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Inhibition of the α-subunit of phosphoinositide 3-kinase (PI3K) in heart increases late sodium current and is arrhythmogenic

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Abbreviations:

AP	action potential
APD	action potential duration
APD_{90}	action potential duration at 90% repolarization
CHO	Chinese hamster ovary
DMEM	Dulbecco's Modified Eagle's Medium
GFP	green fluorescent protein
HERG	older terminology for *KCNH2*
I_{NaL}	late sodium current
KCNE1	gene encoding an I_{Ks} function modifying subunit
KCNE2	gene encoding an I_{Kr} function modifying subunit
KCNH2	gene encoding the I_{Kr} channel
KCNQ1	gene encoding the I_{Ks} channel
$Na_v1.5$	cardiac sodium channel protein
NMDG	N-Methyl-D-glucamine
PI3K	phosphoinositide 3-kinase
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
SCN5A	cardiac sodium channel gene

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Abstract

While inhibition of phosphoinositide 3-kinase (PI3K) is an emerging strategy in cancer therapy, we and others have reported that this action can also contribute to drug-induced QT prolongation and arrhythmias by increasing cardiac late sodium current (I_{NaL}). Previous studies in mice implicate the PI3K-α isoform in arrhythmia susceptibility. Here, we have determined the effects of new anticancer drugs targeting specific PI3K isoforms on I_{NaL} and action potentials (APs) in mouse cardiomyocytes and CHO cells. Chronic exposure (10-100 nM; 5-48hours) to PI3K-α-specific subunit inhibitors (BYL710 (Alpelisib) and A66) and a Pan-PI3K inhibitor (BKM120) increased I_{NaL} in *SCN5A*-transfected CHO cells and mouse cardiomyocytes. The specific inhibitors (10-100 nM for 5 hours) markedly prolonged APs and generated triggered activity in mouse cardiomyocytes (9/12) but not in controls (0/6), and BKM120 caused similar effects (3/6). Including water-soluble PIP3, a downstream effector of the PI3K signaling pathway, in the pipette solution reversed these arrhythmogenic effects. By contrast, inhibition of PI3K-β, -γ and -δ isoforms did not alter I_{NaL} or APs. We conclude that inhibition of cardiac PI3K-α is arrhythmogenic by increasing I_{NaL} and this effect is not seen with inhibition of other PI3K isoforms. These results highlight a mechanism underlying potential cardiotoxicity of PI3K-α inhibitors.

Introduction

Intracellular phosphoinositide 3-kinase (PI3K) is a large heterodimeric protein consisting of a 110 kDa catalytic subunit (p110α, p110β, p110δ, or p110γ) that confers enzyme activity and a regulatory subunit (Damilano et al., 2010). A major product of PI3K activation is the effector phosphatidylinositol-3,4,5-trisphosphate (PIP3). The PI3K pathway is critical for cell survival and growth, and can be activated by growth factors binding to cell-surface receptors (Berenjeno and Vanhaesebroeck, 2009). Activated PI3K signaling is a frequent feature in cancer (Chen et al., 2012), and can lead to abnormal cell growth, uncontrolled cell proliferation, increased cell survival and enhanced cancer cell motility. Therefore, the PI3K pathway is increasingly targeted in human cancers (Agarwal et al., 2010; Courtney et al., 2010; LoRusso, 2016).

In heart, activation of the PI3K-PIP3 pathway has been associated with cardiac hypertrophic growth (Shioi et al., 2000; Matsui et al., 2003; Rigor et al., 2009), and more recently with arrhythmias. Pretorius et al (Pretorius et al., 2009) reported that mice with dilated cardiomyopathy crossed with mice expressing a dominant negative PI3K-α construct displayed increased atrial fibrillation (AF) and in human subjects with AF, PI3K atrial activity was reduced compared to that in patients with normal rhythm. Lu et al (Lu et al., 2012) first reported a role for the PI3K-PIP3 signaling pathway in arrhythmias related to abnormal ventricular repolarization in their studies of the tyrosine kinase inhibitor (TKI) nilotinib, used to treat chronic myelogenous leukemia. The nilotinib label warns that the drug prolongs the QT interval and can thereby cause the polymorphic ventricular tachycardia torsades de pointes. Lu et al. showed that while action potential duration (APD, a cellular surrogate for QT interval) in canine cardiac myocytes acutely exposed to nilotinib was unchanged, chronic (hours) exposure prolonged APD, and the major effect was to enhance "late" (non-inactivating) sodium current (I_{NaL}), an effect well-known to prolong QT and cause arrhythmias (Antzelevitch et al., 2014; Belardinelli et al., 2015). There was

