Differential Effects of Remdesivir and Lumacaftor on Homomorphic and Heteromorphic hERG Channels

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ABSTRACT

The human ether-a-go-go-related gene (hERG) encodes for the pore-forming subunit of the channel that conducts the rapidly activating delayed K+ current (Ito) in the heart. The hERG channel is important for cardiac repolarization, and reduction of its expression in the plasma membrane due to mutations causes long QT syndrome type 2 (LQT2). As such, promoting hERG membrane expression is a strategy to rescue mutant channel function. In the present study, we applied patch clamp, western blots, immunocytochemistry, and quantitative reverse transcription polymerase chain reaction techniques to investigate the rescue effects of two drugs, remdesivir and lumacaftor, on trafficking-defective mutant hERG channels. As our group has recently reported that the antiviral drug remdesivir increases wild-type (WT) hERG current and surface expression, we studied the effects of remdesivir on trafficking-defective LQT2-causing hERG mutants G601S and R582C expressed in HEK293 cells. We also investigated the effects of lumacaftor, a drug used to treat cystic fibrosis, that promotes CFTR protein trafficking and has been shown to rescue membrane expression of some hERG mutations. Our results show that neither remdesivir nor lumacaftor rescued the current or cell-surface expression of homomorphic mutants G601S and R582C. However, remdesivir decreased while lumacaftor increased the current and cell-surface expression of heteromeric channels formed by WT hERG and mutant G601S or R582C hERG. We concluded that drugs can differentially affect homomorphic WT and heteromeric WT+G601S (or WT+R582C) hERG channels. These findings extend our understanding of drug-channel interaction and may have clinical implications for patients with hERG mutations.

SIGNIFICANCE STATEMENT

Various naturally occurring mutations in a cardiac potassium channel called hERG can impair channel function by decreasing cell-surface channel expression, resulting in cardiac electrical disturbances and even sudden cardiac death. Promotion of cell-surface expression of mutant hERG channels represents a strategy to rescue channel function. This work demonstrates that drugs such as remdesivir and lumacaftor can differently affect homomorphic and heteromorphic mutant hERG channels, which have biological and clinical implications.

Introduction

The heartbeat and contraction are controlled through coordinated activity of ion channels (Keating and Sanguinetti, 2001). As such, cardiac ion channel dysfunction can cause cardiac arrhythmias and sudden cardiac death (Keating and Sanguinetti, 2001). One of these ion channels is human ether-a-go-go related gene (hERG)-encoded channel, Kv11.1 (commonly known as hERG) (Sanguinetti et al., 1995; Trudeau et al., 1995). hERG conducts the rapidly activating delayed rectifier potassium current (Ito) in cardiomyocytes, which plays a crucial role in cardiac action potential repolarization (Sanguinetti et al., 1995). On an electrocardiogram, the ventricular action potential duration is reflected by the QT interval. Hundreds of mutations in hERG have been identified in humans (Anderson et al., 2014). A reduction in hERG function due to inherited loss-of-function mutations delays cardiac repolarization and prolongs the QT interval, leading to congenital long QT syndrome type 2 (LQT2), a cardiac ion channelopathy that predisposes affected individuals to the ventricular arrhythmia, Torsade de Pointes, and sudden death (Curran et al., 1995). Broadly, hERG loss-of-function mutations are placed into classes based on the mechanism of dysfunction: impaired hERG synthesis, trafficking, gating, or permeability (Zhou et al., 1998a; Anderson et al., 2006, 2014). After a large-scale analysis of LQT2-linked missense mutations, it was found that 88% exhibited a trafficking-deficient mechanism, suggesting this as the primary mechanism of LQT2 (Anderson et al., 2014). Currently, treatment of LQT2 involves reducing sympathetic stimulation to the heart using β-blockers, an implantable

