ADMINISTRATION OF SEX HORMONES AS DRUGS TO ATTENUATE DRUG-INDUCED LENGTHENING OF VENTRICULAR REPOLARIZATION

by

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This work is dedicated to my husband, John, my parents and my brother. My dad for instilling me from young age with the profound value of higher education and life-long learning, my mom for sharing her passion for medicine and science, my brother for his unconditional support and finally my husband for his continual love and faith in me pursuing my dream to become a clinical scientist.

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LIST OF ABBREVIATIONS

α	Alpha (Intercept)
ACCF	American College of Cardiology Foundation
ADD	Additive
AHA	American Heart Association
AIC	Akaike information criteria
APD	Action potential duration
AP	Action Potential
AUEC	Area under the effect curve
BPH	Benign prostatic hyperplasia
BOV	Between-occasion variability
BSV	Between-subject variability
C	Concentration
CI	Confidence interval
Cl_d	Distribution clearance
Cl_s	Systemic clearance
CPAC	Clinical pharmacology analytical core
CWRES	Conditional weighted residual
DHT	Dihydrotestosterone
Dl	Deciliter
E	Effect
EADs	Early afterdepolarization
EC_{50}	Serum concentration required to achieve 50% of the maximum effect
ECG	Electrocardiogram
E_{max}	Maximum effect
FDA	Food and Drug Administration
FOCE	First order conditional estimation method
GOF	Goodness-of-fit plots
H	Hill coefficient
HFrEF	Heart failure with reduced ejection fraction
HPLC/MS	Reverse-phase high-performance liquid chromatography with mass spectrometry detection
hERG	Human ether-a-go-go-related gene
I_{Ca-L}	L-type calcium
ICRC	Indiana clinical research center
IDS	Investigational drug services
IRB	Institutional review board
I_{kr}	Rapid component of delayed rectifier potassium current

I_{ks}	Slow component of delayed rectifier potassium current
IOV	Inter-occasion variability
IU	Indiana university
LOQ	Lower limit of quantification
LQTS	Long QT syndrome
NOS3	Nitric oxide synthase 3
Ng	Nanograms
Ml	Milliliters
OFV	Objective function value
OR	Odds ratio
P:E	Progesterone-to-estradiol ratio
PI3K	Phosphatidylinositol 3-kinase
QTc	Bazett's heart-rate corrected QT interval
QTcI	Individual-corrected QT interval
QT_F	Fredericia-corrected QT interval
QT_{Fram}	Framingham-corrected QT interval
pcVPC	Prediction corrected visual predicted checks
PD	Pharmacodynamic
PK	Pharmacokinetic
PKPD	Pharmacokinetic-pharmacodynamic
PROP	Proportional
RSE	Residual standard error
RUV	Residual unexplained variability
SEM	Standard error of the mean
SD	Standard deviation
σ	Sigma (Residual variability)
θ	Theta (Population estimate)
TdP	Torsades des pointes
US	United States
Vc	Central compartment volume of distribution
Vp	Peripheral compartment volume of distribution
WT	Weight
ω	Omega (inter-individual variability)

ABSTRACT

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Title: Administration of Sex Hormones as Drugs to Attenuate Drug-Induced Lengthening of Ventricular Repolarization
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Background:

The heart rate-corrected QT (QTc) interval is the electrocardiogram (ECG) representation of ventricular repolarization. Prolongation of the QTc interval is a marker of increased risk of torsades de pointes (TdP), a potentially fatal ventricular arrhythmia. More than 150 commonly used drugs available in the United States (US) can cause QTc interval prolongation and TdP. Few effective strategies have been developed to reduce the risk of drug-induced QTc interval prolongation and TdP, particularly in patients at high risk. In view of the catastrophic consequences of TdP and the widespread use of QTc interval-prolonging medications (including antimicrobials, antidepressants, and other widely-used drug classes), there is an urgent need to identify strategies to reduce the risk in high-risk populations.

The risk of drug-induced QTc interval prolongation and TdP is higher in women than men. Ventricular repolarization occurs more rapidly in men, manifested by shorter QTc intervals, a difference which becomes apparent only after puberty, suggesting that changes in serum sex hormone concentrations are responsible. Post-pubertal differences in QTc intervals may be largely due to the production of testosterone in males, which has been shown to shorten ventricular repolarization. However, although female sex is a risk

factor for drug-induced TdP, 29-46% of reported cases have occurred in men. Older age (> 65-68 years) is an independent risk factor for drug-induced QTc interval prolongation and TdP in both men and women. In men, this may be attributable to age-related declining serum testosterone concentrations.

Higher serum progesterone concentrations have been associated with shorter QT intervals, and preclinical studies indicate that exogenous progesterone administration may protect against drug-induced prolongation of ventricular repolarization, ventricular early afterdepolarizations and arrhythmias. Tisdale et al have previously shown that administration of oral progesterone at a dose of 400 mg once daily attenuates drug-induced QT interval lengthening in young healthy premenopausal women during the menses phase of the menstrual cycle, when endogenous serum estradiol and progesterone concentrations are low. These data provide support for further study of the efficacy, safety and clinical feasibility of oral progesterone administration for reducing the risk of drug-induced QT interval prolongation and TdP in patients with risk factors who require therapy with QT interval-prolonging drugs. However, substantial inter-subject variability was present in this study; some subjects demonstrated a substantial response to progesterone-mediated attenuation of drug-induced QT interval lengthening, while the response was limited or absent in other subjects. Identifying and addressing sources of inter-subject variability in response is essential to optimize future clinical utilization of oral progesterone as a novel therapy to attenuate drug-induced QT interval lengthening in high-risk female patients.

In vitro data indicate that testosterone and progesterone shorten early ventricular repolarization through inhibition of the L-type calcium current ($I_{Ca,\,L}$) and late ventricular

repolarization via enhancement of the slow component of the delayed rectifier potassium current (I_{ks}). However, the *in vivo* effects of testosterone and progesterone on attenuation of drug-induced early versus late ventricular repolarization are unknown. Recently, the novel ECG biomarkers J–Tpeak (corrected for heart rate as J-Tpeakc) and Tpeak-Tend interval (which does not require heart rate correction) have been shown to be accurate biomarkers to assess exogenous effects on different components of ventricular repolarization. Tpeak-Tend/QT is another biomarker that has been used to characterize transmural dispersion of ventricular repolarization, which is associated with an increased risk of TdP. Clinical data have shown that J-Tpeakc interval accurately reflects changes in early repolarization associated with L-type calcium and late sodium (I_{NaL}) currents while the Tpeak-Tend interval reflects late repolarization associated with rapid and slow delayed rectifier potassium currents (I_{Kr} and I_{Ks}). Determination of the effects of administration of testosterone and progesterone on attenuation of early versus late ventricular repolarization is important, as it will allow assessment of whether these hormones can be administered as drugs to attenuate lengthening of QTc interval induced by medications that primarily influence early repolarization, late repolarization, or both.

Objective: In Aim 1, we sought to dermine the influence of transdermal testosterone and oral progesterone on drug-induced QT interval lengthening in older men. In Aim 2, we determined the effect of both transdermal testosterone and oral progesterone on drug-induced lengthening of early vs late ventricular repolarization reflected by J-Tpeak and Tpeak-Tend intervals in older men. Furthermore, we sought to establish the influence of oral progesterone on the J-Tpeak and Tpeak-Tend intervals in premenopausal women. In Aim 2, we also assessed the effect of the administered sex hormones on Tpeak-Tend/QT

to evaluate if progesterone and/or testosterone attenuate dispersion of ventricular repolarization in older men and in premenopausal women. In Aim 3, we sought to develop a pharmacokinetic-pharmacodynamic (PKPD) model with data collected in the previously published study to identify sources of variability in response to oral progesterone for attenuation of drug-induced QT interval lengthening in young premenopausal women.

Methods:

In aim 1 and 2, a prospective, randomized, double-blind, placebo-controlled three-way crossover-design study was conducted, in which 14 healthy male volunteers 65-86 years of age were randomized to receive 7 days of treatment with: a) transdermal testosterone 100 mg and oral placebo once daily, b) oral progesterone 400 mg and transdermal placebo gel once daily or c) transdermal placebo gel and oral placebo once daily (between-phase washout $\geq$ 13 days) . In each phase, on the day after the 7^{th} day of drug/placebo administration, the QT interval-lengthening drug ibutilide 0.003 mg/kg was infused over 10 minutes, after which ECGs were recorded serially for 8 hours. QT intervals, J–Tpeakc and Tpeak–Tend intervals were measured manually from lead II with computerized electronic caliper (EP Calipers 1.6) by one investigator (E.T.M.) who was blinded to the subjects' assigned groups. QT interval was corrected for heart rate with the Fridericia (QT_F) and Framingham (QT_{Fram}) methods and the J-Tpeak interval was corrected for heart rate using the formula introduced by Strauss et al [J-Tpeakc = J-Tpeak / (RR)0.58], respectively. Statistical analyses were performed using repeated-measures ANOVA with *post-hoc* testing using the Bonferroni correction for three group comparisons (QTc interval analysis) (SPSS Inc, Chicago, IL).

In aim 3, data from previously published prospective, randomized, double-blind, placebo-controlled two-way crossover-design study was utilized, in which 15 healthy female volunteers were randomized to receive progesterone 400 mg or matching placebo orally once daily for 7 days during the menses phase of the menstrual cycle. On the morning after the last dose, ibutilide 0.003 mg/kg was infused over 10 minutes and serial ECGs and blood samples for determination of serum ibutilide concentrations were collected for 12 hours. A population PKPD model was developed by linking serum ibutilide concentrations and QT_F intervals. Demographic and clinical data [including progesterone:estradiol serum concentration ratio (P:E)] were evaluated as covariates. Monte Carlo simulations were performed to evaluate the effects of P:E on drug-induced QT_F lengthening.

Results:

In Aim 1, we investigated the effects of transdermal testosterone and oral progesterone on drug-induced QT interval lengthening in a population of older men. Fourteen subjects were enrolled and completed the study. There were no significant differences in maximum serum ibutilide concentrations across the three study phases. Per study design, serum testosterone and progesterone concentrations were significantly higher during the transdermal testosterone and oral progesterone phases, respectively. Mean (±SD) age was 73±6 years (range 65-86); n=13 were white. There was no significant difference between testosterone, progesterone and placebo in pre-ibutilide QT_F (393±19 vs 399±16 vs 399±13 ms; p=0.09). Maximum post-ibutilide QT_F interval was significantly different across the three phases (416±19 vs 425±22 vs 426±18 ms; p<0.001; Bonferroni, p=0.004,

testosterone vs placebo). Area under the effect (QT_F):time curve for 1 hour ($AUEC_{0\text{-}1.17}$) following the 10-minute ibutilide infusion was significantly different across the three phases (471±24 vs 480±24 vs 483±18 ms•hr; p<0.001; Bonferroni, p=0.002, testosterone vs placebo). $AUEC_{0\text{-}8.17}$ was also significantly different across the phases of the study (3255±173 vs 3304±145 vs 3335±142 ms•hr; p<0.01; Bonferroni, p=0.004, testosterone vs placebo). Adverse effects included progesterone-associated fatigue (n=1, 7%) and a mild skin rash associated with transdermal placebo gel (n=1, 7%).

