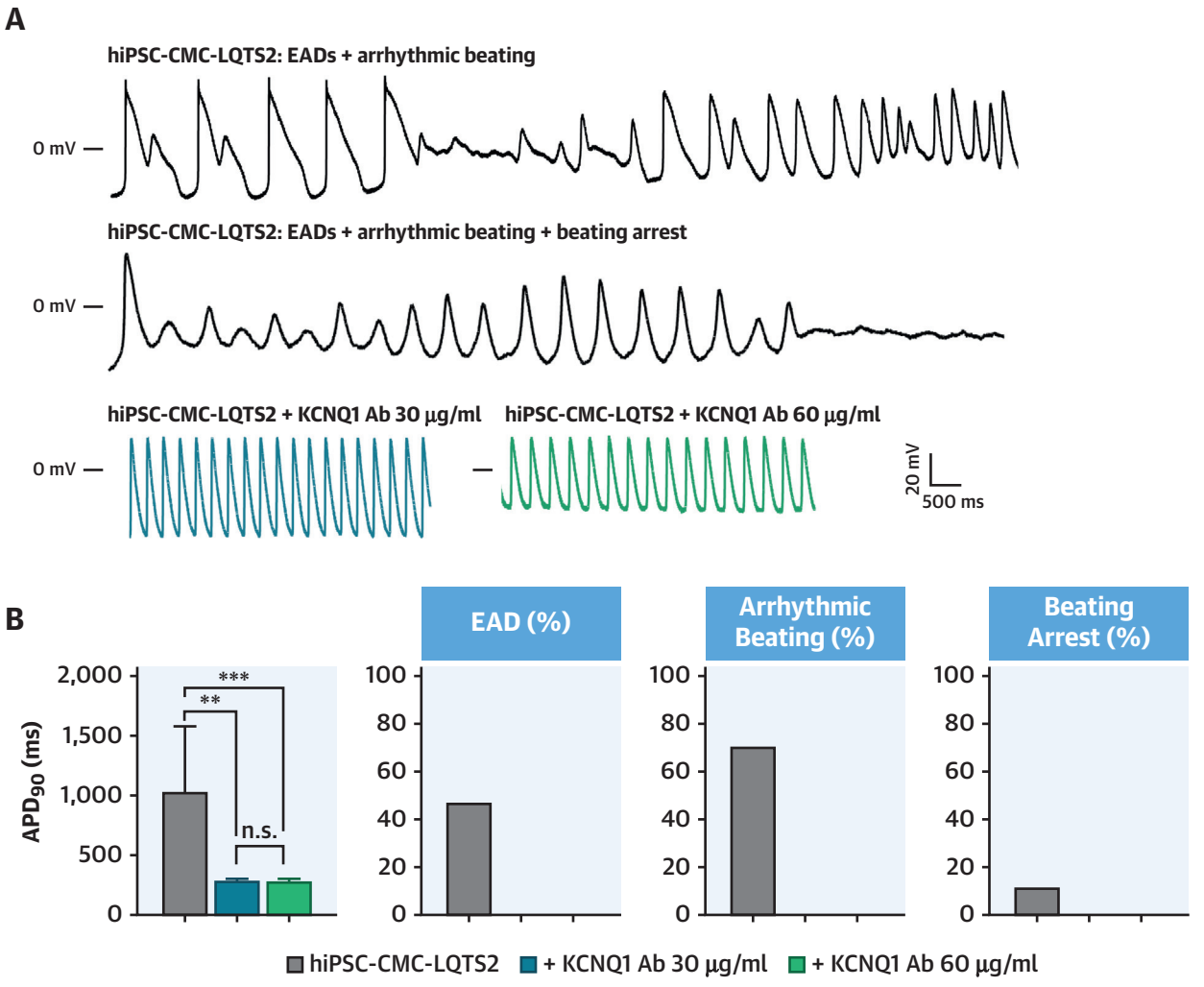


(A) Representative action potentials recorded at 37°C from spontaneously beating cardiomyocytes with (30 µg/ml and 60 µg/ml) and without KCNQ1 antibodies. **(B)** The **bars** represent the mean ± SEM APD_{90} of control cells (n = 10) and cardiomyocytes treated with 30 µg/ml (n = 8) and 60 µg/ml (n = 12) KCNQ1 antibodies. *p < 0.05; ***p < 0.001. Ab = antibody; APD = action potential duration; hiPSC-CMC = human induced pluripotent stem cell-derived cardiomyocyte; n.s. = not significant.