ABBREVIATIONS: ΩC, control; ER, endoplasmic reticulum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HA, hemagglutinin; HEK, human embryonic kidney; hERG, human ether-a-go-go-related gene; hKv11.1, hERG current; Ito, rapidly activating delayed rectifier potassium current; iPSC-CM, induced pluripotent stem cell-derived cardiomyocytes; LQT2, long QT syndrome type 2; LUM, lumacaftor; PCR, polymerase chain reaction; PERK, protein kinase-like ER kinase; RDV, remdesivir; RT-qPCR, quantitative reverse transcription polymerase chain reaction; UPR, unfolded protein response; WGA, wheat germ agglutinin; WT, wild type.
cardioverter-defibrillator, or left cardiac sympathetic denervation (Schwartz et al., 2012). However, these options do not correct the pathologic problem underlying LQT2—defective hERG. As impaired trafficking represents the major LQT2 mechanism, measures to enhance trafficking of mutant hERG represent a valuable area of study. Interestingly, culturing trafficking-defective variants with hERG channel blockers, like E-4031, or at reduced temperature (27°C) can correct trafficking defects (Anderson et al., 2014). Yet, clinical application of these strategies is limited as altering internal body temperature is not feasible, and E-4031 blocks the hERG channel, thereby inhibiting hERG function. As such, there is an unmet need for practical, targeted LQT2 therapy options.

Previously, our group found that remdesivir (RDV), an antiviral drug that gained popularity amid the COVID-19 pandemic, can increase wild-type (WT) hERG surface expression and hERG ral drug that gained popularity amid the COVID-19 pandemic, targeted LQT2 therapy options. hERG function. As such, there is an unmet need for practical, soluble, and E-4031 blocks the hERG channel, thereby inhibiting hERG function. As such, there is an unmet need for practical, targeted LQT2 therapy options.

Materials and Methods

Molecular Biology. WT hERG cDNA was provided by Dr. Gail Robertson (University of Wisconsin-Madison, Madison, WI); hERG mutants R582C, G601S, S624T, N629D, and 42-354 hERG (N-terminal deletion) were constructed using polymerase chain reaction (PCR) overlap extension technique as described previously (Guo et al., 2006; Massuelli et al., 2010). GFP-tagged (to C-terminal) hERG cDNA (Gong et al., 2002) was provided by Dr. Zhengfeng Zhou (Oregon Health and Science University). For the HA-tagged G601S hERG construct, the hemagglutinin (HA)-epitope tag of the sequence 436TEEGPPAT (boldface, insertion; italicized, HA epitope) was inserted into the S1-S2 loop of the G601S mutant hERG construct, thereby inhibiting hERG function. As such, there is an unmet need for practical, targeted LQT2 therapy options.

Each sample was comprised of 15 μg of protein diluted to 40 μL with double-distilled water and 10 μL of 5× Laemmli loading buffer containing 5% β-mercaptoethanol. After boiling, samples were loaded and separated on 8% SDS polyacrylamide electrophoresis gels at 110 V for 2 hours. They were transferred overnight onto polyvinylidene difluoride membranes at 30 V, and membranes were blocked with 5% nonfat milk for 1 hour. The membranes were probed with appropriate primary antibodies (in 5% nonfat milk) for 1.5 hours and then incubated with horseradish peroxidase–conjugated secondary antibodies for 1 hour. Following each period of antibody probing, membranes were washed three times for 5 minutes with 0.1% Tween 20 in Tris-buffered saline. An enhanced chemiluminescence detection kit (Cytiva Life Sciences, Marlborough, MA) was used to visualize and detect protein signals on X-ray film (Fuji, Minato, Tokyo, Japan). Actin was used as a loading control, and molecular masses of proteins were determined using BLUeye prestained protein ladder (GeneDireX, Taiwan). Image Laboratory (Bio-Rad) was used to quantify band intensities, normalized to their respective actin intensities, and then measured relative to their corresponding control.