In Aim 2A/B, we assessed the influence of oral progesterone and transdermal testosterone in older men and of oral progesterone in premenopausal women on attenuation of drug-induced lengthening of early and late ventricular repolarization. We also assessed influence of oral progesterone and transdermal testosterone on Tpeak-Tend/QT in older men. Baseline (pre-ibutilide) J-Tpeakc was significantly different across the three phases of the study (216±23 vs 226±28 vs 227±21 ms; p=0.004; Bonferroni, p=0.01, testosterone vs placebo). There were no significant differences in baseline (pre-ibutilide) Tpeak-Tend intervals between the testosterone, progesterone and placebo phases [70±13 vs 70±8 vs 75±13 ms; p=0.16]. Progesterone did not significantly shorten baseline J-Tpeakc or Tpeak-Tend intervals. The mean maximum J-Tpeakc (246±29 vs 233±22 vs 246±29 ms; p<0.001; Bonferroni, p<0.001 testosterone vs placebo) and Tpeak-Tend intervals [89±18, 80±12 vs 87±15 ms; p=0.008; Bonferroni, p<0.001, testosterone vs placebo] were significantly different across the phases of the study. There were no significant differences in mean maximum J-Tpeakc nor Tpeak-Tend intervals between the progesterone and placebo phases. J-Tpeakc $AUEC_{0\text{-}1.17}$ [273±32 vs 262±25 vs 277±26 ms•hr; p<0.001; Bonferroni, p<0.001, testosterone vs

placebo] and Tpeak-Tend $AUEC_{0\text{-}1.17}$ [92±15 vs 86±13 vs 93±14 ms•hr; p=0.001; Bonferroni, p<0.001, testosterone vs placebo] were significantly different across the testosterone, progesterone and placebo phase. J-Tpeakc $AUEC_{0\text{-}8.17}$ (1797±176 vs 1881±207 vs 1915±191 ms•hr; p<0.001; Bonferroni, p<0.001, testosterone vs placebo) and Tpeak-Tend $AUEC_{0\text{-}8.17}$ (583±79 vs 628±95 vs 626±85 ms•hr; p=0.008, Bonferroni, p<0.001, testosterone vs placebo) were also significantly different across the three phases, indicating both a short-term and more prolonged attenuation of both early and late-phase ventricular repolarization. Oral progesterone did not significantly influence J-Tpeakc and Tpeak-Tend $AUEC_{0\text{-}1.17}$ or $AUEC_{0\text{-}8.17}$ compared to placebo. We established that maximum Tpeak-Tend/QT intervals were significantly different across the three phases of the study (0.2146±0.035 vs 0.1985±0.023 vs 0.2085±0.032; p=0.041; Bonferroni , p=0.084, testosterone vs placebo) in older men. In aim 2C/D it was determined that oral progesterone did not attenuate early or late ventricular repolarization compared to placebo among premenopausal women.

In Aim 3, we developed a pharmacokinetic-pharmacodynamic (PKPD) model to identify sources of variability in response to progesterone for attenuation of drug-induced QT interval lengthening. A two-compartment model with elimination from the central compartment best described ibutilide concentration-time profiles. An Emax model with an intercept term best described the ibutilide concentration-QT_F relationship. The only significant covariate on the maximum effect was P:E (ΔOFV -10.745, p<0.01). Simulations demonstrated that subjects with P:E ≥ 129 achieve clinically relevant progesterone-mediated attenuation of maximum ibutilide-induced QTF lengthening.

Conclusion: In older men, transdermal testosterone attenuates drug-induced QT interval lengthening by shortening both early (J-Tpeakc) and late (Tpeak-Tend) repolarization phases. Transdermal testosterone also significantly attenuates maximum Tpeak-Tend/QT compared to placebo. Oral progesterone does not attenuate drug-induced QT interval lengthening nor lengthening of early and late repolarization in older men. Further research is needed to assess the efficacy, safety and feasibility of transdermal testosterone as a novel therapeutic modality to attenuate drug-induced QT interval lengthening in high-risk older men. Attenuation of late repolarization was only significant after ibutilide administration which implies that testosterone enhances I_{Ks} current only when the human Ether-a-go-go-Related Gene (hERG) is also inhibited. In premenopausal women, oral progesterone attenuated neither early nor late ventricular repolarization compared to placebo.

A PKPD model was developed that describes the relationship between serum ibutilide concentrations and QT intervals in young healthy premenopausal women taking oral progesterone for the purpose of attenuating drug-induced QT interval lengthening. The PKPD model identified the ratio of serum progesterone:estradiol concentrations (P:E) as a predictor of progesterone-mediated attenuation of drug-induced QT interval lengthening and that P:E $\geq$ 129 is associated with clinically relevant attenuation of drug-induced QT interval lengthening.

INTRODUCTION

QT interval prolongation and torsades de pointes

The heart rate-corrected QT (QTc) interval is a measure of duration of both ventricular depolarization and repolarization. According to an American Heart Association (AHA)/American College of Cardiology Foundation (ACCF) Scientific Statement, a prolonged QT interval is defined as a rate-corrected QT (QTc) interval greater than the 99th percentile for adult females and males, which is > 480 ms and > 470 ms, respectively.[1] A prolonged QTc interval reflects prolongation of the repolarization phase of the ventricular action potential[2] and increases the risk of TdP, a polymorphic ventricular arrhythmia that can result in sudden cardiac death.[3] The TdP risk increases markedly when the QTc interval exceeds 500 ms[1] and may be increased when the QTc interval is prolonged > 60 ms compared to the pretreatment value [4]. QTc interval prolongation is widely used in both clinical practice and drug development as a marker for an increased risk of TdP, which typically requires both a trigger as well as a substrate for reentry.[5] Prolongation of ventricular repolarization can lead to oscillations in the membrane potential known as early afterdepolarizations (EADs), which may trigger TdP. EADs can occur as a result of increased calcium or sodium current.[5] Inhibition of hERG current due to exogenous factors such as QTc interval-prolonging drugs can cause significant transmural dispersion of repolarization due to differential expression of ion channels throughout the heart, which can create a substrate for reentry. If dispersion of repolarization across the myocardium is present, an ectopic beat followed by a long pause with a subsequent sinus beat showing marked QTc interval prolongation occurs. The ectopic beat can induce reentrant excitation and TdP.[6] While in many cases TdP

terminates spontaneously, it can degenerate into ventricular fibrillation and result in sudden cardiac death if immediate action is not taken.[6]

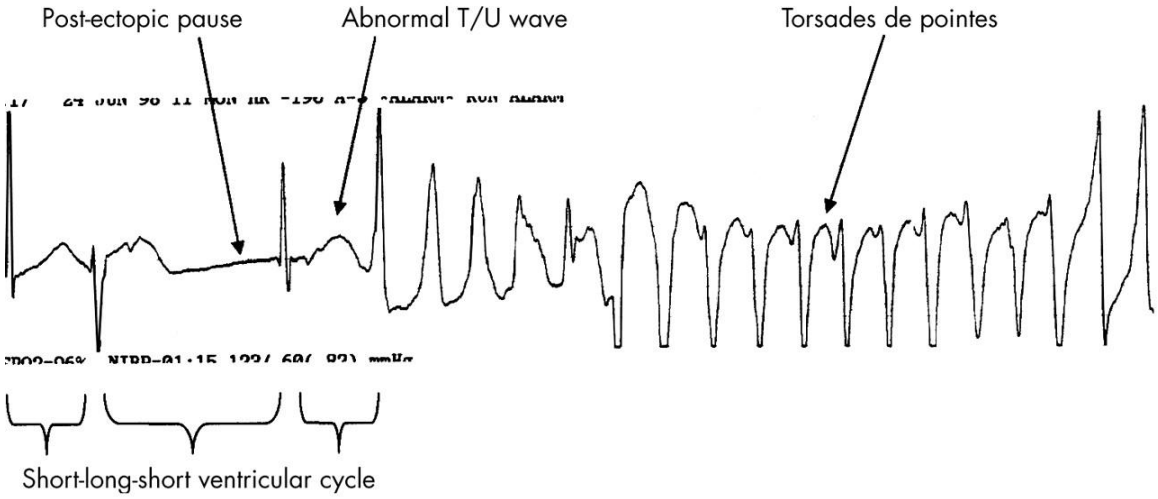

Figure 1 Rhythm strip in a patient with drug induced TdP, characterized by the typical short-long-short initiating ventricular cycle, pause-dependent QT interval prolongation, and abnormal TU wave leading to the classical "twisting of the points" of the cardiac axis during TdP.

Reprinted with permission from: Yap YG, Camm AJ Drug induced QT prolongation and torsades de pointes. Heart 2003;89:1363-1372.

Inherited and acquired long-QT syndrome

QTc interval prolongation can be inherited [congenital long QT syndrome (LQTS)] or acquired. While 17 types of congenital LQTS have been identified[7], LQT1, LQT2 and LQT3 account for the majority of clinical cases. LQT1 is caused by mutations in KCNQ1, which codes the α-subunit for the channel for conduction of the slow component of the delayed rectifier potassium current (I_{Ks}). LQT2 mutations can be found in both the pore units as well as the non-pore-forming units of the channel conducting the rapid component of delayed rectifier potassium current (I_{Kr}), and they account for 35-

45% cases of LQTS. LQT3 is caused by mutations in the late sodium channel gene SCN5A, and account for roughly 8-10% of cases of LQTS.[6]

Acquired QTc interval prolongation is caused most commonly by drugs.[8] Within the past two decades, several high profile drugs, including the urinary antispasmodic agent terodiline, the fluoroquinolone antibiotics sparfloxacin and grepafloxacin, the nonsedating antihistamines terfenadine and astemizole, and the gastrointestinal promotility drug cisapride have been withdrawn from the US market as a result of inducing deaths from TdP.[9] Sertindole was also withdrawn from US market in 1998 due to having caused several cases of sudden cardiac death during a clinical trial.[10] However, more than 150 Food and Drug Administration (FDA)-approved medications that can cause QTc interval prolongation and have the potential to cause TdP remain available in the US.[11]

Mechanisms by which drugs induce QTc interval prolongation

The majority of drugs that prolong the QTc interval do so by blocking the late repolarizing hERG potassium current which plays a critical role in ensuring proper ventricular repolarization.[12] Inhibition of hERG current can lengthen the late phase of ventricular repolarization and cause dispersion of repolarization, which is a substrate of reentry that may lead to the development of TdP.[5] Other drugs such as arsenic oxide, pentamidine and fluoxetine can prolong the QT interval by a different mechanism which is the disruption of KCNH2 protein trafficking.[6] Furthermore, growing evidence has shown that well-established hERG blockers such as dofetilide, haloperidol, thioridazine, erythromycin may also augment late sodium current, which can result in lengthening of early ventricular repolarization and induction of EADs.[13] Newly developed anti-cancer

drugs that inhibit phosphoinositide 3-kinases (PI3Ks) including dasatinib, sunitinib and nilotinib have also been shown to increase the risk of QTc interval prolongation and TdP, however they do so through an indirect effect on cardiac ion currents via a inhibition of the PI3K pathway.[14] By decreasing PI3K/Akt signaling, they indirectly block I_{Kr} and augment late sodium current.[14] The growing number of marketed QTc-interval-prolonging drugs and their divergent multichannel modulatory effects have caused drug-induced QTc interval prolongation to be growing clinical problem.