epicardial and endocardial cell models at all basic cycle lengths, the APD shortening compensated almost fully the APD prolongation induced by the simulated heterozygous LQTS2 (Supplemental Figure 7B). These effects were more prominent with a shift of the steady-state I_{Ks} activation relationship by −28.1 mV, corresponding to 60 µg/ml antibody concentration (Supplemental Figure 8). In particular, this shift compensated the APD prolongation caused by the simulated homozygous LQTS2 in epicardial and endocardial cells and the APD prolongation caused by the simulated heterozygous LQTS2 in mid-myocardial cells. The effects of shifting the steady-state activation relationship of I_{Ks} by −10 mV are further detailed in Supplemental Figure 7C, in which the time courses of I_{Ks} and I_{Kr} are shown jointly with the APs of an epicardial cell paced at a basic cycle length of 1,000 ms. The reduction of I_{Kr} in the heterozygous and homozygous LQTS2 genotypes induced an increase of I_{Ks} (repolarization reserve), which was augmented by the simulated effect of the KCNQ1 antibodies, contributing to the normalization of APD. This effect was considerably more prominent with a shift by −28.1 mV (Supplemental Figure 8). In a transmural model of conduction, shifting the activation relationship of I_{Ks} to more negative potentials shortened the QT interval in pseudo-ECGs for all 3 genotypes (Supplemental Figure 7D). Of note, the shift by −28.1 mV almost fully normalized the QT interval in the simulated heterozygous LQTS2 genotype. These results, therefore, support the notion that by its effect on I_{Ks}, KCNQ1 antibodies may compensate APD and thus QT-interval prolongation in patients afflicted by LQTS2.

KCNQ1 ANTIBODIES SHORTEN APD IN IN VITRO MODELS OF HUMAN CARDIOMYOCYTE LQTS2. With this hypothesis in mind, we next tested whether KCNQ1 antibodies accelerate cardiac repolarization in hiPSC-CMCs. As shown in **Figure 5**, KCNQ1 antibodies caused a concentration-dependent shortening of the APD determined at 90% repolarization (APD_{90}) at 37°C ($p < 0.001$) (Supplemental Table 1). In conjunction with the experimental conditions of CHO cells, patch clamp recordings were also carried out at room temperature. At temperatures below the physiological level, the spontaneous APs of hiPSC-CMCs are

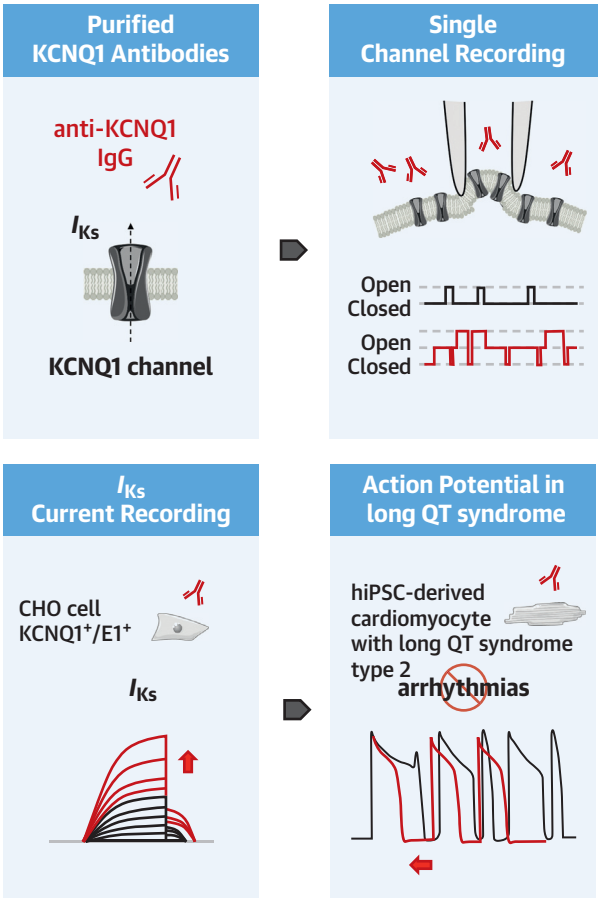
FIGURE 6 Effect of KCNQ1 Antibodies on hiPSC-CMC-LQTS2