Electrophysiological Recordings. The whole-cell voltage-clamp method was used for recordings of IhERG. From a holding potential of −80 mV, cells were depolarized to voltages between −70 mV to 70 mV in 10-mV step increments for 4 seconds, followed by a repolarizing step to −50 mV for 5 seconds, and returned to the holding potential. Peak tail current at −50 mV repolarization step after −50 mV depolarization step was used to quantify IhERG amplitude, which was normalized to the cell capacitance. The pipette solution was comprised of (in mM) 135 KCl, 5 EGTA, 5 MgATP, and 10 HEPES (pH 7.2 with KOH). The bath solution was comprised of (in mM) 135 NaCl, 5 KCl, 10 HEPES, 10 glucose, 1 MgCl₂, and 2 CaCl₂ (pH 7.4 with NaOH). Recordings were performed at room temperature (22±2°C).

Immunofluorescence Microscopy. After culture of hERG-expressing HEK cells grown on glass coverslips, cells were live-membrane stained using Texas Red X–conjugated wheat germ agglutinin (WGA) (5 μg/mL) for 1 minute at room temperature. The cells were then washed with PBS, fixed with ice-cold 4% paraformaldehyde for 15 minutes, and permeabilized with 0.1% triton X-100 for 10 minutes. Afterward, cells were blocked with 5% bovine serum albumin for 1 hour. For detecting G601S hERG labeled with HA tag, cells were exposed to rabbit anti-HA primary antibody (1:100) for 1 hour, followed by Alexa Fluor 350–conjugated donkey anti-rabbit IgG secondary antibody (1:200) for 1 hour. GFP-labeled WT hERG was directly detected. Images were taken with a ZEISS Axio Observer Z1.

Drugs and Reagents. RDV and LUM were purchased from Cayman Chemical Company (Ann Arbor, MI). Both RDV and LUM were dissolved in DMSO to make stock solutions of 50 mM and stored at
-20°C. To exclude any potential vehicle-related effects, the same amounts of the vehicle were applied to control groups. In acute experiments, the highest concentration of DMSO was 0.2%, and for chronic (24-hour) experiments, the highest concentration of DMSO was 0.02%, neither of which affected I_{hERG}. Mouse anti-hERG (F-12) primary antibody was purchased from Santa Cruz Biotechnology (Dallas, TX). Rabbit anti-HA and anti-Kv11.1 primary antibodies were purchased from Sigma-Aldrich (St. Louis, MO). Horseradish peroxidase–conjugated anti-mouse and anti-rabbit secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA). Minimum essential medium, G418, FBS, Alexa Fluor 350-conjugated donkey anti-rabbit IgG secondary antibody, and Texas Red X-conjugated WGA were purchased from Thermo Fisher Scientific (Waltham, MA).

**Statistical Analysis.** Data are expressed using scattered plots with the mean (gray square) ± S.D. (open square), that are overlapped with original data points. A one-way ANOVA with Dunnnett’s post hoc test was used to determine statistical significance between the control and test groups. Analysis was conducted using GraphPad Prism (GraphPad Software, San Diego, CA). A P value of 0.05 or less was considered statistically significant.

**Results**

**Homomorphic G601S Mutant Channels Cannot Be Rescued by RDV or LUM.** I_{hERG} was recorded using the whole-cell voltage-clamp method. hERG channels exhibit unique properties of rapid inactivation; they activate and quickly inactivate during the depolarizing phase (Smith et al., 1996). Upon repolarization, recovery from inactivation to open state occurs much faster than deactivation of open channels. Thus, hERG channels quickly recover to the open state and then gradually deactivate (Smith et al., 1996). This produces unique tail current, which reflects the maximal conductance of the channel following a full activation and is used to measure the amplitude of I_{hERG}. We previously reported that 10 μM RDV did not acutely affect I_{hERG} but chronically (24 hours) increased cell-surface hERG expression and I_{hERG} in HEK293 cells stably expressing G601S hERG (Zhou et al., 1998b; Guo et al., 2007, 2009; Lamothe et al., 2016, 2018). G601S is trafficking defective and thus lacks the 155-kDa protein band and only displays the 135-kDa protein band. Although RDV increased the expression level of the 155-kDa band of WT hERG channels (2.5 ± 0.5-fold versus CTL, n = 4, P < 0.01), LUM had no effect on the expression level of WT hERG channels protein (1.2 ± 0.2-fold versus CTL, n = 4, P > 0.05). Neither RDV nor LUM rescued mature hERG proteins (Fig. 1B). These western blot data are consistent with I_{hERG} data shown in Fig. 1A.