Increased incidence of QTc interval prolongation with specific drugs

The incidence of drug-induced TdP in general population remains largely unknown and it varies significantly based on the population studied and the specific culprit drugs. In one observational study, 3.1% of the patients taking noncardiac medications developed TdP.[10] Another study conducted in Sweden established that the annualized incidence of TdP in the general population is approximately 4 per 100,000.[15] The incidence of TdP associated with specific QTc interval–prolonging drugs varies from 2% to 12% and is highly dependent on the specific drug, the dose administered and the concomitant presence of other risk factors.[16]

Antiarrhythmic agents are the class of drugs that one of the most common cause of drug-induced QTc interval prolongation. For class IA agents (quinidine, procainamide and disopyramide), cases of TdP has been reported at both therapeutic and subtherapeutic doses.[17] Roden et al established that quinidine lengthens the QT interval by 10–15% on average within the first week of treatment and carries a 1.5 to 10% risk of inducing TdP.[18, 19] Another class IA agent, procainamide, was believed to confer a reduced risk of

TdP due to its inhibitory effect on late sodium current;[20] however, cases of TdP in patients using this drug with kidney disease have been reported due to its active metabolite, N-acetylprocainamide, which is an I_{Kr} inhibitor.[20]

Vaughan Williams class III antiarrhythmic agents are potent I_{Kr} inhibitors, with dose-dependent QTc interval lengthening effects.[10] Low heart rates potentiate the potassium blocking effect due to reverse-use dependency.[21] Dofetilide, ibutilide and sotalol exhibit the highest risk for TdP, while amiodarone has the lowest risk due to being a "balanced ion-channel blocker".[5] Sotalol induces TdP in 2–4% of patients, and women are at higher risk for sotalol-induced TdP compared to men.[22] Dofetilide causes TdP in 0.9% of patients with normal LV function and 3.3% in subjects with heart failure.[23] Ibutilide, which activates late sodium current as well as inhibiting I_{Kr} current[24], induced TdP in 1–3% of subjects and the incidence of drug-induced TdP is higher among patients with heart failure, structural heart disease, and electrolyte imbalances.[25]

Several antipsychotic medications are known possess dose-dependent QT interval-prolonging effects. Haloperidol, prescribed to treat schizophrenia and severe agitations, lengthens the QT interval by 15-30 ms by potently inhibiting I_{Kr} current.[26] While in clinical practice it is considered safe to administer up to 2 mg of haloperidol intravenously, the FDA issued a warning for intravenous administration of this drug and has recommended ECG monitoring during its use.[26] Droperidol has a similar QTc prolonging profile to haloperidol and should be administered with caution in subjects with concomitant risk factors for drug-induced QTc prolongation.[10] Phenothiazines, such as chlorpromazine and thioridazine, are known to be cause drug-induced QTc interval prolongation due to their inhibitory effect on late repolarization potassium currents.[10]

Atypical antipsychotics have also been linked to dose-dependent QTc interval prolongation. Ziprasidone exhibit the highest risk, while olanzapine demonstrates the lowest QTc interval-prolonging risk profile.[27]

Among antidepressants, tricyclics have been more commonly associated with prolongation of the QTc interval.[28] Cases of TdP have been reported with amitriptyline, desipramine and imipramine.[29] The selective serotonin-uptake inhibitors citalopram and escitalopram have also been linked to QTc interval prolongation due to their inhibitory effect on I_{Kr} current.[10]

The effect of fluoroquinolones on the QT interval length varies amongst the agents. Grepafloxacin and sparfloxacin were withdrawn from the US market due to cardiovascular safety concerns as they induced a significant lengthening of ventricular repolarization similar to that of class III antiarrhythmics. Others such as gatifloxacin, levofloxacin, and moxifloxacin exhibit QTc interval-prolonging potential but with a lower risk of developing TdP .[30] Macrolide antibiotics, specifically clarithromycin, telithromycin, azithromycin and erythromycin, have also been linked with QTc interval prolongation and TdP.[31, 32] Erythromycin was found to have similar arrhythmogenic profile to class III antiarrhythmic agents due to its potential to induce EADs and transmural dispersion of repolarization. The FDA issued a warning in 2013 stating that use of azithromycin may lead to a potentially fatal irregular rhythm, following a study which established that 5 days long use of azithromycin therapy may result in a small absolute increase in cardiovascular deaths.[33] Finally, several antifungal agents of the azole group exhibit QTc interval prolonging effects and have the potential to induce TdP, particularly in subjects with other concomitant risk factors.[34]

Risk factors for QTc interval prolongation

Risk factors are important with respect to the development of drug-induced QTc interval prolongation and the occurrence of TdP.[35] Compared to patients with no risk factors, the odds ratio (OR) for QTc interval prolongation in individuals with 1 risk factor is 3.2 (95% confidence interval [CI] 2.1-5.5). The risk measured by the OR increases significantly in patients with 2 or ≥3 risk factors (7.3 [4.6–11.7] and 9.2 [4.9–17.4], respectively).[36] In the absence of other risk factors, QTc interval prolongation rarely leads to TdP. Zektser et al established that almost all patients who developed TdP had one risk factor and 71% had more than one risk factor.[37] Among the most significant risk factors are female sex, older age (≥65 years), hypokalemia, hypomagnesemia and hypocalcemia, hepatic dysfunction and kidney disease (for drugs that are hepatically metabolized and renally eliminated, respectively), bradycardia, heart failure, sepsis, pretreatment QTc interval > 450 ms, concurrent use of more than one QT interval-prolonging drug, rapid intravenous infusion of a QT interval-prolonging drug, high serum concentrations of QT interval-prolonging drugs and use of diuretics.[10, 35] Bradycardia predisposes patients to QT interval prolongation and TdP to due reverse-use dependence.[10] Hypokalemia can worsen QT interval prolongation induced by drugs by enhancing drug-induced inhibition of I_{Kr} current.[38] Patients with heart failure are at higher risk for developing a prolonged QT interval due to downregulation of potassium channels and upregulation of L-type Ca^{2+} channels which underlies the pathophysiology of the disease.[39, 40] Liver and kidney disease can lead to increased serum concentrations of QT interval-prolonging drugs due to a decrease in their clearance and elimination. Furthermore, other pharmacokinetic interactions may occur by co-administration of drugs that reduce the clearance of other QT-prolonging drugs.[10] Co-administration of a QT prolonging drug metabolized by

CYP3A4 with CYP3A4 inhibitors such as '-azoles', '-mycins' or grapefruit juice may result in an increased exposure.[41] Simultaneous administration of both haloperidol and thioridazine, which are both metabolized by CYP2D6, results into increased concentrations of both drugs and therefore higher risk of QT interval prolongation.[42]

Female sex as a risk factor for QTc interval prolongation

Female sex is an independent risk factor for TdP in patients with acquired or congenital long QT syndrome.[43, 44] A review of the literature and FDA databases determined that that a much higher percentage of women than men develop TdP after taking a variety of drugs, such as antihistamines (terfenadine, astemizole), antibiotics (erythromycin), antimalarials (halofantrine), antiarrhythmics (quinidine, d-sotalol), and miscellaneous other drugs. Another review of 332 patients describing at least one case of polymorphic ventricular tachycardia (with sex specified) established that women are more prone than men to develop TdP during administration of cardiovascular drugs that prolong cardiac repolarization.[32] QT intervals are longer in women than men; ventricular repolarization occurs faster in men, which is manifested by shorter QT intervals.[45, 46] J-Tpeakc interval, an *in vivo* measure of early repolarization, have also been found to be also significantly longer in women than in men.[47] These differences in both QT and J-Tpeak length become apparent only after puberty, suggesting that increasing testosterone concentration may exert a protective effect in males.[47] In women, QTc interval length fluctuates throughout the menstrual cycle and during pregnancy, suggesting that fluctuations in progesterone and estradiol influence ventricular repolarization.[48] Nakagawa et al determined that QT intervals are 10 ms shorter during the luteal phase of the menstrual cycle compared to the follicular and ovulation phase, suggesting that

progesterone may counteract the effects of the estrogen-induced QT interval prolongation.[48] Burke et al reported that QTc interval is reduced in the luteal phase in the presence of autonomic blockade compared to the follicular and ovulation phase.[49] Rodriguez et al demonstrated that drug-induced QTc interval lengthening is significantly less during the luteal phase compared to the other phases of the menstrual cycle, and that men exhibit significantly attenuated drug-induced QT interval prolongation compared to females.[50]

A variety of studies in postmenopausal women have shown that estrogen replacement therapy prolongs QT intervals.[51-53] A long-term (1 year) study established that use of estrogen replacement therapy increases QT intervals, while a progestin–estrogen replacement therapy does not lengthen ventricular repolarization.[54]

Older age as a risk factor for QT interval prolongation and TdP

As serum testosterone concentrations naturally decline with aging, a lengthening of QT interval is observed in older men compared to their younger counterparts.[55, 56] Further, older men no longer exhibit significantly shorter QTc intervals compared to women of the same age.[45, 47, 57]

The risk of drug-induced TdP in men 55-65 years of age is > 3x higher than in those 45-55 years, and increases in subsequent decades of age.[58] Our group and others have shown that older age is an independent risk factor for QTc interval prolongation and TdP in both sexes.[35, 59-62] In men, this may be partly due to declining serum testosterone/DHT concentrations,[56, 63] as an inverse relationship between serum testosterone concentrations and QT intervals has been demonstrated.[63] Furthermore, J-Tpeakc was determined to be significantly longer in older men compared to younger men; no significant differences in

J-Tpeakc were determined between young and older women.[47] Declining serum testosterone concentrations, therefore, may be partly responsible for the increase in risk of drug-induced TdP in older men and testosterone may exerts its protective effect on the QTc interval length by decreasing duration of early repolarization.[47]

QTc interval prolongation and TdP in men

While female sex is a risk factor for drug-induced TdP[64, 65], 29-46% of all reported cases have occurred in men. The risk of drug-induced TdP in men 55-65 years of age is > 3x higher than in those 45-55 years and increases in subsequent decades of age.[56, 63] In reports of erythromycin-associated cardiac arrhythmias from 1970-1996, 32% of the cases occurred in men.[66] In an analysis of all published cases of TdP associated with noncardiac drugs, 29% occurred in men.[67] Of 100 cases of cisapride-associated TdP reported to the FDA, for which patient sex was specified, 30% occurred in men.[68] Close to half (46%) of reported cases of azimilide-induced TdP occurred in men,[58] while 40% of cases of terfenadine-associated TdP occurred in men.[69] Similar percentages have been reported regarding TdP associated with halofantrine (40% of cases in men)[64] and quinidine (34% in men).[65] Therefore, while female sex is a risk factor, a substantial proportion of drug-induced TdP cases occur in men.