All measurements were performed at 37°C. **(A)** Representative AP traces recorded in hiPSC-CMC-LQTS2 showing prolonged APDs with EADs, degenerating into arrhythmic beating and beating arrest. In the presence of KCNQ1 Ab 30 µg/ml and 60 µg/ml, hiPSC-CMC-LQTS2 cells have shorter APDs and develop no arrhythmia. **(B)** In the **far left panel**, the **bars** represent the mean ± SEM APD_{90} of hiPSC-CMC-LQTS2 (n = 9), treated with 30 µg/ml (n = 9) and 60 µg/ml (n = 9) KCNQ1 antibodies. **p < 0.01; ***p < 0.001. In the **right panels**, the **bars** represent the incidence of EADs, arrhythmic beating, and beating arrest in hiPSC-CMC-LQTS2 (n = 17) and with 30 µg/ml (n = 9) versus 60 µg/ml (n = 9) KCNQ1 antibodies, respectively. Ab = antibody; APD = action potential duration; EAD = early afterdepolarization; hiPSC-CMC-LQTS2 = human induced pluripotent stem cell-derived cardiomyocyte from a patient with congenital LQTS type 2; other abbreviations as in Figure 5.

necessarily prolonged. Nevertheless, the KCNQ1 antibodies significantly shortened the APD_{90} by 21% at 30 µg/ml and by 40% at 60 µg/ml concentration, respectively (Supplemental Figure 9, Supplemental Table 2). E-4031, a selective KCNH2 K^+ channel blocker, was used to create a pharmacological model of LQTS2. The application of E-4031 caused a 4-fold increase in APD_{90} and induced APD alternans as well as early afterdepolarizations (EADs) (Supplemental Figure 10). KCNQ1 antibodies at both concentrations shortened the APD and significantly reduced the incidence of arrhythmic events ($p < 0.05$) (Supplemental Figure 10, Supplemental Table 3). Next, we studied the effect of KCNQ1 antibodies on congenital LQTS2 using hiPSC-CMCs from a patient with an A561P mutation in the KCNH2 gene. HiPSC-CMC-LQTS2 presented markedly prolonged APs (mean APD_{90} 1,033 ± 547.4 ms) and developed frequent arrhythmic events in terms of EADs, arrhythmic beating and beating arrest (**Figure 6**).

CENTRAL ILLUSTRATION **KCNQ1 Antibodies Activate I_{Ks} Channels**



Maguy, A. et al. J Am Coll Cardiol. 2020;75(17):2140–52.

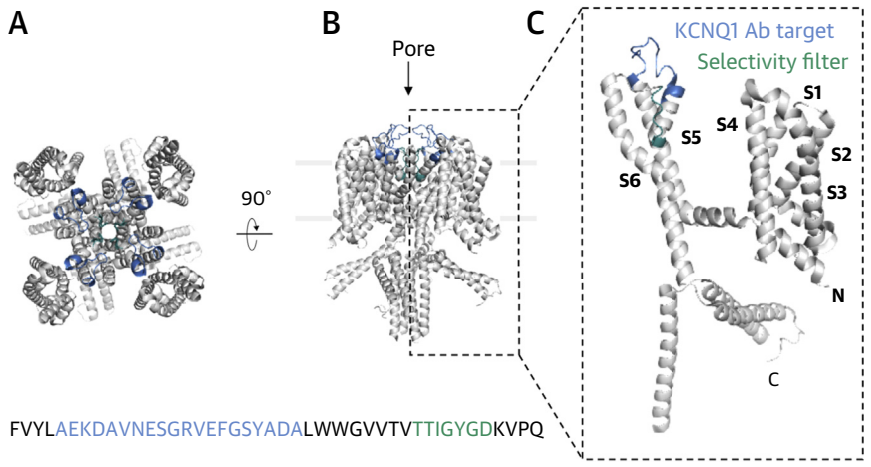
KCNQ1 antibodies activate I_{Ks} channels to shorten action potential duration and to suppress arrhythmias in a cellular model of long QT syndrome type 2.