**RDV and LUM Affect Homo- and Heteromeric hERG Channels in Different Manners.** hERG mutation-associated LQTS is an autosomal dominant disease; only one mutant allele is present, whereas the other allele is normal. A single hERG channel is a tetramer (consisting of four hERG subunits from two alleles) (Vandenberg et al., 2012). To mimic heterozygous phenotypes, we coexpressed WT and mutant hERG to form heteromeric channels. Coexpressed WT+G601S hERG displayed a reduced I_{hERG} compared with WT hERG (56 ± 33 pA/pF from 38 cells in five independent experiments versus 87 ± 43 pA/pF from 37 cells in five independent experiments; P < 0.01 (Fig 2A), consistent with the dominant negative effect of G601S on hERG channels. Acute application of either 10 μM RDV or LUM did not affect the current of WT+hERG channels (data not shown). However, when cells were treated with 10 μM RDV for 24 hours, the heteromeric WT+G601S hERG channel had a reduced I_{hERG} (32 ± 33 pA/pF from 37 cells in five independent experiments; P < 0.05) compared with the control of WT+G601S. On the other hand, cells expressing WT+G601S hERG treated with 10 μM LUM for 24 hours had an increased I_{hERG} (96 ± 56 pA/pF from 21 cells in three independent experiments; P < 0.01) compared with the control of WT+G601S (Fig. 2A).

We further investigated the effect of RDV and LUM on the expression of the WT+G601S hERG heteromeric channels using western blot analysis. Coexpressed WT+G601S hERG had a reduced upper band density (155 kDa) relative to WT hERG (0.33 ± 0.05, n = 4; P < 0.01), consistent with the dominant negative effect of the mutant G601S hERG on the WT channel expression. RDV treatment (10 μM, 24 hours) reduced...
the upper band density of WT+G601S hERG (0.14 ± 0.0, n = 4; P < 0.05) compared with the control of WT+G601S. On the other hand, LUM (10 μM, 24 hours) increased the upper band density of WT+G601S hERG (0.54 ± 0.15, n = 4; P < 0.05) compared with the control of WT+G601S (Fig. 2B).

**Coexpression with WT hERG Promotes Surface Expression of the Trafficking-Defective hERG Mutant G601S.** We hypothesized that the 155-kDa band of WT+G601S hERG reflects heteromeric mature channels, a combination or mixture of both WT and G601S hERG monomers. However, there is a possibility that the 155-kDa band is exclusively WT channels with its expression reduced as G601S may hinder the forward trafficking of WT channels in some capacity. To distinguish these possibilities, using immunocytochemistry, we tracked GFP-tagged WT and HA-tagged G601S protein cellular distribution when expressed alone (homomeric) or coexpressed (heteromeric).

First, we transfected GFP-tagged WT hERG (green) into HEK293 cells and stained the cell membrane with Texas Red–conjugated WGA. WT hERG is expressed throughout the cell, especially on the cell membrane (Fig. 3). Thus, WT hERG is trafficking competent and has surface expression, as expected. Second, we transfected HA-tagged G601S hERG into HEK293 cells and treated it with anti-HA primary antibody. We stained the cell membrane with Texas Red–conjugated WGA and anti-HA antibodies with Alexa Fluor 350–conjugated secondary antibody (blue). G601S hERG is localized intracellularly as it is trafficking defective and does not express on the cell surface (Fig. 3). Third, we coexpressed GFP-tagged WT hERG and HA-tagged G601S hERG in HEK293 cells. We found that cells containing WT+G601S hERG express both WT and mutant channels on the surface (Fig. 3). G601S hERG, which is typically trafficking deficient when expressed alone, can traffic to the cell membrane when coexpressed with WT hERG. These results strongly suggest that WT and G601S form heteromeric channels, which can traffic to the plasma membrane.