Effect of testosterone on ventricular repolarization

A variety of animal studies have provided evidence for the QTinterval -shortening effects of testosterone. It was shown that exogenous administration of dihydrotestosterone (DHT) shortens the QT interval and APD_{90} in orchiectomized rabbits.[70] Liu et al reported the density of I_{K1} and I_{Kr} currents are decreased in female

rabbits compared to males and that administration of DHT increased current densities of I_{K1} and I_{Kr} in orchiectomized male rabbits.[70] DHT administration reduced the incidence of polymorphic ventricular tachycardia and protected against sudden cardiac death in a transgenic rabbit model of LQTS type 2.[71] APD_{30}, epicardial APD_{90}, transmural dispersion of repolarization and incidence of EADs (in the presence of dofetilide) are greater in females than male rabbits presumably due to testosterone's protective effects. Dofetilide-induced ADP_{90} prolongation and EAD incidence was found to be greater in normal female rabbits compared to DHT-treated female rabbits.[72]

Clinical studies have shown that testosterone is associated with reduced QT interval length in men compared to women. Bidoggia et al. proposed the hypothesis that testosterone plays a role in modulating ventricular repolarization.[57] They measured ventricular repolarization in 27 castrated men, 26 women with virilization, and 53 control subjects pair-matched for age and sex. They showed that repolarization in castrated men was longer than that of normal men. Furthermore, they observed that virilized women exhibited a shorter and faster repolarization than normal women and castrated men. Finally, they demonstrated that lengthening in repolarization length observed in castrated men may be reversed by administering exogenous testosterone. Similar findings were demonstrated by Vrtovec et al.[73], Gazi et al.[74] and Abehsira et al.[75]. These studies suggest that testosterone has a shortening effect on QT interval. Several cross-sectional studies involving thousands of men of different ages and varying concentration of testosterone determined that men with higher testosterone concentration have significantly shorter QTc intervals than those with lower concentrations.[63, 76]

Testosterone modulates cardiac repolarization by both a genomic as well as a non-genomic pathway. Testosterone was shown to regulate gene expression of L-type calcium channel in ventricular myocytes by increasing L-type calcium channel mRNA following 8 hour exposure as well as 300 fold increase in androgen receptor abundance after a 6 hour exposure.[77] Bai et al then sought to determine testosterone's influence on action potential duration (APD) and membrane currents in ventricular myocytes though patch-clamp experiments.[78] Testosterone was found to shorten APD in a dose-dependent manner. Testosterone-induced APD shortening is modulated by enhancement of I_{Ks} current at a low concentration (EC50 = 1.1 nmol/L), while at a high concentration (IC50 = 38.8 ± 3.5 nmol/L) by both enhancement of I_{Ks} as well as inhibition of $I_{Ca,L}$ Both I_{Ks} enhancement and $I_{Ca,L}$ suppression were determined to be induced by NOS3 activation and NO production via a nongenomic pathway. Testosterone activates NOS3 through its nongenomic (nonnuclear) pathway, in which binding of testosterone to membrane-localized testosterone receptors activates tyrosine kinase, c-Src, followed by sequential activation of phosphatidylinositol 3-kinase (PI-3 kinase), Akt, and then NOS3. NOS3 then produces NO, which enhances I_{Ks} and inhibits $I_{Ca,L}$ at different concentrations, suggesting that NO acts on these two ion channels with a different mechanism. It was proposed that inhibition of $I_{Ca.L}$ would require cGMP, while enhancement of I_{Ks} does not.[78]

Effect of progesterone on ventricular repolarization

In animal studies, progesterone was also found to influence repolarization length and to exert protective effects against drug-induced lengthening of ventricular repolarization. Progesterone decreases the density of L-type Ca^{2+} currents in rabbit

cardiomyocytes.[71] Progesterone was also shown to induce a concentration-dependent decrease in hERG current density in rat cardiac myocytes.[79] Prepubertal ovariectomized transgenic LQT2 rabbits treated with progesterone exhibited reduced premature ventricular contractions, bigeminy and polymorphic ventricular tachycardia compared to estrogen-treated rabbits; overall progesterone administration protected against sudden cardiac death in a transgenic rabbit model of LQTS type 2.[71] Progesterone pretreatment was also shown to attenuate dofetilide-associated lengthening of QT intervals and reduced the incidence of drug-induced TdP in isolated perfused rabbit hearts.[80]

Clinical data listed in the above paragraphs provides preliminary evidence for the protective effect of progesterone against drug-induced QT interval prolongation. Evidence from Tisdale et al has shown that that administration of oral progesterone at a dose of 400 mg once daily attenuates drug-induced QT interval lengthening in young healthy premenopausal women during the menses phases of the menstrual cycle, when endogenous serum estradiol and progesterone concentrations are low.[81] These data provide support for further study of the efficacy, safety and clinical feasibility of oral progesterone administration for reducing the risk of drug-induced QT interval prolongation and TdP in patients with risk factors who require therapy with QT interval-prolonging drugs. However, substantial inter-subject variability was present in this study; some subjects demonstrated a response to progesterone-mediated attenuation of drug-induced QT interval lengthening, while the response was limited or absent in other subjects. The mechanism by which this protective effect is exerted has been established by Nakamura et al.[82] Through experiments in guinea pig ventricular myocytes it was determined that the effects of progesterone on I_{Ks} were frequency or voltage independent,

while progesterone (100 nmol/L) did significantly reduce $I_{Ca,L}$ current which resulted in APD shortening only under SNS-stimulated conditions. Progesterone-induced I_{Ks} enhancement and reduction of cAMP-induced $I_{Ca,L}$ are mediated by nitric oxide (NO), which is released through the c-Src/PI3-kinase/Akt– dependent eNOS activation.[82] By carrying out simulations in the Faber-Rudy model of the guinea pig myocyte, Nakamura et al also predicted the effects of progesterone on LQTS-associated arrhythmia susceptibility. They determined that at progesterone concentration of 40.6 nmol/L, which is the physiological concentration observed in women during the luteal phase of the menstrual cycle, severe early afterdepolarizations (EADs) induced by 50% block of I_{kr} were completely abolished and the action potential morphology was normalized; a concentration of 2.5 nmol/L, which reflects the serum level in the follicular phase, showed only little improvement in abolishing EADs.[82] Preliminary evidence from another group also advances a possible role of progesterone in regulating early repolarization current via inhibition of the late sodium current. Jackson et al investigated progesterone binding to σ_1- and σ_2- receptors and its associated effect on σ-receptor-mediated modulation on voltage-gated Na^+ channels. They determined that progesterone's inhibition of voltage-gated Na^+ current is mediated by a stronger antagonism of σ_2- receptors.

Indicators of early vs late ventricular repolarization (J-Tpeak and Tpeak-Tend)

While drug-induced QTc interval prolongation is an important risk factor for the development of acquired TdP, not every case of drug-induced QTc interval prolongation carries equal TdP risk. Some QTc interval-prolonging drugs such as verapamil or amiodarone are less likely to cause TdP compare to others, such as dofetilide or

quinidine.[5, 83] This can be explained by their multichannel modulation of cardiac currents of early vs late ventricular repolarization.[5, 83-85] These drug-induced multichannel effects cannot be captured *in vivo* by changes in QTc interval length. Alternative ECG measurements heart-rate corrected J-Tpeak (J-Tpeakc) interval and Tpeak-Tend interval have been recently proposed as biomarkers for the assessment of multichannel drug effects on human ventricular repolarization.[5, 86-88]

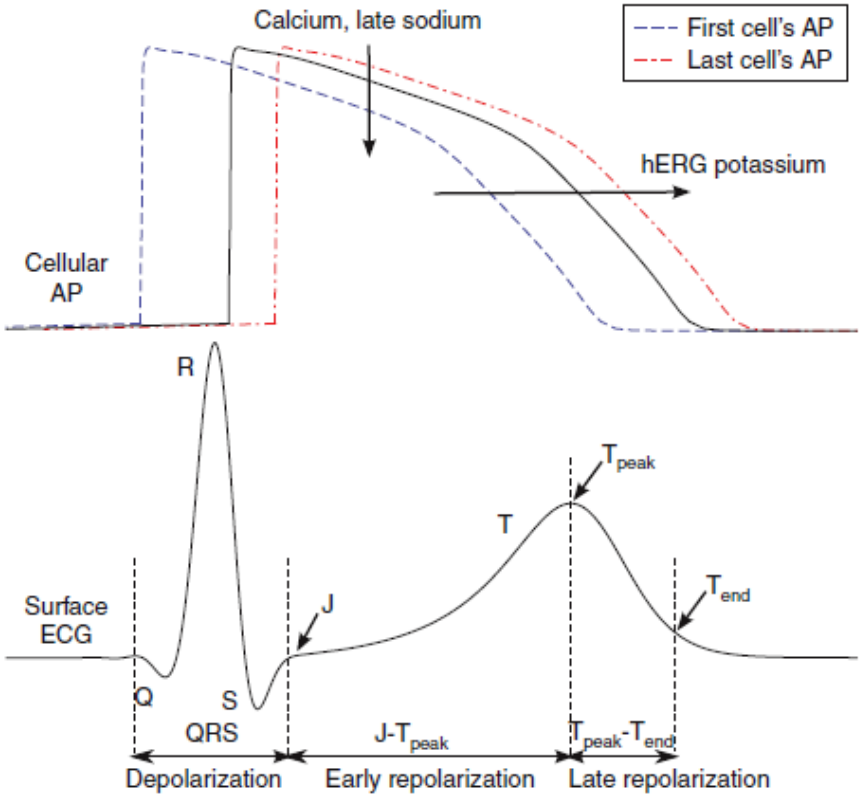

Figure 2 An illustration of a ventricular action potential (AP) and the corresponding surface electrocardiogram (ECG). Arrows pointing into the action potential are inward currents (calcium and late sodium) and arrows pointing out denote outward currents (human ether-à-go-go-related gene (hERG) potassium). Blocking the calcium or late sodium current primarily shortens the early phase of repolarization (J–Tpeak), whereas hERG potassium channel block prolongs both early (J–Tpeak) and late repolarization (Tpeak–Tend).

