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Androgenic Effects on Ventricular Repolarization

A Translational Study From the International Pharmacovigilance Database to iPSC-Cardiomyocytes

Editorial, see p XXX

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BACKGROUND: Male hypogonadism, arising from a range of etiologies including androgen-deprivation therapies (ADTs), has been reported as a risk factor for acquired long-QT syndrome (aLQTS) and torsades de pointes (TdP). A full description of the clinical features of aLQTS associated with ADT and of underlying mechanisms is lacking.

METHODS: We searched the international pharmacovigilance database VigiBase for men (n=6 560 565 individual case safety reports) presenting with aLQTS, TdP, or sudden death associated with ADT. In cardiomyocytes derived from induced pluripotent stem cells from men, we studied electrophysiological effects of ADT and dihydrotestosterone.

RESULTS: Among subjects receiving ADT in VigiBase, we identified 184 cases of aLQTS (n=168) and/or TdP (n=68; 11% fatal), and 99 with sudden death. Of the 10 ADT drugs examined, 7 had a disproportional association (reporting odds ratio=1.4–4.7; $P<0.05$) with aLQTS, TdP, or sudden death. The minimum and median times to sudden death were 0.25 and 92 days, respectively. The androgen receptor antagonist enzalutamide was associated with more deaths (5430/31 896 [17%]; $P<0.0001$) than other ADT used for prostate cancer (4208/52 089 [8.1%]). In induced pluripotent stem cells, acute and chronic enzalutamide (25 μM) significantly prolonged action potential durations (action potential duration at 90% when paced at 0.5 Hz; 429.7±27.1 (control) versus 982.4±33.2 (acute, $P<0.001$) and 1062.3±28.9 ms (chronic; $P<0.001$), and generated afterdepolarizations and/or triggered activity in drug-treated cells (11/20 acutely and 8/15 chronically). Enzalutamide acutely and chronically inhibited delayed rectifier potassium current, and chronically enhanced late sodium current. Dihydrotestosterone (30 nM) reversed enzalutamide electrophysiological effects on induced pluripotent stem cells.

CONCLUSIONS: QT prolongation and TdP are a risk in men receiving enzalutamide and other ADTs.

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Clinical Perspective

What Is New?

- Men receiving androgen deprivation therapy are at increased risk for drug-induced QT-prolongation and torsades de pointes.
- This study supports the growing concept that cardiomyocytes derived from induced pluripotent stem cells can be useful tool to better understand mechanisms underlying diseases and responses to drugs.

What Are the Clinical Implications?

- In men developing acquired long-QT syndrome or torsades de pointes, diagnostic workup should include evaluation of testosterone blood level, androgen deprivation therapy intake, and evaluation for endocrine conditions associated with hypogonadism.
- In men treated with androgen deprivation therapy, other risk factors for torsades de pointes should be sought and corrected, to avoid accumulation of risks.
- In men treated with androgen deprivation therapy, the role of electrocardiographic monitoring to detect QT-prolongation requires further evaluation.

QT interval duration, measured on the electrocardiogram and corrected for heart rate (QTc), represents the duration of ventricular repolarization. Exaggerated QTc prolongation can cause the potentially fatal ventricular tachycardia torsades de pointes (TdP)[1] in both the congenital form of the long-QT syndrome (LQTS) and an acquired form (aLQTS), often drug-induced. A major mechanism for drug-associated LQTS and TdP is block of the repolarizing potassium-current delayed rectifier potassium current (I_{Kr}), which, in addition to prolonging QTc, also generates morphologically distinctive low amplitude bifid T-waves, seen in patients with type 2 congenital LQTS because of reduced I_{Kr}.[2-6] Recently, Yang et al showed that I_{Kr} blockers with the greatest propensity to cause TdP can also augment the late sodium current (I_{Na-L}) within hours of exposure.[7]

In healthy individuals, QTc is longer in women than in men from puberty to menopause, and women are at higher risk of aLQTS and TdP. Several lines of evidence support the contention that this sex specificity is partly attributable to a testosterone effect to shorten QTc.[8-12] QTc prolongation and TdP in men have been linked to hypogonadism, and correction of testosterone deficiency was associated with shortening of QTc in interventional studies,[9–11,13] and absence of TdP recurrence in a small prospective case series.[14] Modest (10–20 ms) QTc prolongation has been seen with androgen deprivation therapies (ADT) in men with prostatism and with prostate cancer, but cases of ADT-associated TdP and sudden death are limited to case reports.[11,15,16] We previously reported an association between ADT and aLQTS and TdP in a European database and a US electronic health record.[14]

Here, we used VigiBase, the World Health Organization's very large global database of individual case safety reports (ICSRs),[17] to further validate a role for ADTs in men presenting with aLQTS, TdP, or sudden death. We then studied the effects of the top implicated drug, enzalutamide, in Chinese hamster ovary (CHO) cells expressing I_{Kr} and cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs).[7]

METHODS

The data that support the findings of this study are available from the corresponding author upon request.

Epidemiological Study

VigiBase is the World Health Organization's global database of >17 million ICSRs. These originate from >130 country members of the World Health Organization Programme for International Drug Monitoring and are reported by diverse sources such as healthcare professionals, patients, and pharmaceutical companies including reports from the US Food and Drug Administration and the European Medicines Agency, with duplicates flagged and dropped.[18] We performed a disproportionality case/noncase analysis that considered reports of adverse drug reactions (ADR) in men contained in the deduplicated VigiBase,[17] from inception (October 1, 1967) to August 9, 2018. This method compares the proportion of specific ADRs reported for the case versus noncase groups. Reactions are based on the medical dictionary for regulatory activities classification of terms for side effects and terms used to define aLQTS, TdP, sudden death, and death are detailed in Table I in the online-only Data Supplement. For each drug, the number of ADRs under study (ie, aLQTS, TdP, sudden death) divided by the total number of all ADRs for that drug (in this case, each ADT) is compared to the proportion of the same specific ADR (aLQTS, TdP, sudden death) over the total number of ADRs for a comparator group. The comparator group used here was the entire database with all other drugs available but restricted to men because ADTs are used only in men. Comparisons are performed using a chi-square test, and results are displayed using a reporting odds ratio ([ROR] Table II in the online-only Data Supplement).[19] When the lower end of the ROR 95% CI is >1, the signal is deemed significant, and the identified association between the specific drug and the reaction is a potential safety signal. This analytical approach has shown, for example, that the magnitude of drug-induced I_{Kr} blockade correlates with risk for aLQTS/TdP/sudden death in VigiBase.[20] Drugs considered as ADTs were the cytochrome P450 17 inhibitor (abiraterone), gonadotropin-releasing hormone agonists (leuprorelin, goserelin, triptorelin) and antagonists (degarelix), nonsteroidal androgen receptor inhibitors (enzalutamide, bicalutamide, flutamide) indicated for prostate cancer, and 5α-reductase inhibitors (finasteride, dutasteride) indicated for androgenic alopecia and prostatism.[21] The standard dosage and indications for these drugs have been recently detailed elsewhere.[21] The drug used as "positive control" for drug-induced LQTS, TdP, and sudden