**The Mutant hERG G601S and Δ2-354 hERG Form Heteromeric Channels.** To directly demonstrate that the trafficking-competent hERG channel subunits can form a heteromeric channel with the trafficking-defective mutant channel subunits, we used an N terminus–deletion hERG mutant, Δ2-354 hERG, which displays unique fast deactivation kinetics (Wang et al., 1998; Gustina and Trudeau, 2009). The G601S hERG channel is trafficking defective and does not generate current in HEK cells under normal culture conditions and treated it with anti-HA primary antibody. We stained the cell membrane with Texas Red–conjugated WGA and anti-HA antibodies with Alexa Fluor 350–conjugated secondary antibody (blue). G601S hERG is localized intracellularly as it is trafficking defective and does not express on the cell surface (Fig. 3).
conditions (e.g., 37°C). However, it can be rescued by conditions such as reduced temperature (e.g., 27°C) for 24 hours and generate robust current with gating kinetics similar to WT hERG (Furutani et al., 1999; Rajamani et al., 2002; Lin et al., 2010). Thus, as currents from Δ2-354 hERG and rescued G601S display different gating kinetics, we were able to determine whether a current is from homomeric Δ2-354 hERG, homomeric G601S, or heteromeric Δ2-354+G601S hERG. As can be seen in Fig. 4, Δ2-354 hERG alone generated \(I_{\text{hERG}}\) with fast deactivation, consistent with the previous studies (Wang et al., 1998; Gustina and Trudeau, 2009). G601S hERG generated no current as it is trafficking defective. However, \(I_{\text{hERG}}\) of G601S recorded after a reduced temperature culture at 27°C (instead of normal 37°C) for 24 hours displayed kinetics similar to that of WT hERG (Furutani et al., 1999; Rajamani et al., 2002; Lin et al., 2010). Δ2-354+G601S hERG generated \(I_{\text{hERG}}\) with clear tail current that deactivated much slower than that of Δ2-354 but faster than that of rescued G601S (Fig. 4A). These data indicate that Δ2-354 and G601S can form heteromeric channels that are trafficking competent and thus functional.

The expression of heteromeric Δ2-354+G601S hERG was studied using western blot analysis. Δ2-354 hERG is trafficking competent. Since it is truncated at the N terminus, the immature core-glycosylated and mature fully glycosylated hERG channel proteins are smaller in size, 95 kDa and 115 kDa, respectively.
respectively (Fig. 4B). The trafficking-defective hERG mutant G601S displays only the immature band (135 kDa) without the mature band (155 kDa). However, coexpression of Δ2-354 and G601S generated four bands (95, 115, 135, and 155 kDa), indicating that the coexistence of Δ2-354 hERG enables G601S to mature (to the 155-kDa protein form). These data are also consistent with our electrophysiological data showing that Δ2-354 and G601S form functional heteromeric channels (Fig. 4A).