Reprinted with permission from: Johannesen L, Vicente J, Mason JW, et al. Differentiating drug-induced multichannel block on the electrocardiogram:

By dividing the QTc interval into components of early (J-Tpeakc) and late repolarization (Tpeak-Tend), it is possible to differentiate the multichannel effects of a drug.[5, 87, 89] In the presence of hERG potassium channel block, changes in J-Tpeakc interval length reflect the effect of a QTc prolonging drug on early repolarization due to modulation of the inward late sodium and/or calcium currents. On the other hand, changes in Tpeak-Tend interval length reflect late repolarization via a drug's modulation of outward potassium currents I_{ks} and I_{kr}.[5] Strauss et al demonstrated that these new electrocardiographic biomarkers of ventricular repolarization are effective in assessing *in vivo* multicurrent block induced by a series of QTc prolonging drugs such as dofetilide, quinidine, verapamil and ranolazine.[5] Recent evidence has shown that dofetilide and other known "pure" I_{kr} blockers, are not "pure" as they also augment late sodium current.[13, 14, 90] These new finding are reflected *in vivo* by the equal lengthening of early and as well as late ventricular repolarization phase assessed by the J-Tpeakc and Tpeak-Tend interval length.

The ECG biomarkers J-Tpeakc andTpeak-Tend interval have been shown to successfully differentiate between predominant hERG block and hERG block with inward current block in a variety of clinical trials.[86-88, 91] J- Tpeakc interval is also the only known ECG biomarker being able to improve detection of evident late sodium current block in the presence of hERG block compared to using QTc interval alone.[86] Tpeak-Tend interval as a biomarker of late ventricular repolarization was also validated in study conducted by FDA.[89] This interval has also been extensively used as a proarrhythmic index and a measure of ventricular arrhythmogenesis in several clinical studies and risk stratification for ventricular tachycardia/fibrillation (VT/VF). [87, 92-95]

Another clinical biomarker that has been recently proposed to predict subjects' risk of developing ventricular tachycardia/fibrillation (VT/VF) is the raio of the Tpeak-Tend divided by the uncorrected QT.[96] This biomarker has been sugeested to be a novel marker of arrhythmogenesis in patients with Brugada Syndrome and Type 2 diabetes mellitus.[97, 98] Higher Tpeak-Tend/QT was linked to VT/VF inducibility in patients with Brugada syndrome.[96]

Study rationale

The overarching concept of this work is that the FDA-approved drugs testosterone and progesterone could be repurposed for attenuating drug-induced QT interval lengthening and reducing the risk of drug-induced TdP in patients who require therapy with one or more QT interval-prolonging drugs and who are at high risk for drug-induced QT interval prolongation and TdP. Furthermore, determination of the influence of oral progesterone or transdermal testosterone on specific phases of drug-induced lengthening of ventricular repolarization will provide important information regarding the potential clinical use of these agents for attenuation of drug-induced QTc interval prolongation; i.e., whether progesterone and testosterone may attenuate QTc interval prolongation induced by drugs that primarily lengthen early repolarization, late repolarization, or both. Finally, identifying and addressing sources of inter-subject variability in progesterone-mediated attenuation of drug-induced QT interval lengthening is necessary to optimize future clinical utilization of oral progesterone as a novel therapy to attenuate drug-induced QT interval lengthening in high-risk female patients. The proposed research will ultimately allow optimization of the clinical utilization of sex hormones as drugs for

prevention of drug-induced QTc interval prolongation and associated reduction in risk of TdP.

Study aims & objectives

The overall objective was to assess the effects of administration of sex hormones on attenuation of drug-induced QT interval lengthening, attenuation of distinct phases of drug-induced lengthening of ventricular repolarization and to identify sources of inter-patient variability in progesterone-mediated attenuation of drug-induced QT interval lengthening. Towards this objective, the following specific aims were pursued:

Specific aim 1: Determine the influence of transdermal testosterone and oral progesterone on drug-induced QT interval lengthening in older men. This objective was carried out by conducting a prospective, randomized, double blind, placebo controlled, three-way crossover-design study in human subjects.

Hypothesis 1: Transdermal testosterone and oral progesterone administration attenuate drug-induced QT interval lengthening in older men.

Specific aim 2: Determine the influence of 2A) transdermal testosterone and oral progesterone administration on drug-induced lengthening of early ventricular repolarization in older men, 2B) transdermal testosterone and oral progesterone on drug-induced lengthening of late ventricular repolarization and Tpeak-Tend/QT in older men, 2C) oral progesterone on drug-induced lengthening of early ventricular repolarization in premenopausal women, 2D) oral progesterone on drug-induced lengthening of late ventricular repolarization in premenopausal women

Hypothesis 2: Transdermal testosterone and oral progesterone attenuate drug-induced lengthening of both early and late ventricular repolarization, as measured using the ECG markers of J–Tpeakc, Tpeak–Tend intervals and Tpeak-Tend/QT, respectively

Specific aim 3: Identify determinants of inter-subject variability in response to oral progesterone for attenuation of drug-induced QTc interval lengthening in premenopausal women. This objective utilizes a PKPD modeling approach and the utilization of NONMEM and R for model development and graphical analysis, respectively.

Hypothesis 3: The clinical variables weight, age, serum progesterone concentration, serum estradiol concentration, and the ratio of serum progesterone:estradiol concentrations explain inter-subject variability in progesterone-mediated attenuation of drug-induced QT interval lengthening in premenopausal women.

METHODS

SPECIFIC AIM 1

Study subjects

Men ≥ 65 years of age were enrolled. Exclusion criteria were:

- Prostate cancer, history of prostate or breast cancer, benign prostatic hyperplasia
    - *Administration of exogenous testosterone is contraindicated in patients with these conditions*
- Weight < 60 kg or > 135 kg
    - *Weight limits are necessary for ssafety reasons, to ensure that subjects do not receive inappropriately high ibutilide doses*
- Serum potassium < 3.6 mEq/L, serum magnesium < 1.8 mg/dL
    - *Hypokalemia and hypomagnesemia are risk factors for drug-induced QT interval prolongation and TdP*
- Hematocrit < 26%
    - *Due to multiple blood sample collection, it is important to ensure that study subjects are not anemic at time of the study*
- Hepatic transaminases > 3x upper limit of normal

- *Ibutilide, progesterone and testosterone are metabolized primarily in the liver*

- Baseline Bazett's-corrected QTc interval > 450 ms
    - *Prolonged QTc interval at baseline is a risk factor for drug-induced QT interval prolongation and TdP. Bazett's correction is used for this exclusion criterion as it is the most commonly used heart rate correction in clinical practice and a Bazett's-corrected QTc interval of 450 ms is a widely accepted cutoff point as an exclusion criterion for studies such as this*

- Heart failure with reduced ejection fraction (left ventricular ejection fraction < 40%) or HFrEF
    - *HFrEF is a risk factor for drug-induce QT interval prolongation and TdP*

- Family or personal history of long QT syndrome, arrhythmias or sudden cardiac death
    - *Family or personal history of long QT syndrome, arrhythmias or sudden cardiac death are risk factors for drug-induced QT interval prolongation and TdP*

- Permanently paced ventricular rhythm
    - *Ventricular pacemakers widen the QRS complex and render accurate interpretation of the QT interval difficult*

- Concomitant use of any QT interval–prolonging drugs
    - *This increases the risk for subject to develop drug-induced QT interval prolongation and TdP*
- Concomitant use of any strong non-QT interval-prolonging cytochrome P450 3A inhibitors or inducers.[99]
    - *Strong cytochrome P450 3A inhibitors or inducers could result in unwanted increase or decrease of serum concentrations of progesterone and testosterone, which are metabolized by CYP3A4/5.*

This study was approved by the Indiana University (IU) Institutional Review Board (IRB). Subjects provided written informed consent.

Study procedures

This was a prospective, randomized, double-blind, placebo-controlled, three-way crossover-design study conducted in the Indiana Clinical Research Center (ICRC) in Indianapolis (**Figure 3**). Recruitment began in July 2015, and procedures were completed on the last enrolled subject in October 2017. There were three treatment phases: transdermal testosterone and oral placebo; oral progesterone and transdermal placebo; transdermal placebo and oral placebo. Prior to enrollment, all subjects underwent a screening physical examination including a blood sample to determine laboratory values.

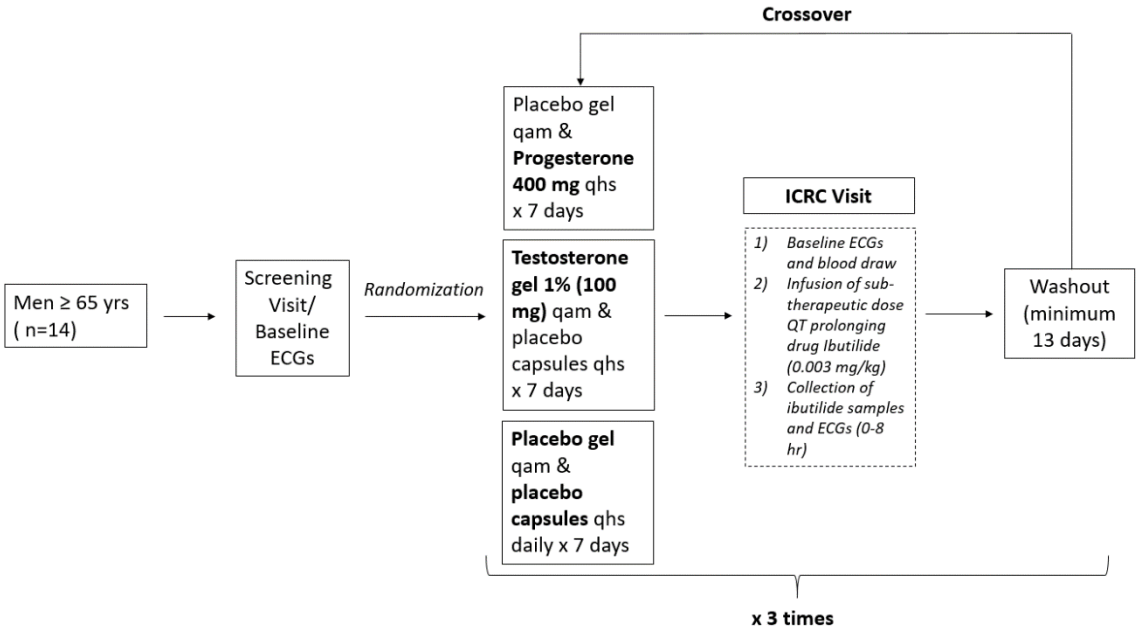

Figure 3 Design of Randomized, Double-Blind, Placebo-Controlled, Three-Way Crossover Study of Efficacy of Transdermal Testosterone and Oral Progesterone for Attenuation of Drug-Induced QT Interval Lengthening

Subjects who met inclusion criteria and met no exclusion criteria were randomized in double-blind fashion to receive 7 days of treatment with: a) Transdermal testosterone 100 mg (1% Androgel®, AbbVie, North Chicago, Illinois) once every morning and 2 placebo capsules once every evening, b) Oral progesterone 400 mg (2 x 200 mg capsules, Teva Pharmaceuticals, North Wales, Pennsylvania) once every evening and placebo transdermal gel once every morning, or c) Placebo transdermal gel once daily morning and 2 placebo capsules once every evening. Transdermal testosterone 100 mg delivers approximately 10 mg testosterone systemically over 24 hours.[100] This transdermal testosterone formulation was selected as it provides continuous transdermal delivery for 24 hours.[100] This testosterone dose was selected as it results in average serum testosterone concentration of 713 ± 209 ng/dL and maximum serum testosterone concentration of 1083 ± 434 ng/dL [100], which are in the upper range of normal. The half-life of testosterone is 100 minutes; therefore 7 days is sufficient to reach steady state. Placebo will consist of a gel comprised of the inactive ingredients of the testosterone gel: carbomer 980, ethanol 67.0%, isopropyl myristate, purified water, and sodium hydroxide. Oral progesterone 400 mg once daily is the dose that was effective for attenuating drug-associated QT interval response in premenopausal women, and therefore is the dose that was selected for this study.[81] Transdermal testosterone, oral progesterone, placebo transdermal gel (hydroalcoholic carbomer gel, Letco Medical, Decatur, Alabama) and oral placebo (lactose) capsules were prepared and dispensed in double-blind fashion by the IU Health Investigational Drug Service (IDS).