also modest reduction in the potassium currents I_{Kr} and I_{Ks}, attributed to decreased cell surface expression. Subsequently, we showed (Yang et al., 2014) that multiple antiarrhythmic and other drugs thought to prolong QT interval by blocking I_{Kr} also increased I_{NaL} during chronic (hours-long) exposure and were arrhythmogenic in cardiomyocytes from adult mice, which lack I_{Kr} and I_{Ks}. This effect was blocked by including PIP3 in the patch pipette and was most prominent with drugs thought to be highly arrhythmogenic like dofetilide which produces long QT related arrhythmias in >1% of treated patients (Abraham et al., 2015), and much less prominent or absent with QT-prolonging drugs like moxifloxacin or thioridazine that cause arrhythmias much more rarely. In that study, we also showed that dofetilide inhibited the phosphorylation of Akt, a downstream target of PI3K, whereas moxifloxacin did not (Yang et al., 2014). QT intervals are prolonged in diabetes, and Lu et al. have shown AP prolongation due to increased I_{NaL} in myocytes from mice models of diabetes (Lu et al., 2013).

The identification of PI3K as a target in cancer has led to the development of isoform-specific inhibitors. In this study, we used these reagents to identify the specific arrhythmogenic pathway *in vitro*. These findings not only dissect a novel arrhythmogenic pathway in drug-induced arrhythmias and possibly other clinical settings, but also have implications for development and use of PI3K inhibitors in cancer (Yap et al., 2015) (Massacesi et al., 2016).

Materials and Methods

Isolation of mouse ventricular cardiomyocytes. Given the recognition of a gender effect on repolarization in mice (Lowe et al., 2012), these experiments were conducted in isolated ventricular myocytes from adult female C57 mice (3 hearts in each group, 2-3 cells from each heart). Ventricular myocytes were isolated by a modified collagenase/protease method, as previously reported (Yang et al., 2012; Yang et al., 2014). In this study, at least three mouse hearts were isolated for collection of electrophysiological data for each study group. All procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

FuGENE6-mediated ion channel expression and CHO cell transfection. In Chinese hamster ovary (CHO) cells, transient transfections were performed as previously reported (Yang et al., 2012) to express cardiac ion channels: 2 μg of the cDNA encoding human *SCN5A* (encoding the cardiac sodium channel) or *KCNH2 (HERG)+KCNE2* (*Mirp1*) (encoding I_{Kr}), *KCNQ1+KCNE1* (encoding I_{Ks}), *CACNA1C+CACNB2* (encoding the L-type calcium channel) and 0.5 μg of a plasmid for green fluorescent protein (GFP, as a marker to identify successfully transfected cells for subsequent patch clamp experiments) were mixed with 10 μl FuGENE6 (Roche) in 0.5 mL serum-free DMEM medium and incubated for 30 minutes, after which the standard medium was replaced for a 48-hour incubation. The cells were harvested by brief trypsinization and stored in standard medium for use within 10 hours. Electrophysiological data in CHO cells were collected from 2-3 separate cell transfections in each experimental group.