death was sotalol.[1,6] The use of confidential electronically processed patient data was approved by the Vanderbilt University Medical Center institutional review board (IRB#181337).

In Vitro Electrophysiology

FuGENE6-Mediated SCN5A and KCNH2 Channel Expression and Cell Transfection

Recombinant cDNA for human *SCN5A* (2 μg, encoding the α-subunit of cardiac sodium channel Nav1.5) or for human *KCNH2* (2 μg, also known as *HERG*, human ether-a-go-go-related gene encoding the α-subunit of cardiac potassium channel Kv11.1) were transiently transfected in CHO cells, as previously reported.[3,7] In brief, *SCN5A* and *KCNH2* DNA were subcloned into the pRc-CMV vector (Stratagene) and transiently transfected into cultured CHO cells using FuGENE6 (Roche Applied Bioscience). To identify transfected cells for electrophysiological study, 0.5 μg plasmid encoding the enhanced green fluorescent protein (pEGFP-N3; BD Bioscience Clontech) was cotransfected. Cells were studied at 48 hours after transfection with or without drug exposures.

Reprogramming and Generating iPSC-CMs

iPSC-CM lines were developed using the episomal vector method from 3 men with normal QTc duration.[7] Briefly, episomal vectors were transfected into erythroblasts via nucleofection. Cells were then plated onto mouse embryonic fibroblast–coated plates. iPSC-like colonies were picked up at approximately day 20 posttransfection. The matrix sandwich method was used to generate iPSC-CM from human iPSCs.[7] Single iPSCs were plated onto Matrigel coated 6-well plates, and growth factors (Activin-A, BMP4, and basic fibroblast growth factor) were added sequentially to differentiate the iPSCs into cardiomyocytes. iPSC-CMs were then replated onto matrigel-coated plates and incubated at 37°C for 30 to 35 days postinduction. Spontaneously beating iPSC-CMs were used for action potential recordings in current-clamp mode. Single cardiomyocytes were used for ion current recordings in voltage-clamp mode after brief trypsinization. This study was approved by Vanderbilt University Medical Center review committee (IRB#040551), and the subjects gave informed consent.

Action Potential Recordings

Action potentials in iPSC-CMs were recorded from spontaneously beating cells at days 30 to 35 postinduction. For these experiments, the bath (extracellular) solution contained (in mmol/L) NaCl 135, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, and glucose 10, with a pH of 7.4 (adjusted by NaOH). The pipette-filling (intracellular) solution contained (in mmol/L) KCl 130, ATP-K_2 5.0, $MgCl_2$ 1.0, $CaCl_2$ 1.0, a calcium-specific aminopolycarboxylic acid 0.1, and HEPES 5.0, with a pH of 7.3 (adjusted by KOH). Microelectrodes with tip resistances of 3 to 5 mΩ were used. Ten successive traces were averaged for analysis of action potential durations (APDs) at 90% repolarization (APD_{90}). Action potentials were recorded before and after acute (15 minutes) or chronic (5 hours) exposure to drugs (enzalutamide and dihydrotestosterone) as detailed in Results.

I_{Kr}, Peak Sodium Current, and $I_{Na\text{-}L}$ Recordings

Whole-cell voltage clamp experiments were conducted at room temperature (22–23°C). To record sodium currents, 2 extracellular bath solutions were used. In CHO cells and iPSC-CMs, the external solution contained (in mmol/L) NaCl 135, KCl 4.0, $MgCl_2$ 1.0, $CaCl_2$ 1.8, glucose 10, and HEPES 10; the pH was 7.4, adjusted with NaOH. The pipette (intracellular) solution contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 10, and HEPES 10; the pH was 7.4, adjusted with CsOH. To eliminate L- and T-type inward calcium currents, as well as outward potassium currents in iPSC-CMs, 1 μmol/L nisoldipine, 200 μmol/L $NiCl_2$, and 500 μmol/L 4-aminopyride were added into the bath solution, respectively. Glass microelectrodes were heat-polished to tip resistances of 0.5 to 2 MΩ. Cells were held at –120 mV, and sodium current was elicited with a single 200-ms pulse from –120 to –30 mV, at which maximal peak inward sodium current is usually observed.

To obtain *KCNH2*-encoded I_{Kr} current–voltage relations, activating current was elicited with a 2-s voltage clamp protocol from a holding potential of –80 mV to 60 mV with 10-mV steps, and deactivating tail current was measured upon a 2-s returning pulse to –40 mV. The cycle time between pulses was 15 s or slower to accommodate pulse durations. Under these conditions, I_{Kr} was stable for >60 minutes in the absence of a drug intervention.[3]