Subjects were instructed to apply testosterone and placebo gel to intact, clean skin of the upper shoulders or arms, wash their hands following application, and allow the

application area to dry for 15 minutes before covering it with clothing. Subjects were asked to exercise and/or shower prior to gel application in the morning or in the late afternoon/evening to maximize absorption.

Randomization was performed by two investigators using a computerized random number generator. The blinded randomization scheme was maintained in an IDS randomization log. Participants were assigned to testosterone, progesterone or placebo in each phase by IDS personnel. Testosterone, progesterone and matching placebo were delivered to the ICRC by IDS personnel; investigators, subjects, and ICRC personnel were blinded to treatment assignments during data collection and analysis.

Subjects were instructed to initiate testosterone, progesterone or dual-matching placebo 7 days before the day of the next scheduled ICRC visit. Between each study phase, there was a minimum washout period of 13 days, which is sufficient for elimination of testosterone, dihydrotestosterone (DHT) and progesterone, which possess half-lives of 100 minutes, 2.8 hours, and 5 hours, respectively.[100, 101] Subjects were reminded when to start the next treatment phase and were monitored for adverse effects during each study phase via daily phone calls from an investigator.

On the morning after the 7th day of administration of transdermal testosterone, oral progesterone or dual-matched placebo, subjects presented to the ICRC for an approximately 10-hour stay. Blood was obtained to ensure that serum potassium concentrations were above 3.6 mEq/L prior to ibutilide administration. Three 10-second 12-lead electrocardiograms (ECGs) (Marquette Mac 5500, GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania) were obtained ~ 1 minute apart for baseline (pre-ibutilide) measurements. If the serum potassium was > 3.6 mEq/L and the QTc interval was <450

ms, one peripheral indwelling intravenous catheter was inserted into each arm. Blood (4.5 mL) for determination of serum testosterone and progesterone concentrations was collected in gold-top serum separator tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey). Subjects then received a single intravenous dose of the QTc interval-lengthening drug ibutilide 0.003 mg/kg diluted in 50 mL normal saline and infused over 10 minutes. Three 12-lead ECGs were obtained ~ 1 minute apart immediately at the end of the infusion and at 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 4, 6, and 8 hours post-infusion. Pre-ibutilide ECGs were obtained between 6:30 AM and 9:30 AM, and ibutilide was administered immediately after baseline ECGs were obtained. Blood (10 mL) for determination of serum ibutilide concentrations was obtained from the catheter contralateral to that into which ibutilide was infused and collected in red-top tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, New Jersey) at the same times that ECGs were obtained. Subjects underwent continuous ECG monitoring for 6 hours post-ibutilide administration. Subjects were discharged after the 8-hour ECG and blood sample.

Ibutilide was selected as the QT interval-prolonging probe for this study because it is an antiarrhythmic drug used to terminate atrial fibrillation (AF) and flutter[102]. Ibutilide prolongs QT_c interval dose-dependently, with a rapid onset and return to baseline within 2-6 hours.[103] Serum concentrations decline rapidly, and there are no active metabolites. Ibutilide prolongs QT interval via inhibition of the rapid component of the delayed rectifier potassium current (I_{Kr}), and activation of slow inward sodium current [24, 104]. The ibutilide dose proposed for use (0.003 mg/kg) is 20% of the therapeutic dose. Ibutilide has been administered safely at this dose to men,[81] and safely prolonged the maximum

QT_c interval by 46±16 ms,[81] which is sufficient to demonstrate a significant reduction in QT interval associated with testosterone or progesterone. Therefore, ibutilide was selected as an appropriate QT-prolonging probe for this study.

QT interval measurement

QT intervals were measured from lead II by one investigator (E.T.M.) who was blinded to the subjects' assigned treatment phases. QT intervals were measured using computerized high-resolution electronic calipers (EP Calipers 1.6). QT and RR intervals at each time point were averaged over 3 consecutive complexes. The end of the T-wave was determined via the tangent method.[29] Only clearly discernable QT intervals were measured. QT intervals were corrected using the Fridericia (QT_F)[30] and the Framingham methods (QT_{Fram})[105]. Bazett's method, the most common QT interval correction formula used in clinical practice, was not employed in this study, as it overcorrects the QT interval at more rapid heart rates and under-corrects at slower heart rates [106].

Determination of serum hormone and ibutilide concentrations

Serum testosterone and progesterone concentrations were determined in the IU Health Pathology Laboratory using electrochemiluminescence and chemiluminescence immunoassays, respectively.[107] Venous blood (4.5 mL, Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey) for determination of serum progesterone concentrations was collected in gold-top serum separator tubes on the morning after the 7th day of testosterone, progesterone and placebo phases prior to ibutilide administration.

Blood (10 mL) for determination of serum ibutilide concentrations was obtained from the indwelling catheter in the arm contralateral to that into which ibutilide was

infused and collected in red-top tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey). Serum was separated from whole blood via centrifugation and then stored at -70°F until analysis.

Serum ibutilide concentrations were determined in the IU Clinical Pharmacology Analytical Core (CPAC) Laboratory, IU School of Medicine, using reverse-phase high-performance liquid chromatography with mass spectrometry detection[81]. A method to quantify ibutilide from serum was developed in the CPAC, using temazepam as the internal standard and HPLC-MS/MS (Agilent 1290 pump, Eskigent Autosampler, and 5500 QTRAP® Sciex). In brief, ibutilide and temazepam were separated on an Agilent Eclipse Plus C18 RRHD 50X2.1 mm 1.8µm column with acetonitrile: 5mM NH4OAc; 50:50v/v mobile phase delivered isocratically at 200µL/min. The mass spectrometer utilized an electrospray ionization probe run in positive mode. The multiple reaction monitoring (MRM) Q1/Q3 (m/z) transitions for ibutilide and temazepam were 385.1/144.0 and 301.1/255.0, respectively. For the serum sample extraction, 200µL of sample or standard, was transferred to glass tubes and temazepam was added as the internal standard (20µL of 0.01ng/µL), followed by the addition of methyl tertiary butyl ether. The samples were then vortexed, centrifuged, and the organic layer was transferred to a clean glass tube, and evaporated to dryness. The samples were then reconstituted with mobile phase (50µL) and an aliquot (10µL) was injected into the HPLC-MS/MS. The lower limit of quantification (LOQ) was 3pg/mL.

Study outcome measures

QT intervals

1) Baseline (pre-ibutilide) QT_F and QT_{Fram} intervals; 2) Maximum post-ibutilide QT_F and

QT_{Fram} intervals; 3) Post-ibutilide % change from baseline in maximum QT_F and QT_{Fram} intervals; 4) Area under the QT_F and QT_{Fram} interval vs time curves from 0 to 1.17 hours ($AUEC_{0\text{-}1.17}$) and from 0 to 8.17 hours ($AUEC_{0\text{-}8.17}$) after ibutilide administration (times are presented as 0-1.17 and 0-8.17, rather than 0-1 and 0-8, to account for the 10-minute ibutilide infusion).

Adverse Effects

Subjects were monitored for adverse effects during the study treatment phases through daily phone calls from one of the study investigators.

Sample size calculation

In this three-way crossover study, we anticipated that ibutilide 0.003 mg/kg would lengthen the QT_F interval by 30±15 ms in the absence of testosterone or progesterone.[22] A sample size of n=14 was required to detect a difference in maximum QT_F interval of 15 ms at a two-sided α level of 0.05 and a power of 0.80.

Statistical analysis

Outcomes were assessed using the intention-to-treat approach. AUECs were calculated using the linear trapezoidal rule. Statistical analysis was performed using SPSS 22.0 (SPSS Inc, Chicago, IL). Normality of data was determined using the Kolmogorov-Smirnov test. Comparison of the outcome measures across the three phases (testosterone vs. progesterone vs. placebo) was performed using repeated measures analysis of variance (ANOVA) with *post-hoc* Bonferroni correction to determine between-phase differences. The Friedman test was used to compare the means of the three phases for data that were non-normally distributed data. Comparisons were performed using a 2-

sided α of 0.05. Continuous data are presented as mean ± [standard deviation (SD)] unless otherwise indicated.