Treatment with KCNQ1 antibodies completely reversed the pathological and proarrhythmogenic AP lengthening of LQTS2 (mean APD_{90} 289.7 ± 12.9 ms and 284.2 ± 17.2 ms with 30 and 60 μg/ml, respectively; $p < 0.001$) (Supplemental Table 4) and abolished all arrhythmic activities (**Figure 6**). Of note, in the presence of 60 μg/ml KCNQ1 antibodies, we found a less negative maximum diastolic potential (MDP) of hiPSC-CMCs and hiPSC-CMC-LQTS2 (Supplemental Tables 1 and 4). This depolarization of the MDP is possibly driven by a net inward current induced by KCNQ1 antibodies at the higher concentration, consistent with the inward current recorded in CHO cells at 60 μg/ml, but not 30 μg/ml, antibody concentration at the test potential of −50 mV. As KCNQ1 antibody-induced I_{Ks} activation shortens APD, the beating rate of cardiomyocytes necessarily increased and explains our observation of higher frequencies when exposed to KCNQ1 antibodies (Supplemental Tables 1 to 4)

DISCUSSION

Patients with LQTS are predisposed to VT and sudden cardiac death (1). Current treatment options for LQTS include beta-blockers, the implantation of a cardioverter-defibrillator and the left cardiac sympathetic denervation (3). However, there is a substantial interest and well-recognized need for developing specific drugs able to activate the repolarizing currents in order to normalize the abnormally prolonged repolarization of the heart in LQTS. This is particularly true for LQTS patients who enter periods of electrical storms resistant to standard therapy with

FIGURE 7 Model of the KCNQ1 Channel Tetramer (PDB: 5VMS)



(A) Top view of the I_{Ks} channel. **(B)** Side view of the I_{Ks} channel. **(C)** Model of 1 subunit of the I_{Ks} channel, the transmembrane segments S1 to S6 are labeled (PyMol Molecular Graphics System, v2.0.7 Schrödinger, New York, New York). The targeted peptide sequence **(blue)** and selectivity filter **(green)** are highlighted. Ab = antibody.

repeated defibrillation shocks and increased mortality (2). Moreover, patients with the type 2 form of LQTS respond less well to conventional treatment than LQTS1 individuals (10–15). In the present study, we provided the first proof of concept that purified KCNQ1 antibodies act as agonists on the I_{Ks} channel (**Central Illustration**). We found a concentration-dependent response: doubling the KCNQ1 antibody concentration increased the I_{Ks} current density by 2-fold in CHO $KCNQ1^{+}/KCNE1^{+}$ cells. Patch clamp recordings disclosed a dual effect of KCNQ1 antibodies: a negative shift in voltage sensitivity and a marked slowing of I_{Ks} channel deactivation. To better understand the mechanisms underlying the I_{Ks} increase, we performed single-channel recordings. At +40 mV, I_{Ks} activation is near maximal in both conditions (control vs. KCNQ1 antibodies). We therefore chose to study single-channel behavior at this particular potential, thereby reducing the impact of the shift in activation. In the presence of 60 μg/ml KCNQ1 antibodies, we found that the observed gain of function of KCNQ1 channels results mainly from an increased mean open dwell time as well as open probability, in addition to the shift in activation. The contribution of the single-channel conductance to the I_{Ks} increase is less evident, because it is essentially the consequence of a shift in reversal potential reflecting a change in ion selectivity. In line with this observation, an inward current could be recorded at the whole-cell level at a potential below the shifted reversal potential in the presence of 60 μg/ml KCNQ1 antibodies. As illustrated in **Figure 7**, the peptide sequence targeted by the KCNQ1 antibodies is located only 8 amino acids away from the selectivity filter of the I_{Ks} channel (TTIGYGD motif), leading us to reasonably speculate that KCNQ1 antibodies can alter ion selectivity when present in high concentrations, potentially due to steric hindrance (16). When reducing the dose of KCNQ1 antibodies down to 30 μg/ml, the ion selectivity remained unaffected (equilibrium potential within normal range for K^{+}). With 30 μg/ml KCNQ1 antibodies, the longer mean open dwell time is the predominant mechanism underlying the observed I_{Ks} increase, whereas the open probability was on average higher compared with control cells. At a concentration of 30 μg/ml, KCNQ1 antibodies do not affect single-channel conductance.