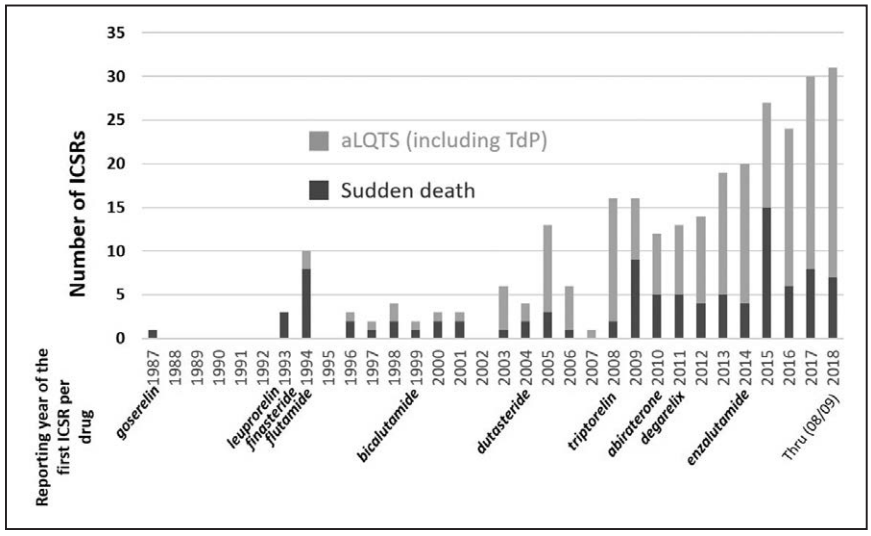

Figure 1. Number of acquired long QT syndrome (aLQTS) including torsades de pointes (TdP) and sudden death associated with androgen deprivation therapies (ADT) reported over time within VigiBase.
Each ADT is noted below the year corresponding to its first associated individual case safety report (ICSR) related to aLQTS, TdP, or sudden death reported in VigiBase.

Table 1. Details Concerning Men With aLQTS and/or TdP (n=184) and Men With Sudden Death (n=99) Associated With ADT in VigiBase

Characteristic		
Reported condition	aLQTS and/or TdP	Sudden death
Age, years	n=146 available	n=82 available
Median [interquartile range]	75 [70–82]	77 [68–85]
Minimum–maximum	[11–94]	[30–95]
Time to onset, days	n=43 available	n=33 available
Median [interquartile range]	170 [37–473]	92 [21–390]
Minimum–maximum	[7–4884]	[0.25–4984]
Indication, n/N (%)		
Prostate cancer	70/91 (77%)	35/44 (80%)
Prostatism	17/91 (19%)	8/44 (18%)
Androgenic alopecia	3/91 (3%)	1/44 (2%)
Sexual disorders	1/91 (1%)	0/44 (0%)
Region of reporting, n/N (%)		
Americas	85/184 (46%)	38/99 (38%)
Europe	85/184 (46%)	44/99 (45%)
Asia, Oceania	14/184 (8%)	17/99 (17%)
Concurrent reported drugs at known TdP risk, n/N (%)[24]		
None	115/184 (62%)	87/99 (88%)
1 other	55/184 (30%)	11/99 (11%)
≥2 others	14/184 (8%)	1/99 (1%)
Concurrent reported drugs at conditional, possible or known TdP risk, n/N (%)[24]		
None	63/184 (34%)	59/99 (60%)
1 other	43/184 (23%)	18/99 (18%)
≥2 others	78/184 (43%)	22/99 (22%)
Proton pump inhibitors	54/184 (29%)	15/99 (15%)
Diuretics (potassium lowering)	45/184 (24%)	18/99 (18%)
Antidepressants	42/184 (23%)	12/99 (12%)
Antiarrhythmics	28/184 (15%)	4/99 (4%)
Antiinfectious	25/184 (14%)	4/99 (4%)
Neuroleptics	21/184 (11%)	1/99 (1%)
Opioids	12/184 (7%)	3/99 (3%)
Antiemetics	12/184 (7%)	3/99 (3%)
Anticancer drugs	12/184 (7%)	1/99 (1%)
Antihistamines	3/184 (2%)	1/99 (1%)
Anti–α1-adrenergics	4/184 (2%)	1/99 (1%)
Other	18/184 (10%)	3/99 (3%)
ADT regimen, n/N (%)		
Monotherapy	155/184 (84%)	82/99 (83%)
Combination therapy	29/184 (16%)	17/99 (17%)
Seriousness, n/N (%)		
Serious	159/159 (100%)	99/99 (100%)
Fatal	12/159 (8%)	99/99 (100%)
Concurrent reported condition favoring aLQTS/TdP, n/N (%)		
Hypokalemia	13/184 (7%)	0/99 (0%)
Hypocalcemia	16/184 (9%)*	0/99 (0%)
Diabetes	32/184 (17%)	7/99 (7%)
Uncontrolled hypertension	13/184 (7%)	0/99 (0%)
Cardiac ischemia or heart failure	22/184 (12%)	9/99 (9%)
Bradycardia or conductive disorders	26/184 (14%)	0/99 (0%)
Atrial fibrillation	33/184 (18%)	1/99 (1%)
Acute kidney injury	23/184 (13%)	0/99 (0%)
Infection (bacteria, fungus, or parasite)	35/184 (19%)	7/99 (7%)
Acute stroke or epilepsy	12/184 (7%)	2/99 (2%)
Reporting year, n/N (%)		
1987–1997	4/184 (2%)	15/99 (15%)
1998–2007	28/184 (15%)	14/99 (14%)
2008–2018	152/184 (83%)	70/99 (71%)

ADT indicates androgen deprivation therapy; aLQTS, acquired long-QT syndrome; and TdP, torsades de pointes.

*11/16 (69%) were taking denosumab.

Data acquisition was carried out using an Axopatch200B patch-clamp amplifier and pCLAMP-9.2 software (MDS-Inc, Canada). Currents were filtered at 5 kHz (–3 dB, 4-pole Bessel filter) and digitized using an analog-to-digital interface (DigiData1322A, MDS-Inc). To minimize capacitive transients, capacitance and series resistance were corrected ~80%. In some experiments, peak sodium current (I_{Na}) magnitudes were expressed in units of picoamperes per picofarad after normalization to cell sizes generated from the cell capacitance calculated by Membrane Test (OUT 0) in pClamp9.2. Clamp protocols used are shown in the figures. $I_{Na\text{-}L}$ (expressed as a percentage of I_{Na}) was measured in a 3-ms time window (195–198 ms after the pulse) before the capacity transient at the end of a 200-ms depolarizing pulse. Electrophysiological data were analyzed using pCLAMP-9.2 software, and the figures were prepared by using Origin 8.5.1 software (OriginLab Corp, USA) to generate figures. Data were recorded before and/or after acute (15 minutes) or chronic (24 or 48 hours) exposure to drugs (enzalutamide and dihydrotestosterone) as detailed in Results.