Late sodium current (I_{NaL}) recording. Endogenous late sodium current (I_{NaL}) in cardiac cells is very small (~0.5% of peak sodium current, with a physiological concentration of extracellular sodium) (Saint et al., 1992; Ju et al., 1994). We used previously described methods (Yang et al., 2012; Yang et al., 2014) to record late sodium current (I_{NaL}) in whole-cell voltage

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clamp mode at room temperature (22-23°C). To optimize I_{NaL} recordings in mouse ventricular myocytes, the external bath solution was K^+- and Ca^{2+}-free and contained (in mM) NaCl 135, $MgCl_2$ 1.0, glucose 10, and HEPES 10; the pH was 7.4, adjusted with CsOH. To rule out the possibility that during the test pulse the large rapid sodium current in mouse ventricular myocytes could be out of voltage clamp control and alter the measurement of I_{NaL}, in some experiments we lowered the external sodium concentration to 50 mmol/L to better control peak I_{Na} and using NMDG to replace 85 mM sodium in the above solution. In *SCN5A*-transfected CHO cells, peak and late sodium currents were recorded with external Tyrode's solution (in mmol/L): NaCl 135, $CalCl_2$ 1.8, KCl 4.0, $MgCl_2$ 1.0, HEPES 5, glucose 10, with a pH of 7.35 (adjusted by NaOH). The pipette (intracellular) solution contained (in mmol/L) NaF 5, CsF 110, CsCl 20, EGTA 10, and HEPES 10; the pH was 7.4, adjusted with CsOH. To eliminate possible L-/T-type inward calcium currents and transient outward potassium current (I_{TO}) in mouse ventricular myocytes, nisoldipine (1 μM), $NiCl_2$ (200 μM) and 4-aminopyride (4-AP, 500 μM) were added into the bath solution.

Because previous studies (Lu et al., 2012; Yang et al., 2014; Qiu et al., 2016) have demonstrated that hours of drug exposure are required to demonstrate increased I_{NaL}, data were collected after acute (10 minutes) and chronic (5-48 hours) exposure. To record I_{NaL}, a 200-ms voltage clamp protocol from the holding potential of -120 mV to a test potential to -30 mV was used. I_{NaL} was measured in a 3-ms time window before the capacity transient, 195-198 ms after the start of the pulse, and expressed as the percentage of peak sodium current.

Action potential (AP) recordings. In current-clamp mode, APs from isolated mouse ventricular myocytes were elicited by injection of a brief stimulus current (1-2 nA, 2-6 ms) at stimulation rates of 1 Hz and 0.1 Hz. For AP experiments, the bath (extracellular) solution was

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normal Tyrode's, containing (in mmol/L): NaCl 135, KCl 4.0, $CaCl_2$ 1.8, and $MgCl_2$ 1.0, HEPES 5.0, glucose 10, with a pH of 7.4. The pipette-filling (intracellular) solution contained (in mmol/L): KCl 130, ATP-K_2 5.0, $MgCl_2$ 1.0, $CaCl_2$, 1.0, BAPTA 0.1 and HEPES 5.0, with a pH of 7.3 (adjusted by KOH). Ten successive AP traces elicited with a stimulation rate of 1 Hz were averaged for analysis of action potential durations at 90% repolarization (APD_{90}). Under chronic (5 hours) exposure to PI3K inhibitors, abnormal APs were defined as those with early afterdepolarizations (EADs), delayed afterdepolarizations (DADs) or triggered activity at a slow stimulation rate (0.1 Hz) which enhances late sodium current (Wu et al., 2011).

For the experiments to observe the effect of intracellular PIP3, water-soluble PIP3 (1 μM) was added to the pipette (intracellular) solution. Data were continuously collected at 0.1 Hz for 3-5 minutes.

Recording of cardiac potassium and L-type calcium currents. To determine whether PI3K inhibition influences other cardiac repolarizing potassium currents (such as *KCNH2*-encoded I_{Kr} or the transient outward current I_{TO}), *KCNH2*-transfected CHO cells (for I_{Kr}) and adult mouse cardiomyocytes (for I_{TO}) were exposed to PI3K-α inhibitors acutely and chronically. Experiments were also conducted in transfected CHO cells as above. The drug concentrations and voltage clamp protocols used are shown in the figures. All electrophysiological data were analyzed using pCLAMP version 9.2 software and the figures were prepared by using Origin 8.5.1 software (OriginLab Corp., Northampton, MA, USA).