Next, we used a mathematical model to predict the effect of KCNQ1 antibodies on human ventricular APs, thereby simulating 3 different genotypes (wild-type, heterozygous, and homozygous LQTS2). Because LQTS1 is due to a mutation in the KCNQ1 gene, it seems unlikely that antibodies targeting these defective channels would increase I_{Ks}. Our investigations, therefore, focused on LQTS2 that are more likely to benefit from KCNQ1 antibodies by compensating for the loss of the I_{Kr} repolarizing current through I_{Ks} up-regulation. In our simulations, incorporating the shift in steady-state I_{Ks} activation induced by the KCNQ1 antibodies shortened the APD

and, most importantly, normalized APD in simulated LQTS2. Moreover, the shift in steady-state I_{Ks} activation normalized the QT interval of pseudo-ECGs in a transmural conduction model of heterozygous LQTS2. The shift of the steady-state activation function of I_{Ks} was sufficient to produce these effects via enhancement of I_{Ks}. In line with these in silico findings, we then studied the therapeutic potential of KCNQ1 antibodies both in a pharmacological and congenital model of LQTS2 using hiPSC-CMCs. As expected, the APD was significantly prolonged in both experimental LQTS2 models. In addition, we observed frequent arrhythmic activities of the cardiomyocytes, well-known precursors of life-threatening VT in LQTS patients. Treatment with KCNQ1 antibodies at 30 μg/ml was sufficient to restore electrical stability in LQTS2. KCNQ1 antibodies normalized the APD, indicating that KCNQ1 antibodies are able to compensate for the prolonged repolarization caused by I_{Kr} inhibition (enhanced repolarization reserve). Moreover, KCNQ1 antibodies completely suppressed spontaneous arrhythmias. Of note, the aforementioned concern of depolarized MDP is only observed with the higher concentration of KCNQ1 antibodies. The altered ion selectivity presumed with 60 μg/ml KCNQ1 antibodies is reasonably the underlying cause for the depolarization of the MDP. Because hiPSC-CMCs have an inherent deficiency of the inward rectifier K^+ current I_{K1}, an increase of nonselective ion conductance as with 60 μg/ml KCNQ1 antibodies would result in unopposed depolarization. With our dynamic clamp system, electronic I_{K1} expression established a physiological resting membrane potential to recapitulate human mature ventricular cardiomyocytes. In this physiological context, I_{K1} functionally counterbalances the changed ion selectivity with 60 μg/ml KCNQ1 antibodies (Supplemental Table 5). This is an important observation, because in view of a therapeutic application, even at the higher concentration, the intrinsic I_{K1} current of mature cardiomyocytes (as in patients) can compensate for possible altered ion selectivity.

STUDY LIMITATIONS. In current-clamp experiments, we were not able to pace the hiPSC-CMCs due to technical limitations. The maximum stimulus the EPC-10 amplifier can apply (1 nA/10 ms) is below the diastolic current threshold for hiPSC-CMCs. The purified antibodies were polyclonal and not monoclonal immunoglobulins. However, in view of the development of a therapeutic vaccine for the LQTS, our intent was to prove that the polyclonal antibody population resulting from immunization is effective as I_{Ks} activator. Future studies on animal models will be important to investigate the effect on the whole-heart level. We do not completely rule out a potential heart rate acceleration by the KCNQ1 antibodies in patients with LQTS. Moreover, clinical trials will be needed to determine the kinetics of antibody response in humans and to formulate vaccination protocols to induce and maintain a therapeutic antibody level.

CONCLUSIONS

Taken together, this is the first proof-of-concept study providing evidence that KCNQ1 antibodies are potent I_{Ks} activators. We elucidated the mechanisms of action of KCNQ1 antibodies on the single-channel level. Moreover, we demonstrated the therapeutic potential of KCNQ1 antibodies by enhancing repolarization reserve and restoring electrical stability in LQTS2. In contrast to previous studies on antibodies with almost exclusively inhibitory effects on channels, the KCNQ1 antibody is unique in its kind because it induces a gain-of-function of the KCNQ1 channel. KCNQ1 antibody therapy thus presents an innovative, unprecedented treatment approach for patients with LQTS (17).

ACKNOWLEDGMENTS The authors thank Dr. Gildas Loussouarn (l'Institut du Thorax, Nantes, France) for providing the dynamic clamp system. The authors also thank PD Dr. Jean-Sébastien Rougier for sharing his expertise in sodium and calcium currents.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: KCNQ1 antibodies activate I_{Ks} channels, accelerate ventricular repolarization, and suppress arrhythmias in an in vitro cellular model of long QT syndrome.

TRANSLATIONAL OUTLOOK: Clinical studies are now needed to evaluate the efficacy and safety of KCNQ1 antibodies in patients with long QT syndrome type 2.

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KEY WORDS cardiac arrhythmias, I_{Ks}, immunotherapy, KCNQ1 antibody, long QT syndrome

APPENDIX For an expanded Methods section, and supplemental figures and tables, please see the online version of this paper.