Chemicals Used for Electrophysiological Studies

Enzalutamide and dihydrotestosterone were purchased from SelleckChem and Sigma-Aldrich, respectively. Stock solutions for the tested drugs were prepared according to the vendors' instructions and then diluted for studies, as needed. The concentrations used for dihydrotestosterone (30 nM) and enzalutamide (25 μM) were within human physiological and therapeutic ranges, respectively.[22,23]

Descriptive Statistical Analysis

Results were described in terms of mean±SD (clinical data), ±SEM (preclinical data), or medians (interquartile range) for quantitative variables, and in terms of number and proportion for qualitative variables. Comparisons used unpaired *t* test or Mann-Whitney tests for quantitative variables, and χ^2 test for qualitative variables (Prism-7; GraphPad). Statistical significance was accepted for $P<0.05$.

RESULTS

Clinical Characteristics of Patients With aLQTS, TdP, and Sudden Death Associated With ADT

In VigiBase, 283 ICSRs of aLQTS, TdP, or sudden death associated with ADT were identified (Figure 1), and their clinical characteristics are detailed in Table 1. These ADT were mainly used to treat prostate cancer, with prostatism and androgenic alopecia as less common indications. Most cases of aLQTS/TdP (115/184 [62%]) and sudden death (87/99 [88%]) associated with ADT were reported without exposure to other drugs known to confer TdP risk. The times to onset between ADT introduction and cardiac events were scattered, ranging from few hours with ADT being the only suspected intervention to years after ADT introduction in the context of multiple other risk factors or other drugs conferring TdP risk (Table 1).[24] Among concurrent reported risk factors for aLQTS/TdP (Table 1), hypocalcemia was present in 16/184 (9%) of ICSRs, usually seen with denosumab (11/16 [69%]), which may reflect its use in patients with bone metastases. Hypokalemia was present more often in abiraterone cases (6/21 [29%]) versus other ADT (7/163 [4%]; $P<0.0001$), consistent with the drug's known action to generate hypermineralocorticism.[25]

Table 2. Number (n) of ICSRs in Men in VigiBase by ADT, With Sotalol (Positive Control) and in the Entire Database Through August 9, 2018

	n_{death}/n_{total}, (%)	aLQTS, n*	TdP, n*	Sudden Death, n*	$n/n_{aLQTS±TdP±Sudden-death}$, (%) With ADT Considered Suspect by Reporter**
Enzalutamide	5430/31 896 (17%)	19	4	13	30/32 (93.8%)
Abiraterone	1240/14 261 (8.7%)	19	7	10	29/31 (92.5%)
Bicalutamide	724/10 144 (7.1%)	23	16	11	28/41 (68.3%)
Leuprorelin	1871/22 113 (8.5%)	33	16	18	28/55 (50.9%)
Finasteride	1062/33 877 (3.1%)	52	20	32	20/87 (23%)
Goserelin	471/5821 (8.1%)	8	2	15	17/22 (77.3%)
Degarelix	82/2787 (2.9%)	7	4	3	10/11 (90.9%)
Triptorelin	52/1517 (3.4%)	6	3	2	5/8 (62.5%)
Dutasteride	248/15 177 (1.6%)	26	7	11	5/38 (13.2%)
Flutamide	163/4075 (4.0%)	4	2	3	3/7 (42.9%)
Sotalol	210/9541 (2.2%)	134	152	29	NA
Entire database	161 130/6 560 565 (2.5%)	7288	2769	4880	NA

ADR indicates adverse drug reaction; ADT, androgen deprivation therapy; aLQTS, acquired long-QT syndrome; ICSR, individual case safety reports; NA, not available; ROR, reporting odds ratio; and TdP, torsades de pointes.

*These numbers in ADT and sotalol rows correspond to the A values in the contingency table displayed in Table II in the online-only Data Supplement, explaining how ROR (=AD/BC) is calculated. For example, there were 9541 ADRs reported with sotalol and 152 cases of TdP (1.59%) as compared to 6 551 176 total ADRs on all other drugs in men, including 2617 cases of TdP (0.04%). This results in a ROR of 40.51 for the association between sotalol and TdP (see Table 3).

**The numerator is the number of ICSRs where the ADT was considered by the reporter to be suspect of directly inducing the aLQTS or TdP or sudden death. The denominator is the number of ICSRs where the ADT was associated to a drug-induced aLQTS or TdP or sudden death, in which the reporter may have considered the ADT as suspect, interacting, or concomitant.

Disproportionality Analysis in VigiBase

The number of total ICSRs reported in men in VigiBase on each of the 10 ADTs analyzed and the subsets of those with aLQTS, TdP, sudden death, and death are detailed in Table 2. Overall, we found 184 cases presenting aLQTS (n=168) and/or TdP (n=68; 11% fatal), and another 99 who developed sudden death associated with ADT. Analysis of VigiBase reports in men through August 9, 2018, revealed 6 560 565 ICSRs (n=7288 aLQTS; n=2769 TdP; n=4880 sudden death) for the full database from >130 countries. Seventy percent of ADT (7/10) had a disproportional association (ROR, 1.4–4.7; $P<0.05$) with aLQTS, TdP, or sudden death (Table 3). Sotalol was used as positive control and had a significant disproportional association with

Table 3. Association of ADTs With the ROR for aLQTS, TdP, and Sudden Death in VigiBase (Through August 9, 2018)

	ROR [CI_{95}]		
	aLQTS	TdP	Sudden Death
Enzalutamide	(-)	(-)	(-)
Abiraterone	(-)	(-)	(-)
Bicalutamide	2.1 [1.4–3.1]	3.8 [2.3–6.1]	(-)
Leuprorelin	(-)	1.7 [1.1–2.8]	(-)
Finasteride	1.4 [1.1–1.8]	(-)	(-)
Goserelin	(-)	(-)	3.5 [2.1–5.8]
Degarelix	2.3 [1.1–4.8]	3.4 [1.3–9.1]	(-)
Triptorelin	3.6 [1.6–8]	4.7 [1.5–14.6]	(-)
Dutasteride	1.6 [1.1–2.3]	(-)	(-)
Flutamide	(-)	(-)	(-)
Sotalol	13.03 [10.97–15.48]	40.51 [34.36–47.77]	4.11 [2.85–5.93]

Significantly increased ROR and 95% confidence interval (CI_{95}) when comparing reporting rate for aLQTS, TdP, and sudden death associated with sotalol (positive control) or ADTs versus the full database. Associations not significantly increased are displayed by (-). ADT indicates androgen deprivation therapy; aLQTS, acquired long-QT syndrome; ROR, reporting odds ratio; and TdP, torsades de pointes.