Glass microelectrodes were heat polished to tip resistances of 2-3 MΩ. Data acquisition was carried out using an Axopatch 200B patch-clamp amplifier and pCLAMP version 9.2 software (MDS Inc., Mississauga, Ontario, Canada**)**. Currents were filtered at 100 kHz (-3 dB, four-pole Bessel filter) and digitized using an analog-to-digital interface (DigiData 1322A, MDS Inc.). To

minimize capacitive transients, capacitance and series resistance were corrected ~80%. Cell size (in pF) was calculated by Membrane Test (OUT 0) in pClamp 9.2. Clamp protocols used are shown on the figures.

Drugs. 10 mM stock solutions of PI3K-isoform inhibitors were purchased from Selleckchem (Houston, TX, USA). During experiments, the desired test concentrations were prepared according to the vendor's instructions, as needed. BYL716 (alpelisib)/A66, TGX-221, AS252424, and idelalisib are inhibitors of PI3K-α (Juric et al., 2018; Park and Kim, 2018), -β (Mao et al., 2017), -γ (Jin et al., 2014) and -δ (Jin et al., 2016) subunits respectively, and BKM120 is an inhibitor of all four isoforms (Bashash et al., 2017). The concentrations used were 10-100 nM, while the concentrations used to inhibit PI3K isoforms in cell assays are ≥1 μM (Xu et al., 2013; Costa et al., 2015; Aragoneses-Fenoll et al., 2016).

Statistical analysis. Results are presented as mean±SEM. For comparisons of paired and unpaired groups, nonparametric Wilcoxon Signed Rank Sum and Mann-Whitney *U* tests were used. For comparisons of three more groups, Kruskal-Wallis (K-W) One-Way ANOVA test was first performed to determine if there was a significant difference among means. If K-W test displayed a significant difference of $P<0.05$, then Dunnett's T3 Post Hoc test was further used to examine the differences of the mean values between two groups among multiple samples. Statistical analyses were carried out with SPSS software version 22 (IBM, NY, USA), OriginPro 8.5.1 (OriginLab, Northampton, MA, USA), and Prism 7 (GraphPad Software, La Jolla, CA, USA). A value of $P<0.05$ was considered statistically significant.

Results

Chronic effects of multiple inhibitors of PI3K subunits and Pan-PI3K on adult mouse cardiac action potentials (APs). We first determined the effects of a series of PI3K subunit inhibitors on action potentials (APs) from mouse ventricular myocytes drug-exposed for 5 hours. Before AP recordings, freshly-isolated mouse ventricular myocytes were pretreated for 5 hours with individual inhibitors. As shown in Fig.1A-C, the two "pure" PI3K-α subunit inhibitors, BYL719 and A66, as well as the Pan-PI3K inhibitor BKM120 caused AP abnormalities (varying from prolonged APs and triggered activity, described below). At a stimulation rate of 1 Hz, this AP prolongation appeared more prominent during late repolarization with either 100 nM BYL719 or 100 nM A66, and action potential prolongation was evident at concentrations as low as 10 nM. Fig. 1D summarizes action potential duration at 90% repolarization (APD_{90}) by 100 nM of the all inhibitors, and 10 nM of the α-specific ones. Inhibition of PI3K-β, -γ and -δ subunits did not alter APD_{90}.

PI3K-α inhibitors are arrhythmogenic. AP prolongation by the two selective PI3K-α subunit inhibitors was exaggerated when a slower stimulation rate (0.1 Hz) was used. Compared to non-drug treated mouse ventricular myocytes (Fig. 2A), drug-treated cells displayed abnormal APs with triggered activity (Fig. 2B and D). Further, these AP abnormalities were seen only at initial recording after cell break-in, and resolved within minutes when the PI3K effector molecule PIP3 was included in the pipette solution (Fig. 2C and E). Summary results are presented in Fig. 2F. In the absence of internal PIP3, abnormal APs included multiple forms (EADs, DADs and triggered activity), while in the presence of PIP3, abnormal APs when seen were only in the form of single triggered upstrokes. Abnormal APs were also seen in 3/6 cells with the Pan-PI3K inhibitor BKM120 but no abnormal APs were seen with the β-, γ-, and δ-inhibitors.