Patch-clamp and western blot analysis were used to investigate the effects of RDV and LUM on Δ2-354, G601S, and Δ2-354+G601S hERG channels. Like WT I_{hERG} (Fig. 1), Δ2-354 I_{hERG} was increased by RDV treatment (10 μM, 24 hours) but was not affected by LUM treatment (10 μM, 24 hours) (CTL: 34 ± 16 pA/pF from 17 cells, RDV: 61 ± 31 pA/pF from 12 cells, P < 0.01 versus CTL; LUM: 43 ± 33 pA/pF from 20 cells, P > 0.05 versus CTL; each group of cells were recorded from three independent experiments) (Fig. 5). As shown in Fig. 1A, G601S expressed alone was trafficking defective, and neither RDV nor LUM rescued its function. Like I_{hERG} of WT+G601S heteromeric channels (Fig. 2), I_{hERG} of Δ2-354+G601S was decreased by RDV treatment but increased by LUM treatment (CTL: 41 ± 22 pA/pF from 17 cells, RDV: 11 ± 10 pA/pF from 11 cells, P < 0.01 versus CTL; LUM: 64 ± 38 pA/pF from 16 cells, P < 0.05 versus CTL; each group of cells were recorded from 3 to 4 independent experiments) (Fig. 5). At protein expression levels, treatment of Δ2-354+G601S hERG with RDV (10 μM) for 24 hours caused a reduction in both 115- and 155-kDa bands, which correspond to the mature forms of Δ2-354 hERG and G601S hERG, respectively. On the other hand, treatment of Δ2-354+G601S hERG with LUM (10 μM) for 24 hours caused an increase in expression of mature (115- and 155-kDa) bands of Δ2-354+G601S hERG channels (Fig. 6). Thus, alteration of the mature channel expression levels underlies the changes of current amplitudes induced by RDV or LUM treatment.

Effects of RDV and LUM on WT+R582C hERG Heteromeric Channels. Our results have shown that RDV and LUM differentially affect homomeric and heteromeric WT and G601S. To address whether this phenomenon applies to other trafficking-defective mutant channels, we coexpressed WT and R582C hERG into HEK293 cells. R582C is also a trafficking-deficient mutation (Fougere et al., 2011). Our patch clamp and Western blot data showed that neither RDV nor LUM rescued homomeric R582C function and mature band expression (data not shown). R582C displayed a dominant negative effect, which resulted in reduced I_{hERG} (Fig. 7A) and decreased surface expression (Fig. 7B) of WT+R582C hERG compared with WT hERG. Like WT+G601S channels, WT+R582C current (Fig. 7A) and mature band expression (Fig. 7B) were reduced by RDV treatment (10 μM, 24 hours) but increased by LUM treatment (10 μM, 24 hours).

Effects of RDV and LUM Treatment on hERG mRNA Levels. To determine if RDV or LUM alters hERG transcription, RT-qPCR was used to quantify hERG mRNA levels. HEK cells stably expressing homomeric WT or mutant hERG channels were cultured without (control) or with 10 μM RDV or LUM for 24 hours. Total RNA was extracted and converted to cDNA with reverse transcriptase. cDNA was then subjected to real-time PCR, and Ct values were obtained. Ct values were averaged for each duplicate and then normalized to that of reference gene GAPDH for internal control. Ct values from treated cells were then normalized to controls to obtain the fold changes in hERG mRNA expression, which are displayed in boxplots with means and original data points superimposed (Fig. 8). LUM did not affect mRNA levels of either WT or G601S hERG. On the other hand, RDV treatment increased mRNA levels of WT hERG by 3.7- ± 1.1-fold (n = 9; P < 0.01) and G601S mutant hERG channels by 1.8- ± 0.7-fold (n = 6; P < 0.01). The RDV-mediated increase of mRNA level of G601S was less than that of WT hERG G601S (P < 0.01) (Fig. 8A). WT hERG is trafficking competent, whereas G601S mutant hERG is trafficking