SPECIFIC AIM 2

Study subjects

This specific aim incorporated all ECG data from the study of men ≥ 65 years of age described in specific aims 1 as well as ECG data from 11 of the 15 healthy premenopausal female volunteer subjects who completed a previously conducted study by Tisdale et al.[81] Exclusion criteria for the pre-menopausal study were:

- Weight < 45 kg
    - *Weight limits are necessary for safety reasons, to ensure that subjects do not receive inappropriately high ibutilide doses*
- Serum potassium < 3.6 mEq/L, serum magnesium < 1.8 mg/dL
    - *Hypokalemia and hypomagnesemia are risk factors for drug-induced QT interval prolongation and TdP*
- Hematocrit < 26%
    - *Due to multiple blood sample collection, it is important to ensure that study subjects are not anemic at time of the study*
- Hepatic transaminases > 3x upper limit of normal

- *Ibutilide, progesterone and testosterone are metabolized primarily in the liver*

- Baseline Bazett's-corrected QTc interval > 450 ms
    - *Prolonged QTc interval at baseline is a risk factor for drug-induced QT interval prolongation and TdP. Bazett's correction is used for this exclusion criterion as it is the most commonly used heart rate correction in clinical practice and a Bazett's-corrected QTc interval of 450 ms is a widely accepted cutoff point as an exclusion criterion for studies similar to this*
- History of hypertension, coronary artery disease, heart failure, or liver or kidney disease
    - *Our goal was to enroll healthy volunteers for this study.*
- Serum creatinine >1.5 mg/dL
    - We desired healthy volunteers with normal kidney function
- Use of hormonal contraceptives
    - *Hormonal contraceptives contain estradiol and/or progesterone-derivatives that may have altered the serum progesterone and estradiol concentrations during the study*
- Family or personal history of long QT syndrome, arrhythmias or sudden cardiac death

- *Family or personal history of long QT syndrome, arrhythmias or sudden cardiac death are risk factors for drug-induced QT interval prolongation and TdP*

- Permanently paced ventricular rhythm
    - *Ventricular pacemakers widen the QRS complex and render accurate interpretation of the QT interval difficult*

- Pregnancy
    - *During pregnancy, progesterone and estradiol concentrations change significantly; furthermore, animal studies have revealed that ibutilide has evidence of embryocidal and teratogenic effects on the fetus.*[103]

- Unwillingness to use nonhormonal forms of birth control during the study period

- Concomitant use of any QT interval–prolonging drugs
    - *This increases the risk for subject to develop drug-induced QT interval prolongation and TdP*

Study procedures

The data utilized for the J-Tpeakc and Tpeak-Tend measurements were collected in a clinical study, the methodology for which has been published previously [81], but is described in brief in the following sections. This was a prospective, randomized, double-blind, placebo-controlled, crossover-design study consisting of a pre-randomization phase followed by two phases during which subjects were randomized to receive oral

progesterone 400 mg (2 x 200 mg capsules; Teva Pharmaceuticals, North Wales, Pennsylvania) or two placebo capsules once daily at bedtime for 7 days. Each subject was studied twice, serving as her own control, with an average between-phase washout period of 49 days. A progesterone dose of 400 mg daily was selected as this dosage is commonly used for management of polycystic ovary syndrome and for prevention of preterm birth [81]. The study was approved by the Indiana University (IU) Institutional Review Board. All subjects provided written informed consent.

The pre-randomization phase consisted of a 12-hour stay at the Indiana Clinical Research Center (ICRC), during which subjects underwent three 12-lead ECGs (Marquette Mac 5500, GE Healthcare Bio-Sciences, Pittsburgh, Pennsylvania) ~ one minute apart at pre-specified time points that were matched with the dosing phases: 0, 15, 30 minutes, 1, 2, 4, 6, 8 and 18 hours. On the morning after the 7th day of oral progesterone/placebo, subjects presented to the ICRC. After collection of a venous blood sample for the determination of serum progesterone and estradiol concentration and three baseline (pre-ibutilide) 12-lead electrocardiograms (ECGs) ~ one minute apart, subjects received a single intravenous dose of ibutilide 0.003 mg/kg diluted in 50 mL normal saline and infused over 10 minutes via infusion pump. Three ECGs (~ one minute apart) and venous blood samples for determination of serum ibutilide concentrations were obtained immediately at the end of infusion and at 5, 10, 15, 20, 30, and 45 minutes and 1, 2, 4, 6, 8, and 12 hours post-infusion. All three phases of the study were initiated between 7 and 9 am and finished between 7 and 9 pm. Study phases during which subjects received progesterone or placebo were timed to the beginning of the menses

phase for each individual subject to minimize the effects of endogenous progesterone and estradiol.

J-Tpeak and Tpeak-Tend interval measurement

J–Tpeak and Tpeak–Tend intervals were measured from lead II by one investigator (E.T.M.) who was blinded to the subjects' assigned treatment phases. J–Tpeak and Tpeak–Tend intervals were measured using computerized high-resolution electronic calipers (EP Calipers 1.6). The J point was assessed as the QRS offset annotation while the T peak was assessed as the intersection point of two drawn tangents of the up and down slope of the T-wave.[32] The J-Tpeak intervals were heart-rate corrected due to heart-rate dependency using the formula:[25] $J\text{-}Tpeakc = J\text{-}Tpeak /(RR)^{0.58}$. Tpeak-Tend intervals were measured from the peak of T wave to the end of T wave. The end of the T-wave was defined as intersection of tangent to the down slope of T wave and isoelectric line and was determined via tangent method.[29] Tpeak-Tend intervals were not corrected for heart-rate based on data showing that Tpeak- Tend interval does not exhibit heart-rate dependency.[25] The Tpeak-Tend/QT interval was calculated by diving the Tpeak-Tend by the uncorrected QT interval.[98]

Study outcome measures

<u>J-Tpeakc and Tpeak-Tend intervals</u>

1) Baseline (pre-ibutilide) J-Tpeakc and Tpeak-Tend intervals; 2) Maximum post-ibutilide J-Tpeakc and Tpeak-Tend intervals; 3) Post-ibutilide % change from baseline in maximum J-Tpeakc and Tpeak-Tend intervals; 4) Area under the J-Tpeakc and Tpeak-

Tend interval vs time curves from 0 to 1.17 hours (AUEC0-1.17) and from 0 to 8.17 hours (AUEC0-8.17) after ibutilide administration.

Tpeak-Tend/QT interval ratio

Baseline (pre-ibutilide) Tpeak-Tend/QT interval ratio; 2) Tpeak-Tend/QT interval ratio at end of infusion; 3) Maximum Tpeak-Tend/QT ratio.

Statistical analysis

Outcomes were assessed using the intention-to-treat approach. AUECs were calculated using the linear trapezoidal rule. Statistical analysis was performed using SPSS 22.0 (SPSS Inc, Chicago, IL). Normality of data was determined using the Kolmogorov-Smirnov test. Comparison of the outcome measures across the three phases (testosterone vs. progesterone vs. placebo) in the study of older men was performed using repeated measures analysis of variance (ANOVA) with *post-hoc* Bonferroni correction to determine between-phase differences. Friedman test was utilized instead of repeated measures ANOVA for non-normally distributed data.

Comparison of the outcome measures across the two phases (progesterone vs. placebo) in the study of premenopausal women was performed using paired t-tests. Comparisons were performed using a 2-sided α of 0.05. Continuous data are presented as mean $\pm$ [standard deviation (SD)] unless otherwise indicated.

SPECIFIC AIM 3

Healthy premenopausal women

For the development of the PK/PD model for specific aim 3, ibutilide concentration and QT data from all 15 premenopausal subjects described in aim 2 were incorporated into the model. Study procedures are described under specific aim 2.

QT interval measurements and Baseline QT Interval Correction

QT intervals were measured from lead II by one investigator (HAJ) who was blinded to the subjects' assigned groups. The average QT interval length of three ECGs per time point was determined. QT intervals were corrected for heart rate using the Fridericia method (QT_F) [108]. To account for potential circadian variation in QT interval length, time-matched QT_F interval measurements from the screening day without ibutilide were subtracted from the QT_F interval measurements from both the placebo and progesterone phase. This allowed the determination of a (1) time-matched baseline corrected QT_F interval ("baseline-corrected QT_F") for the PKPD model [109] = Treatment day (QT_F at each time-point) – Screening day (QT_F at each time-point)

Determination of serum hormones and ibutilide concentrations

Serum estradiol and progesterone concentrations were determined in the IU Health Pathology Laboratory using chemiluminescence immunoassays [19,20]. Blood (10 mL) for determination of serum ibutilide concentrations was obtained from the indwelling catheter in the arm contralateral to that into which ibutilide was infused and collected in red-top tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey).

Serum was separated from whole blood via centrifugation and then stored at -70°F until analysis. Serum ibutilide concentrations were determined in the IU CPAC Laboratory using the method described under Specific Aim 1.

Software for population pharmacokinetic-pharmacodynamic analysis

The analysis was performed with a nonlinear mixed-effects approach using NONMEM® (Version 7.2.1, GloboMax LLC, Hanover, Maryland).[110] Pirana (version 2.9.2) was used as graphical user interface.[111] Models were estimated using first order conditional estimation method (FOCE). R 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism 7 (San Diego, California) were utilized for graphical explorations.[112]

Structural pharmacokinetic model development

The population PK model was developed to describe ibutilide serum concentration versus time profiles. Non-compartmental analysis was performed in PKSolver[113] (within Microsoft Excel Office 365) to determine preliminary pharmacokinetic parameters. Based on previous published literature on ibutilide pharmacokinetics[114, 115] and visual inspection of the data, two and three- compartment pharmacokinetic models with first-order elimination from central compartment were tested in NONMEM.

Pharmacokinetic-pharmacodynamic model development

The population PKPD model was developed to describe the relationship between serum ibutilide concentrations and changes in baseline-corrected QT_F intervals during the

placebo and progesterone phases. The population PKPD model was constructed in a sequential manner by incorporating individual PK parameters from the final PK model into a dataset in which baseline-corrected QT_F intervals represented the dependent variable [116]. Baseline-corrected QT_F interval exploratory plots were used to determine the initial structure of the PD model [109] (Figure 1). An exploratory plot of individual and mean baseline-corrected QT_F and serum ibutilide concentrations versus time was created to investigate possible equilibration delays. The PKPD data was fitted to a linear (Equation 2), E_{max} (Equation 3) and sigmoid E_{max} model with and without an hypothetical effect compartment. The equations describing the models are shown below:

$$E = \alpha + (S \bullet C) \quad (1)$$

$$E = \alpha + \frac{E_{Max} \cdot C^h}{EC_{50}^h + C^h} \quad (2)$$

E is the observed effect (QT_F interval lengthening), α represents the intercept, C is the ibutilide concentration, S is the slope of the relationship between E and C, E_{max} is the maximum QT_F interval effect attributable to ibutilide, EC_{50} is the concentration that produced one-half of the maximum effect, and h is the Hill coefficient affecting the sigmoid shape of the curve. For the E_{max} model, h is equal to 1. For the hypothetical compartment models, C represents the ibutilide concentration in the hypothetical effect compartment; otherwise, C represents the serum ibutilide concentration.

Random effects

Inter-subject variability of PK and PD parameters was modeled using an exponential interindividual variability model assuming log-normal distribution of the between-subject variability in population parameter estimates (Equation 4). Therefore, each subject's estimated PK and PD parameter was related to the corresponding population estimate using the following equation:

$$P_j = P_{TV} \cdot \exp(^{\eta p}) \quad (3)$$

where P_j is the jth individual parameter estimate, P_{TV} is the typical value (population estimate) of the parameter P, and $\exp(^{\eta p})$ is the interindividual variability for this parameter[117]. To account for individual PK parameter changes between the two phases of the study, we added between-occasion variability (BOV) in our model based on the following equations (equation 5)[118]:

If (Occasion = 1) BOV = η_1

If (Occasion = 2) BOV = η_2

BSV = η_3

$$P_j = \theta_1 \cdot \exp (BSV + BOV) \quad (4)$$

Residual unexplained variability (RUV) (ε) was assumed to be normally distributed with a mean of zero and a variance of σ^2. RUV was modeled by testing three residual error models (additional, proportional, and combined). For the final PK model, a proportional error term (equation 6) was chosen, whereas for the final PK/PD model an additive error term (equation 7) was utilized according to the following equations[118]:

$$y_{ij} = y_{ij} + (\hat{y}_{ij} \cdot \varepsilon_{ij}) \quad (5)$$

$$y_{ij} = \hat{y}_{ij} + \varepsilon_{ij} \quad (6)$$

Where y_{ij} is the i_{th} observed concentration for the j_{th} individual, $\hat{y}_{ij}$ is the i_{th} model-predicted concentration for the j_{th} individual, and ε_{ij} is the residual error term for the i_{th} observation of the j_{th} individual[117].