aLQTS, TdP, and sudden death (Table 3). For example, there were 9541 ADRs reported with sotalol, and 152 cases of TdP (1.59%) as compared to 6 551 176 total ADRs on all other drugs in men, including 2617 cases of TdP (0.04%). This results in a ROR of 40.51 for the association between sotalol and TdP (P<0.0001). Enzalutamide was associated with the highest rate of death (5430/31 896 [17%]; P<0.0001; with the numerator being the number of ICSRs with a death outcome and the denominator being the overall number of ICSRs associated with enzalutamide) compared to the other ADTs used for prostate cancer (4208/52

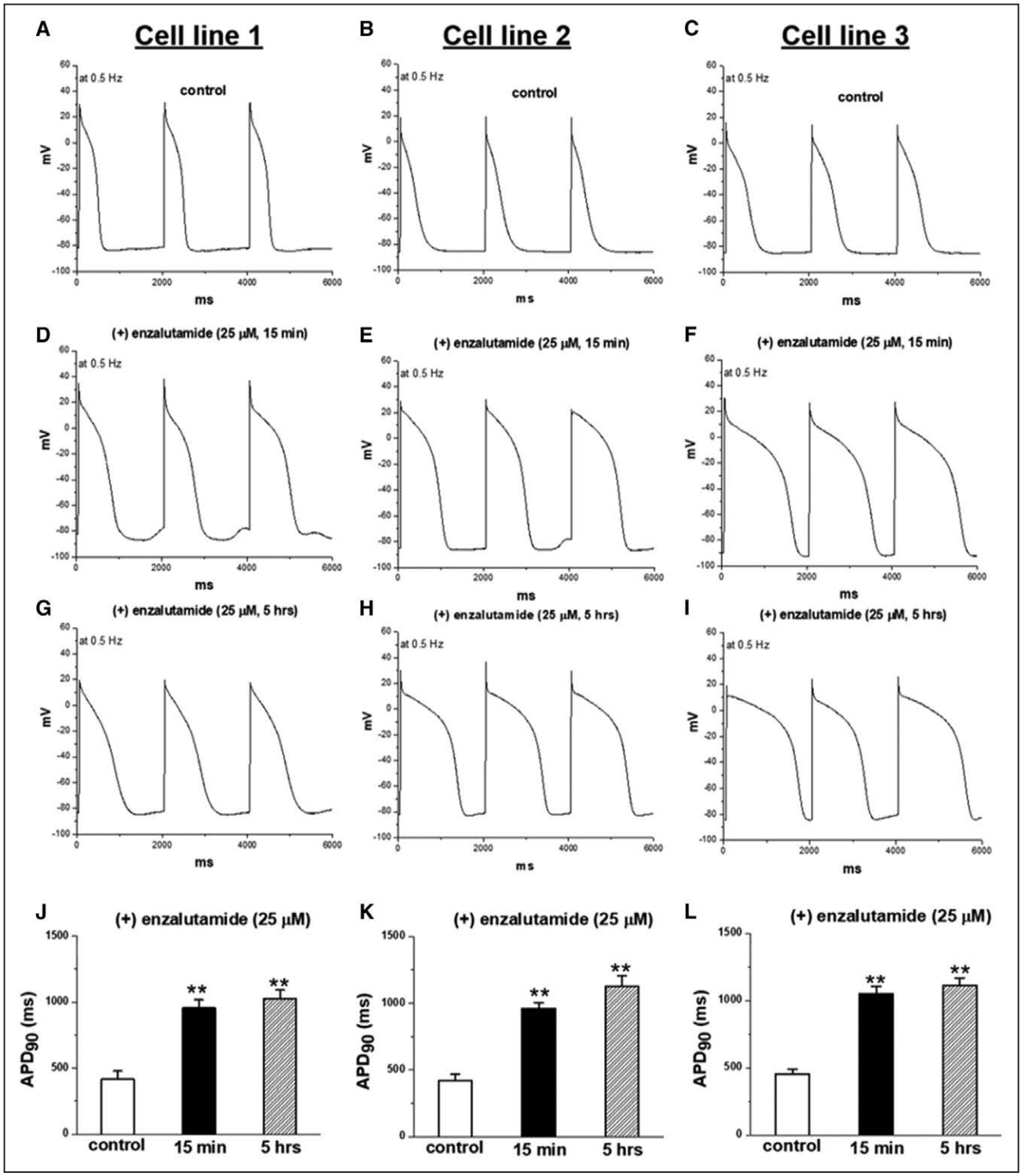

Figure 2. Electrophysiological effects of enzalutamide on IPSC-CMs action potential duration.
In cardiomyocytes derived from induced pluripotent stem cells from 3 different male lines (**A** through **C**), acute (**D** through **F**) and chronic (**G** through **I**) exposure to enzalutamide prolonged action potential duration (**J** through **L**). **P<0.01 versus control (n=5–7 each). APD_{90} indicates action potential durations at 90% repolarization.

089 [8.1%]; degarelix, abiraterone, flutamide, bicalutamide, goserelin, leuprorelin, triptorelin; Table 2) or prostatism (1303/48 720 [2.7%]; dutasteride, finasteride). Enzalutamide was associated with a total of 32 aLQTS, TDP, or sudden death, and enzalutamide was almost always considered a responsible drug by the reporter (n=30/32 [93.8%]; Table 2).

Androgen Effects on Ventricular Repolarization

In these experiments, we studied enzalutamide, the ADT most strongly associated with death. In IPSC-CMs from 3 male subjects, acute and chronic exposure to enzalutamide prolonged APD_{90} recorded during stimulation at 0.5 Hz from 429.7±27.1 (control) to 982.4±33.2 (acute; $P<0.001$) and 1062.3±28.9 ms (chronic; $P<0.001$), and early-/delayed-afterdepolarizations and/or triggered activity were elicited in enzalutamide treated cells (11/20 acutely and 8/15 chronically versus 0/15 in nontreated cells; $P=0.001$; Figure 2). Acute dihydrotestosterone exposure reversed APD_{90} prolongation observed on acute and chronic enzalutamide exposure at a stimulation of 0.5 Hz from 933±105 ms (acute enzalutamide) to 397±113 ms (dihydrotestosterone acute; $P<0.01$) and 863±86 ms (chronic enzalutamide) to 275±57 ms (dihydrotestosterone acute; $P<0.01$; Figure 3). Acute dihydrotestosterone exposure in non–enzalutamide-treated cells also shortened APD_{90} from 439.5±79.2 ms to 189.2±23.3 ms ($P<0.01$; Figure 3). In CHO cells transfected with KCNH2, acute and chronic exposure to enzalutamide decreased tail current measured after pulses to +20 mV from 1050±253 at baseline to 492±108 (acute; $P<0.01$) and 307±57 pA (chronic; $P<0.01$; Figure 4). Conversely, exposure to dihydrotestosterone increased tail current from baseline 760±77 to 1051±79 (acute; $P<0.05$) and 1698±218 pA (chronic; $P<0.01$; Figure 4). In IPSC-CMs, chronic exposure