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**Fig. 5.** Effects of RDV and LUM on I_{hERG} of homomeric Δ2-354, G601S, and heteromeric Δ2-354 hERG channels. Representative current tracings (upper) and amplitudes (lower) of I_{hERG} after 24 hours of RDV or LUM treatment in HEK293 cells expressing Δ2-354 hERG, G601S hERG (G601S), or Δ2-354+G601S are shown. RDV increased homomeric Δ2-354 hERG I_{hERG}, did not affect homomeric G601S I_{hERG}, and decreased Δ2-354+G601S hERG channels. LUM did not affect either homomeric WT I_{hERG} or homomeric G601S I_{hERG}, but increased Δ2-354+G601S I_{hERG}. *P < 0.05; **P < 0.01.
HEK cells, demonstrate that N629D hERG is trafficking-competent mutant S624T and trafficking-defective N629D hERG channels. S624T is a mutant hERG channel that traffics normally and displays altered sensitivity to extracellular Na⁺ and K⁺ levels (Mullins et al., 2002, 2004; Guo et al., 2009; Massaeli et al., 2010). N629D hERG is a human missense LQT2 mutation (Satler et al., 1998). Although studies in Xenopus oocyte expression system show that N629D expressed in HEK cells only displayed extracellular Na⁺ current, its inward current was reduced when expressed in mammalian cell line, studies in the mammalian cell line, hERG displays altered gating properties (Lees-Miller et al., 2000; Teng et al., 2003), and N629D hERG is a homo-meric WT hERG mutant with reduced cell-surface expression and IhERG in trafficking-defective homomeric hERG channels (Fig. 1, 2, and 5–7). On the other hand, RDV increased the mRNA level of N629D hERG channels (0.7±0.2 fold (n = 3; P < 0.01). In contrast, RDV slightly decreased the mRNA level of N629D hERG channels (0.7±0.2 fold (n = 3; P > 0.05).

Discussion

Defective trafficking represents a major mechanism that underlies hERG mutation-induced channel dysfunction and associated LQT2 (Anderson et al., 2014). In the present study, we investigated the potential of RDV and LUM to rescue cell-surface expression and function of trafficking-defective homo- and heteromeric mutant hERG channels in HEK293 cells. Our data show that WT, homomeric mutant, and heteromeric hERG channels respond to drugs in different and unique ways. Neither RDV nor LUM rescued the G601S homomeric mutant hERG function or expression (Fig. 1). RDV increased cell-surface expression and IhERG in trafficking-competent homomeric hERG channels but decreased cell-surface expression and IhERG in trafficking-deficient mutant-containing heteromeric channels (Figs. 1, 2, and 5–7). Thus, in heteromeric channels containing trafficking-deficient hERG mutations, RDV may decrease IhERG and thus exacerbate LQT2, whereas LUM may increase IhERG and thus mitigate LQT2. Given that LQT2 is an autosomal disease, and most hERG mutant carriers possess heteromeric hERG channels, our findings are clinically relevant.

RDV has been authorized by the US Food and Drug Administration for emergency use to treat COVID-19 patients hospitalized with severe forms of the disease. Our study suggests that the use of RDV may warrant close monitoring, especially in patients harboring hERG mutations, particularly since many variants are silent and/or remain unidentified (Anson et al., 2004). In fact, prolongation of the QT interval and associated Torsade de Pointes following RDV administration during the COVID-19 pandemic has been reported by several independent groups (Gupta et al., 2020; Haghjoo et al., 2021; Michaud et al., 2021; Singla et al., 2022; Fung et al., 2023). It would be...
and our results, for the present study, we investigated the blocking effects of LUM on (Guimbellot et al., 2020), equivalent to 4.2 ivacaftor/lumacaftor as CFTR modulator therapy, measured et al., 2018; O

LUM is a drug approved by the Food and Drug Administrers (Ficker et al., 2002), limiting their therapeutical potential. shortened action potential duration in iPSC-CMs with hERG LUM increased mature hERG membrane localization and heteromeric hERG channels containing traf

and traf

get misfolded or defective proteins for degradation, which quality control mechanisms exist to screen proteins and tar-

nels warrant further investigation. For RDV, alterations in protein synthesis and induction of endoplasmic reticulum (ER) stress may be involved. Under normal conditions, ER protein synthesis and induction of endoplasmic reticulum

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involves chaperone-mediated ubiquitination and ER-associated degradation (Gong et al., 2005; Vandenberg et al., 2012). For trafficking-defective hERG mutants, ER-associated degradation results in retention of channels in a microtubule-dependent subcompartment of the ER (Smith et al., 2011). This accumulation of misfolded proteins in the ER can induce ER stress, which alters the homeostatic balance in the ER and impairs its folding capacity (Olsowski and Urano, 2011). ER stress can trigger the unfolded protein response (UPR) to relieve the load of misfolded proteins and restore homeostasis by enhancing mRNA degradation, inhibiting protein translation, and accelerating protein degradation (Liu et al., 2022). Specifically, one branch of the UPR is initiated by the protein kinase-like ER kinase (PERK), which becomes phosphorylated and activates phosphorylation of eukaryotic initiation factor 2a (eIF2a).