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PI3K-α inhibition increases I_{NaL}. In untreated mouse ventricular myocytes, control I_{NaL} at the end of a 200-ms pulse to -30 mV from a holding potential of -120 mV was small, ~0.2% of the peak sodium current (Fig. 3A). In myocytes pretreated with either BYL719 or A66 for 5 hours, however, I_{NaL} was increased 3-5-fold (Fig. 3B-E) in a concentration-dependent fashion. Summary data are shown in Fig. 3F. To exclude the possibility that this result was attributable to loss of voltage control, we repeated the experiment with a lower extracellular sodium concentration (50 mmol/L, see Methods) and observed the same result (Supplemental Fig.1). In separate experiments, we also demonstrated that all five PI3K subunit inhibitors did not affect peak sodium current (Supplemental Fig. 2).

In order to determine whether this increased I_{NaL} is specific for the PI3K-α inhibition, we compared the effects of 48 hour exposures of the isoform selective inhibitors and of BKM120 on I_{NaL} in *SCN5A*-transfected CHO cells. As in untreated mouse ventricular myocytes, control I_{NaL} level was ~0.2% of the peak sodium current (Fig. 4A). BYL719 (1 μM, 48 hours) still enhanced I_{NaL} (Fig. 4B) and this effect was very similar to that seen in mouse myocytes (Fig. 3B and C). The Pan-PI3K inhibitor (BKM120) also augmented I_{NaL} (Fig. 4C). By comparison, the PI3K-β, -γ, and -δ inhibitors did not affect I_{NaL} (Fig. 4D-F). In addition, the action of BYL719 to increase I_{NaL} under these conditions was reversed by including PIP3 in the pipette solution (Fig. 4G). Summary data for *SCN5A*-transfected CHO cells are presented in Fig. 4H. The drug-induced increase in I_{NaL} by chronic A66 (100 nM) was suppressed by ranolazine (10 μM), a known blocker of I_{NaL} that produced minimal effects on peak sodium current (Supplemental Fig. 3). All these data further support the hypothesis that I_{NaL} contributes to the abnormal cardiac action potentials caused by PI3K-α inhibitors.

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No effect on I_{Kr} or I_{TO} of acute or chronic PI3K-α inhibition. We also tested other potential mechanisms for AP prolongation. To date, the mostly widely accepted explanation for drug-induced QT prolongation has been I_{Kr} inhibition. Therefore, we tested the acute (10 minutes) and chronic (48 hours) effects of 10 and 100 nM of the two PI3K-α inhibitors (BYL719 and A66) on *KCNH2*-expressed current in CHO cells. As shown in Supplemental Fig.4, the drugs had no effect on I_{Kr} in these experiments. We also observed no effect of a high concentration (3 μM) in cells expressing *KCNH2* and *KCNE2 (Mirp1),* a putative I_{Kr} subunit (Supplemental Fig. 5).

In mouse myocytes, acute (10 min) or chronic (5 hours) exposure to the two inhibitors had no effect on transient outward potassium current (I_{TO}) in adult mouse ventricular myocytes (Supplemental Fig. 6 and 7). In addition, we found that including PIP3 in the intracellular (pipette) solution had no effect on I_{CaL}, I_{Kr}, or I_{Ks} studied in CHO cells (Supplemental Fig. 8).