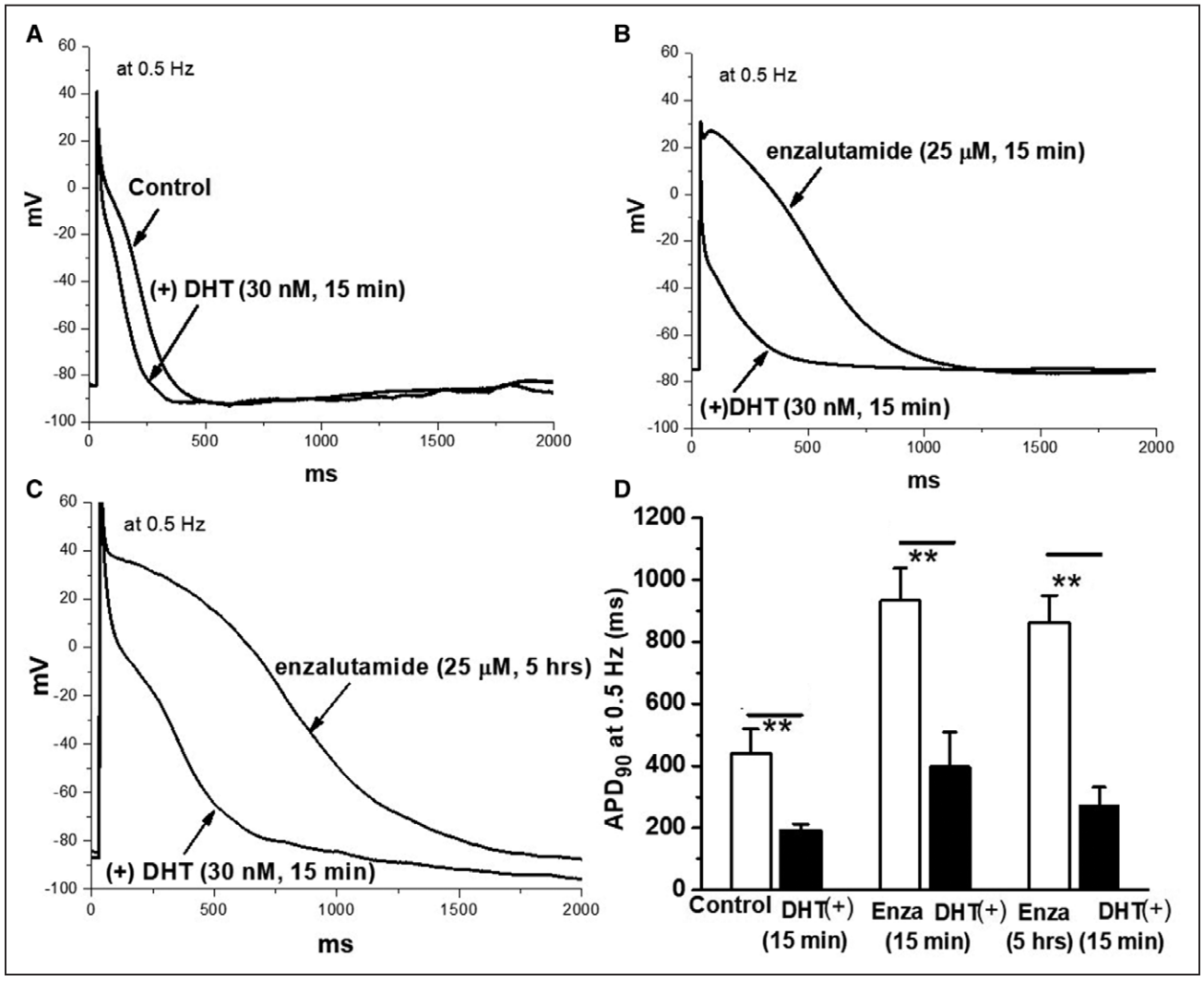

Figure 3. Electrophysiological effects of dihydrotestosterone on IPSC-CMs action potential duration.
A through **C** are typical action potential traces in 3 groups of men cardiomyocytes derived from induced pluripotent stem cells in the absence and presence of dihydrotestosterone (DHT). **D**, Summary of these groups of cells. Acute and chronic enzalutamide (Enza) prolonged action potentials whereas DHT had opposite effect. $**P<0.01$ (n=4–5 each). APD_{90} indicates action potential durations at 90% repolarization.