This PERK branch is known to downregulate cardiac ion channels, including hERG, by increasing mRNA degradation and decreasing protein translation as phosphorylated eIF2a inhibits ribosomal mRNA interactions (Liu et al., 2018, 2022). ER stress-associated activation of transcription factor 6x (ATF6x) is also known to increase hERG protein degradation (Keller et al., 2005; Liu et al., 2022). Our data showed that following culture with RDV, WT hERG mRNA levels increased more than G601S hERG mRNA levels. Similarly, S624T hERG mRNA levels increased, but N629D hERG mRNA levels slightly decreased. We believe that RDV may upregulate hERG mRNA levels to a similar extent in both WT and mutant hERG-expressing cells; the discrepancy between trafficking-competent and incompetent hERG channels in responses to RDV may be a result of the different levels of ER stress induced by the differing degrees of mutant subunits in the hERG channels. In fact, RDV-induced promotion of ER stress and activation of the PERK-mediated UPR has been reported in glioblastoma cells (Chen et al., 2023). Thus, WT hERG may demonstrate increased expression and function following RDV administration due to minimal ER stress, whereas heteromeric mutant hERG channels may show diminished expression and function due to enhanced ER stress-related mRNA reduction and protein degradation.

LUM did not affect the levels of mRNA of WT or G601S. It is likely that LUM exerts its action as a pharmacological chaperone promoting protein trafficking to plasma membrane. The effect of LUM on WT is negligible, probably because WT is already trafficking competent. Mechanisms underlying LUM-mediated rescue are currently unknown. For rescuing CFTR, it has been proposed that LUM's large, hydrophobic 1,3-benzodioxol-5-yl-cylopropane carboxamide (BCC) head group inserts into a hydrophobic pocket formed by transmembrane domains 1, 2, 3, and 6 in a lock-and-key fit, and its binding is dependent on interactions with surrounding amino acid residues that correctly position the transmembrane domains (Fiedorczuk and Chen, 2022). Our finding that LUM could rescue heteromeric channels but had no effect on homomeric mutant hERG suggests that LUM's mechanism of action may depend on the presence of trafficking-competent hERG subunits. This is in line with mutational analyses demonstrating that pharmacological correction of trafficking-deficient hERG variants is improved when mutant and WT hERG are coexpressed (Anderson et al., 2014). The cryo-EM structure of hERG reveals that it has four deep hydrophobic pockets below its selectivity filter that participate in the binding of hERG blockers (Dickson et al., 2020). hERG activators have been found to insert hydrophobic moieties into a
hydrophobic pocket formed by F619 and L646 that overlaps part of the blocker binding site but is shifted laterally to not impede ion permeation (Dickson et al., 2020). Given LUM’s hydrophobic interaction with CFPTR and that LUM did not block I_{hERG} of rescued channels, its BCC headgroup may bind to hERG in a similar manner to these activators. The conformational changes induced by mutations may affect the binding of LUM, and heteromeric channels containing mutant and WT hERG may possess conformations for LUM binding.

In conclusion, our study demonstrated that RDV and LUM can affect homomeric and heteromeric channels in distinctively different manners. These findings may have clinical implications, especially in patients harboring hERG mutations.

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Data Availability
The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions
Participated in research design: Campagna, Guo, Li, Yang, Zhang. Conducted experiments: Campagna, Wall, Lee, Guo, Li, Yang. Performed data analysis: Campagna, Guo, Li, Yang. Wrote or contributed to the writing of the manuscript: Campagna, Wall, Lee, Guo, Li, Yang, Baranchuk, El-Diasty, Zhang.

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