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Discussion

The PI3K-PIP3 signaling pathway has been recognized to be crucial in tumorigenesis and has therefore stimulated efforts for oncology drug development (Samuels et al., 2004; Yap et al., 2015; Massacesi et al., 2016). Although a role for PI3K in cardiac hypertrophy and contractile activity has been investigated in detail (Shioi et al., 2000; Dorn and Force, 2005; Rossello et al., 2017), the effects of the PI3K-PIP3 pathway in cardiac electrical activity and arrhythmogenesis have only recently begun to be defined. An initial study implicated PI3Kα in atrial fibrillation susceptibility in the mouse and human heart (Pretorius et al., 2009). More recently, a number of reports have implicated PI3K inhibition as a pathway to QT prolongation and subsequent arrhythmias (McMullen et al., 2014; Ballou et al., 2015; Ezeani and Elom, 2017). This is of great interest to the drug development community since drug-induced QT prolongation is a major liability during drug development (Townsend, 2014). The usually-accepted mechanism for drug-induced prolongation of cardiac action potentials and QT interval is inhibition of the rapidly-activating potassium current I_{Kr} conducted by the Kv11.1 channel, encoded by *KCNH2*, also known as *HERG* (Roden, 2008). However, acute exposure of canine cardiac myocytes to nilotinib (whose label carries a "black box" warning for QT prolongation) did not change action potentials (Lu et al., 2012), suggesting mechanism beyond acute I_{Kr} block. Chronic (hours) exposure to nilotinib reduced cell surface expression of a range of ion channels (including Kv11.1) and increased the amplitude of the persistent ("late") sodium current I_{NaL}, an effect known to prolong QT interval. These actions were reversed by including PIP3 in the patch pipette, providing strong evidence the mechanism of the electrophysiologic effect of nilotinib was reduced PI3K activity, attributed to block of the receptor tyrosine kinase(s) that nilotinib targets. Other work implicated the I_{NaL} effect in prolonging repolarization in animal models of diabetes (Lu et al., 2013), a clinical setting associated with increased QT intervals, and we have shown that some but not all drugs known to

block I_{Kr} can prolong APs and generate arrhythmogenic afterdepolarizations through this pathway (Yang 2014).

In order to determine which PI3K isoform(s) drive altered cardiac electrophysiology and arrhythmogenesis, in the present study we examined the effects of multiple PI3K inhibitors on cardiac action potentials (APs), late sodium current and other major repolarizing outward potassium currents in isolated adult mouse cardiomyocytes (which lack I_{Kr} and I_{Ks}) and transfected CHO cells. The experimental results are summarized here: (1) chronic (5 hours) exposure to PI3K-α subunit inhibitors (BYL716 and A66) and the Pan-PI3K blocker BKM-120 caused AP prolongation and abnormalities (EADs, DADs and triggered activity) and these effects were reversed when PIP3 was included in the pipette solution; (2) this chronic PI3K-α inhibitor exposure markedly increased late sodium current in mouse cardiomyocytes and in *SCN5A*-transfected cells, and the increased late current was abolished by ranolazine, a blocker of late sodium current; (3) chronic exposure to inhibitors of PI3K-β, -γ and -δ did not alter mouse cardiac APs or late I_{Na}; and (4) acute and chronic exposures to selective PI3K-α inhibitors did not affect two major repolarizing potassium currents (I_{TO} and *HERG*).

These data are compatible with the hypothesis that inhibition of PI3K-α subunit in the heart contributes to cardiac arrhythmogenesis by increasing late sodium current. The possibility that PI3Kα also affects other channels in the human heart, particularly over days or longer, cannot be excluded but the present data highlight the effects of the α isoform as primary in effecting the changes we reported here. The mechanism whereby PIP3 inhibits late sodium current has not been defined but a direct interaction with the channel seems likely given the very rapid onset of action when PIP3 is included in the pipette solution. The mechanisms for the delay in onset of the effect

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with exposure to the kinase blockers has not been defined but a time lag in driving down PIP3 concentrations is one possibility.