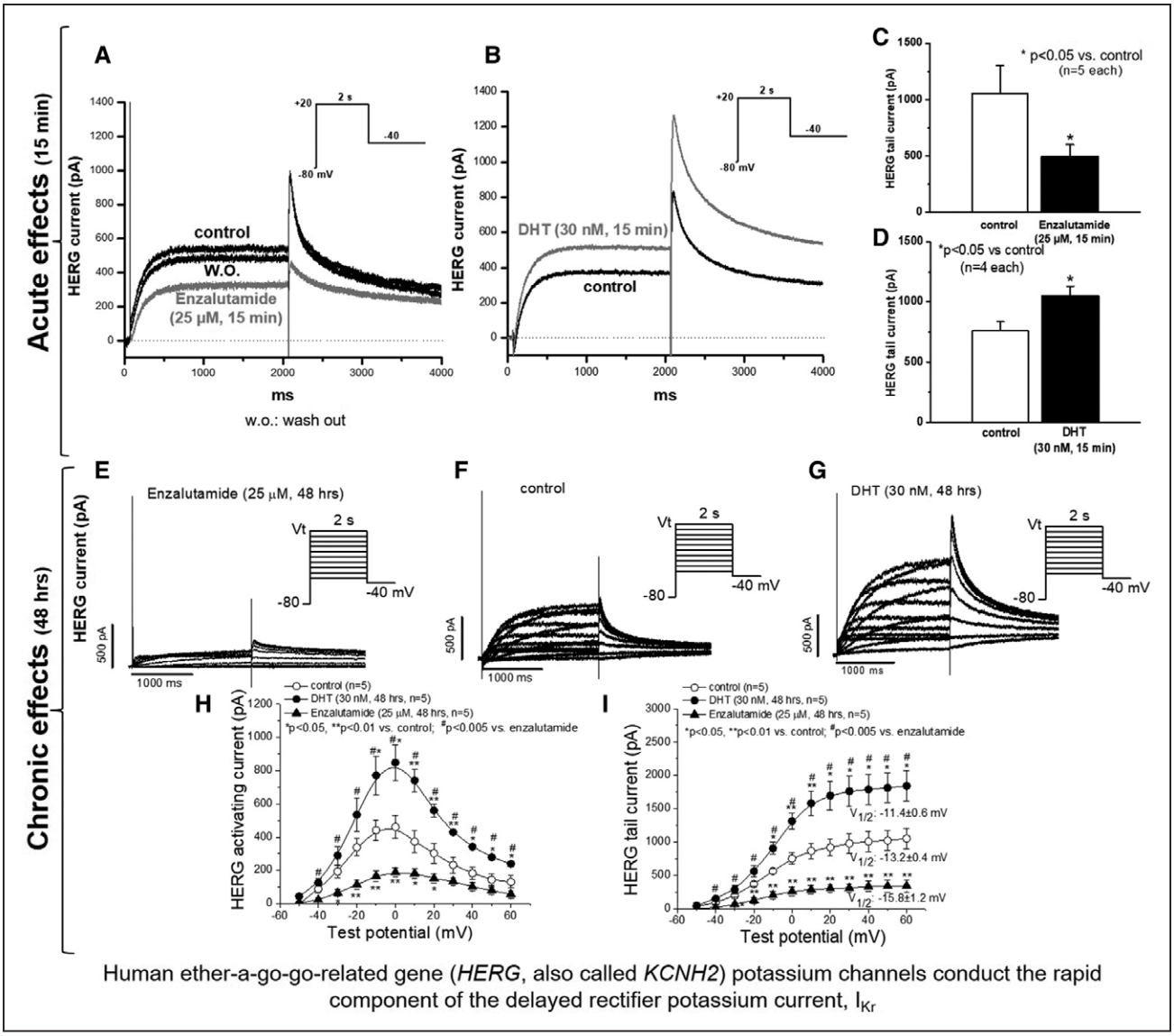

Figure 4. Enzalutamide and dihydrotestosterone effects on I_{Kr}.
In Chinese hamster ovary cells, acute (**A** through **D**) and chronic (**E** through **I**) exposure to enzalutamide (**A**, **C**, **E**, **H**, **I**) decreased I_{Kr}. Conversely, exposure to dihydrotestosterone (DHT) increased I_{Kr} (**B**, **D**, **G**, **H**, and **I**). P<0.005 was deemed significant to account for multiple comparisons (Bonferroni adjustment; **H** and **I**). **P*<0.05 versus control; ***P*<0.01 versus control; #*P*<0.005 versus enzalutamide (n=5 each). pA indicates picoamperes; and Vt, the voltage at the pipette tip.

to enzalutamide significantly increased both peak I_{Na} from 169±12 to 293±23 picoamperes per picofarad (*P*<0.01), and I_{Na-L} from 0.2±0.04% to 2.1±0.5% of peak current (*P*<0.01; Figure 5). In CHO cells transfected with SCN5A, similar results were seen (Figure I in the online-only Data Supplement). Figures 2 and 5 summarize acute and chronic effects of enzalutamide on APD_{90} (Figures 2A through 2D), and peak I_{Na}/I_{Na-L} (Figures 5A through 5D) of IPSC-CMs. Figure 3 summarizes acute dihydrotestosterone effects on APD_{90} of IPSC-CMs from men subjects already exposed acutely or chronically on enzalutamide. Figure 4 summarizes acute and chronic effects of dihydrotestosterone (Figures 4B, 4D, and 4G through 4I) and enzalutamide (Figures 4A, 4C, 4E, 4H, and 4I) on I_{Kr} in CHO cells.

DISCUSSION

Taken together, our analyses by multiple translational approaches consistently support the concept that ADT is a cause of aLQTS and TdP. The in vitro work here provides further support for this concept and specifically for the idea that treatment of hypogonadism by testosterone replacement therapy can shorten QTc duration and treat and/or prevent TdP.[9–11,13,14] These results provide a strong justification for a clinical recommendation to systematically investigate the possibility of hypogonadism and ADT intake when men are evaluated for aLQTS or TdP and suggest electrocardiographic monitoring may have a place in the surveillance of men with known hypogonadism or when treated with ADT.