PI3K is the most frequently mutated signaling pathway in multiple tumor types, stimulating intense efforts to target PI3K or downstream kinase targets for the treatment of cancer (Samuels et al., 2004; Costa et al., 2018). Early efforts focused on specific PI3K inhibitor targeting δ-(Gopal et al., 2014) and γ-(Evans et al., 2016) isoforms, whose expression is generally restricted to hematopoietic cells. Idelalisib, a PI3K-δ inhibitor, was the first inhibitor approved for multiple hematological malignancies. Current efforts have focused on pan-PI3K inhibitors or alpha specific inhibitors that might have broader efficacy (LoRusso, 2016). To date, mechanisms for drug-induced QT interval prolongation have been assumed to be acute and almost inevitably due to drug targeting of I_{Kr}. As a result, regulatory agencies recommend that all drug candidates undergo *in vitro* testing for drug effects on I_{Kr} in early development (Authier et al., 2010; Goineau et al., 2013; Vicente et al., 2016). The present data lead to a prediction that inhibitors of PI3Kα will prolong QT while inhibitors of other isoforms may not (assuming no interaction with I_{Kr}) and that the QT prolongation would not be addressed appropriated during pre-clinical testing. To date, clinical trials have not reported systematically collected QT intervals in large numbers of drug-exposed subjects (Juric et al., 2017; Juric et al., 2018).

For new drug evaluations and drug safety pharmacology, our work reinforces the suggestion that that drug effects should be investigated over time in *in vitro* and *in vivo* biological systems that include all of the components of normal and perhaps abnormal cardiac repolarization (Vicente et al., 2018).

Authorship contributions

Participated in research design: Tao Yang, Dan M. Roden

Conducted experiments: Tao Yang

Performed data analysis: Tao Yang, David F. Meoli

Contributed to the writing of the manuscript: Tao Yang, David F. Meoli, Javid Moslehi, Dan M. Roden

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Footnotes

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Figure legends

Fig. 1. Chronic PI3K-α and Pan-PI3K inhibitors caused action potential (AP) prolongation and abnormal APs in adult mouse ventricular myocytes. A, chronic BYL719 (5 hours) prolonged APs in a concentration-dependent manner at a stimulation rate of 1 Hz. **B**, chronic A66 (5 hours) prolonged APs in a concentration-dependent manner at a stimulation rate of 1 Hz. **C**, chronic the Pan-PI3K inhibitor BKM120 (5 hours) prolonged AP at a stimulation rate of 1 Hz. **D**, summary of the effects on APD_{90} by all inhibitors at 1 Hz (n=5-8 each).

Fig. 2. Internally applied PIP3 antagonized action potential (AP) abnormalities by chronic PI3K-α inhibitors in adult mouse ventricular myocytes. A, control AP traces at a stimulation rate of 0.1 Hz. **B**, chronic BYL719 induced triggered activity. **C**, inclusion of PIP3 in the internal pipette solution dramatically attenuated BYL719-induced abnormal action potentials (arrow). **D**, chronic A66 caused triggered activity. **E**, inclusion of PIP3 in the internal pipette solution dramatically attenuated A66-induced abnormal action potentials (arrow). **F**, summary of the numbers of myocytes with abnormal APs in each groups.

Fig. 3. Chronic PI3K-α inhibitors increased late I_{Na} in adult mouse ventricular myocytes. A, control current traces recorded with the protocol shown. **B** and **C**, effects of two concentrations of BYL719 (10 and 100 nM) on late I_{Na} after 5-hr treatment. **D** and **E**, effects of two concentrations of A66 (10 and 100 nM) on late I_{Na} after 5-hr treatment. **F**, summary of the concentration-

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dependent increases in cardiac late I_{Na} in mice by the two inhibitors (n=6-7 each group). The two inhibitors increased late I_{NaL} to same extent.

Fig. 4. Chronic PI3K-α and Pan-PI3K inhibitors increased late I_{Na} in *SCN5A*-transfected CHO cells. **A**, control I_{Na} recorded with the protocol shown. **B**, current trace showing that chronic PI3K-α inhibitor BYL719 increased late I_{Na}. **C**, current traces showing that chronic Pan-PI3K inhibitor BKM120 increased late I_{Na}. **C-F**, current traces showing that the inhibitors targeting PI3K-β, -γ and -δ did not alter late I_{Na}. **G**, current trace showing that internally-adding PIP3 antagonized chronic BYL719-increased late I_{Na}. **H**, summary (n=6-8 each group).