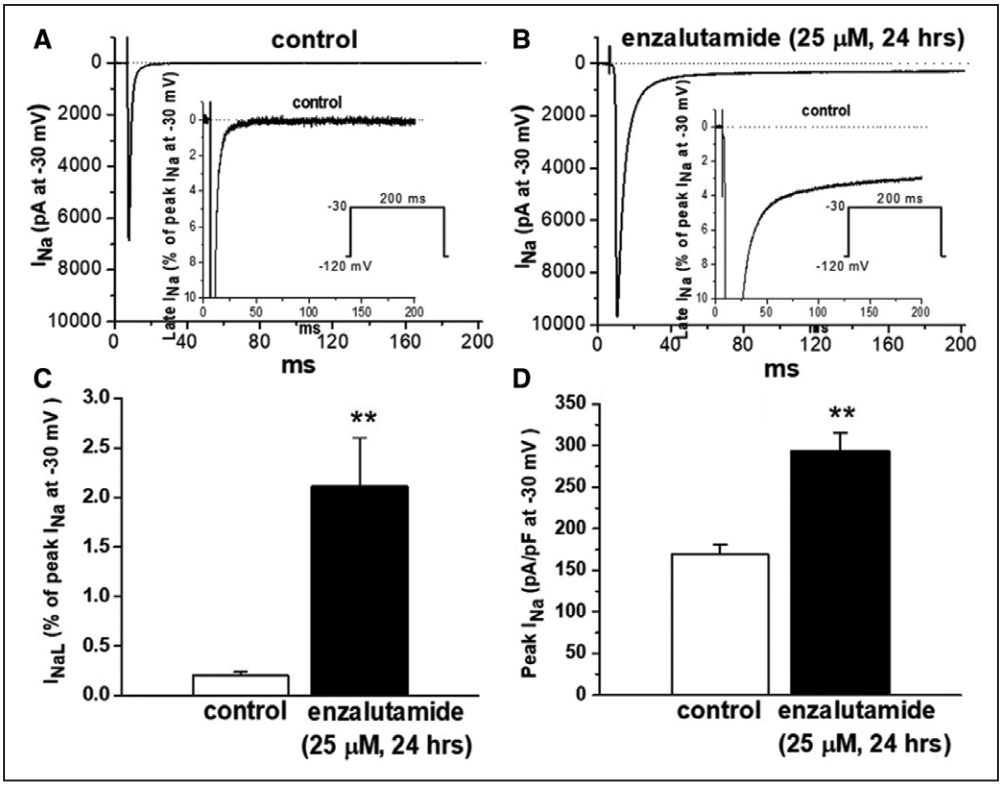

Figure 5. Enzalutamide effects on I_{Na-L}.
Chronic exposure to enzalutamide increased ventricular peak (I_{Na}) and late (I_{Na-L}) sodium current in cardiomyocytes derived from induced pluripotent stem cells (**A** through **D**). ***P*<0.01 versus control (n=4 each). pA indicates picoamperes; and pF, picofarad.

The findings in the experiments do provide insights into mechanisms whereby enzalutamide, a competitive androgen antagonist, and dihydrotestosterone modulate APD_{90}.[26] Dihydrotestosterone shortened APD acutely, and this appears to be related to I_{Kr} enhancement. Conversely, enzalutamide prolonged APD both acutely and chronically. The acute effect likely reflects I_{Kr} blockade, while late I_{Na-L} enhancement may contribute chronically. This dual time-dependence has also been seen with other potent QT-prolonging drugs causing TdP, such as dofetilide[27] and terfenadine.[28] The electrocardiographic effects[29] observed in a case series of 7 men with aLQTS/TdP associated to androgen deficiency (decreased T-wave maximal amplitude and notching) are also consistent with a predominant role for I_{Kr} block.[14] Gagliano-Jucá et al recently showed in a 6-month prospective cohort study that ADT shortened QRS and prolonged QTc in men with prostate cancer starting ADT versus a control group of men who previously underwent prostatectomy for cancer and were not receiving ADT.[15] The clinical finding of shortened QRS is consistent with the increase in peak I_{Na} we observe hereafter in chronic exposure to enzalutamide. Other groups have studied effects of androgens in vitro on multiple systems and with heterogeneous and discordant results that have been summarized recently.[21] In this study, we used physiological doses of dihydrotestosterone, as well as therapeutic concentrations of enzalutamide. Enzalutamide is a second-generation androgen receptor antagonist active in prostate cancer that has become resistant to first generation androgen receptor antagonists (flutamide, bicalutamide, nilutamide),[30,31] which are weaker blockers of the androgen receptor.[32] We selected enzalutamide for in vitro experiments since the pharmacovigilance signal was the largest in terms of absolute numbers of suspected ADR associated with aLQTS, TdP, sudden death, and death. Of note, the association of enzalutamide with death was strikingly higher (17% of total ICSRs) than that for other ADT (1.6% to 8.7%) or entire database (2.5%); this may result in a competition (termed "masking bias") between sudden death and aLQTS/TdP, accounting for the absence of a positive ROR between enzalutamide and aLQTS, TdP, or sudden death.[33]

ADT are the cornerstone of treatments for prostate cancer or adenoma, and may be also used for androgenic alopecia in younger men. There is no mention in the latest European Society of Cardiology and American Heart Association position papers on cancer treatments and cardiovascular toxicity that ADT use might lead to aLQTS, and no specific caution is recommended.[34,35] Degarelix and leuprolide are the only ADT considered at possible risk for TdP according to the reference website http://www.CredibleMeds.org, which presents TdP risk classification of drugs.[24] Guidelines will need to be developed to appropriately monitor and manage this

risk, particularly knowing that other anticancer and non–cancer-related drugs used in combination carry additional TdP risk.[1,36] Interestingly, all classes of ADT, even those with mild ADT effects (eg, 5α-reductase inhibitors),[37] appeared to be associated with ventricular arrhythmic events. The risk of TdP with 5α-reductase inhibitors is particularly noteworthy since these drugs are indicated in benign conditions, including prostatism and androgenic alopecia.[37]

Importantly, this study supports the growing concept that IPSC-CMs can contribute to the understanding and possible novel management of clinical conditions such as drug-induced diseases and to move further toward personalized medicine. This concept is just beginning to receive initial support from translational studies such as this work, incorporating a clinical part and an experimental one, using IPSC-CMs. For example, using a similar strategy, a recent study showed that lumacaftor/ivacaftor—drugs developed to improve cell surface trafficking of mutant proteins in cystic fibrosis—significantly shortened the QTc in 2 LQTS type 2 patients with a trafficking defect; these in vivo findings supported in vitro data suggesting improved trafficking of mutant *KCNH2* in iPSC-CMs.[38]

A limitation of the analyses of the pharmacovigilance databases is that the data come from uncontrolled sources. Nevertheless, the preclinical mechanistic studies, the case series,[14] the literature,[21] and the population analyses of ADT and of the positive control sotalol provide cross-validation for the causal—and treatable—relationship we postulate between male hypogonadism, because of either endocrine conditions or ADT and TdP risk. Further mechanistic studies are needed to better decipher mechanisms downstream of androgen receptor pathway leading to I_{Kr} and $I_{Na}/I_{Na\text{-}L}$ modulation, but these studies were beyond the objective of this work aiming at raising awareness concerning ADT use and TdP risk, as well as potential therapeutic use of testosterone for aLQTS and TdP.

CONCLUSIONS

Cautious prescription and electrocardiogram monitoring should be considered in men on ADT, particularly when at risk of TdP. Androgens might be useful to prevent or treat TdP in men.

ARTICLE INFORMATION

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Disclosures

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