Covariate analysis

Covariates including weight, age, race, serum progesterone and estradiol concentration as well as P:E were evaluated as predictors of response and determinants of inter-patient variability. Serum progesterone:estradiol concentration ratios were calculated by converting ng/mL and pg/mL concentrations to nanomolar concentrations (equation 7).

$$\frac{P}{E} = \frac{Molar\ Progesterone\ Concentration}{Molar\ Estradiol\ Concentration} \quad (7)$$

The final structural PK and PKPD models were used to test the effects of the subject covariates on the model parameters. Covariate analysis was performed using a likelihood ratio test. The effects of weight, age, race, progesterone and estradiol serum concentrations and P:E were tested on final PK (CL_s, V_c, V_d and Cl_d) and PKPD (E_{max}, EC_{50}, α) model parameters. Covariates were considered statistically significant if the objective function value (OFV) decreased by > 3.84 units after addition of each covariate ($\chi 2$ distribution; $P < 0.05$; df =1). Similarly, only covariates that increased the OFV by > 6.63 units ($\chi 2$ distribution; $P < 0.01$; df =1) during backward elimination were kept in the model[118]. The relationship between continuous covariates and each parameter was

described using an additive, proportional and exponential model after centering with the population median estimate of each covariate value according to the following equation:

$$P_{TV} = \theta_1 + \frac{COVARIATE}{MEDIAN\ OF\ COVARIATE} \cdot \theta_2 \quad (8)$$

where θ_1 is the typical value (population estimate) of the parameter in a subject, COVARIATE is the abbreviation of each covariate tested in the model and MEDIAN OF COVARIATE is the study population median value of each specific covariate value of individual i. This was used to test the effect of the other continuous variables such as weight, age, progesterone and estradiol serum concentration and P:E on the PKPD population estimate. The relationship between the categorical covariate and study phase (placebo phase =0, progesterone phase = 1) and PKPD parameters was also tested using an additive model according to the following equation:

$$P_{TV} = \theta_1 \cdot \text{PHASE} + \theta_2 \cdot (1\text{-PHASE}) \quad (9)$$

The relationship between the categorical covariate race (Caucasian = 1, African American = 2, Middle Eastern = 3) and PKPD parmaters was tested using the following equation:

If (race.eq.1) then $P_{TV} = \theta_1$

If (race.eq.2) then $P_{TV} = \theta_2$

If (race.eq.3) then $P_{TV} = \theta_3$ (10)

Model Evaluation

Model evaluation criteria included OFV, Akaike information criteria (AIC), goodness-of-fit (GOF) plots, plausibility, stability and precision of parameter estimates (relative

standard error percentage) and observed vs. model-predicted dependent variable versus time plots. A $p < 0.05$ was considered significant, meaning that an OFV drop of >3.84 was considered a significant improvement [118]. Bootstrapping and visual predictive checks (VPCs) were further utilized to validate the final model. Prediction corrected visual predictive checks (pcVPCs) were created for both the final PK and PKPD model by simulating 500 individuals using the respective final models. The median and 2.5th and 97.5th percentiles of the simulated data were estimated and overlaid with the observed data points. The model was considered to appropriately describe the data if most of the observed data points fell within the 2.5th and 97.5th percentile interval and were equally distributed around the median. The robustness of the model was established via non-parametric bootstrap resampling. The final NONMEM parameter estimates of both the PK as well as PKPD model were compared with the median parameter values and the 2.5th to 97.5th percentiles of the 500 non-parametric bootstrap replicates.

Model evaluation

Model evaluation criteria included OFV, goodness-of-fit (GOF) plots, plausibility, stability and precision of parameter estimates (relative standard error percentage) and observed vs. model-predicted dependent variable versus time plots. A $p < 0.05$ was considered significant, meaning that an OFV drop of >3.84 was considered a significant improvement[118]. Bootstrapping was performed to test the model's stability and visual predictive checks (VPCs) were further utilized for the internal validation of the model. Prediction corrected visual predictive checks (pcVPCs) were created for both the final PK and PK/PD model by simulating 500 individuals using the respective final models. The median and 2.5th and 97.5th percentiles of the simulated data were estimated and overlaid

with the observed data points. The model was considered to appropriately describe the data if most of the observed data points fell within the 2.5^{th} and 97.5^{th} percentile interval and were equally distributed around the median. The robustness of the model was established via non-parametric bootstrap resampling. The final NONMEM parameter estimates of both the PK as well as PK/PD model were compared with the median parameter values and the 2.5^{th} to 97.5^{th} percentiles of the 500 non-parametric bootstrap replicates.

Monte-carlo simulations

Monte Carlo simulations were performed in NONMEM with the "$SIMULATION" function to evaluate the protective effects of different P:E against ibutilide-induced baseline-corrected QT_F interval lengthening. A mean decrease in baseline-corrected QT_F interval ≥ 5 ms with a decrease in lower bound 90% CI of 10 ms was considered clinically significant[109]. Each population simulation corresponding to a specific P:E consisted of 1,000 simulations obtained using the model-estimated population parameters (E_{max}, EC_{50} and α) and interindividual variability terms [119]. The simulations were used to generate time-baseline-corrected QT_F interval profiles (means ± 90% CI) over the time period of 1 and 12 hours after ibutilide administration. Box-plots of the mean simulated ibutilide-induced baseline-corrected QT_F interval lengthening at the end of the infusion were determined to compare the effect of different P:E on the maximum ibutilide-induced baseline-corrected QT_F interval lengthening. Five P:E values were chosen for the simulations, corresponding to the median P:E of the placebo phase (15) and the progesterone phase (129), the median of the entire study population (45), and the 75^{th} (278) and 95^{th} percentiles P:E (412) in the progesterone phase.

RESULTS

Specific Aim 1: Determine the influence of transdermal testosterone and oral progesterone on drug-induced QT interval lengthening in older men.

Study subjects

Fourteen subjects were enrolled, each of whom completed all study phases (**Figure 4**). Mean age was 73 ± 6 years, (range 65-86; Table 1). Thirteen subjects were white and n=1 was black. Mean weight was 90 ± 16 kg and the mean ibutilide dose was 0.27 ± 0.05 mg.

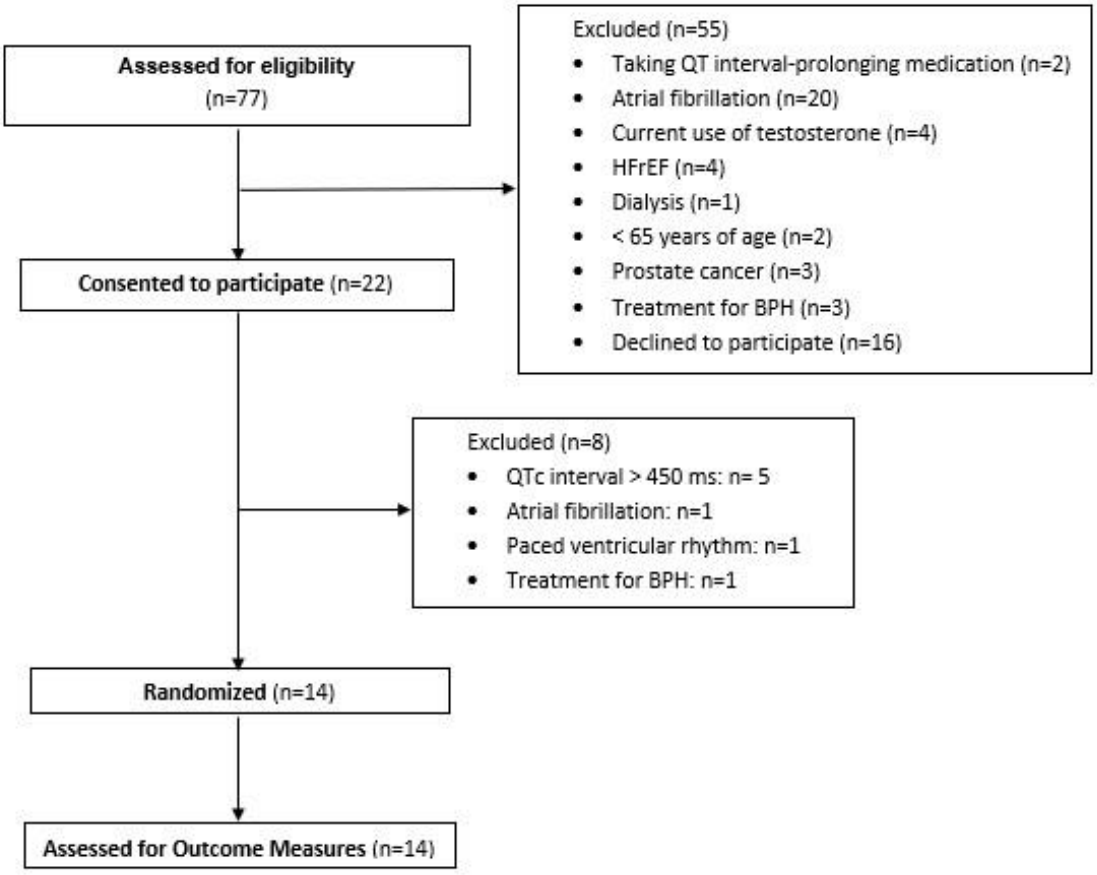

Figure 4 Recruitment and Enrollment for Randomized, Double-Blind, Placebo-Controlled, Three-Way Crossover Study of Efficacy of Transdermal Testosterone and Oral Progesterone on Drug-Induced QT Interval Lengthening

Subjects' chronic conditions and concomitant (non-QT interval-prolonging) medications are listed in Table 1. All subjects were taking the same medications at the same doses during all phases of the study. The median between-phase washout period was 15.5 days (range 13-28 days).

Table 1 Study Subjects' Baseline Characteristics

Characteristics	**n (±SD or %)**
Age (years)	73 ± 6
Weight (kg)	90 ± 16
Race	
White	13 (93%)
Black	1 (7%)
Comorbidities	
Hypertension	14 (100%)
Hyperlipidemia	8 (57%)
Diabetes mellitus	5 (36%)
Osteoarthritis	4 (28%)
Sleep apnea	1 (7%)
Chronic obstructive pulmonary disease	1 (7%)
Hypothyroidism	1 (7%)
Co-Medications	
Statins	7 (50%)
Angiotensin-converting-enzyme inhibitors	6 (43%)
Aspirin (81 mg)	6 (43%)
Thiazide diuretics	6 (43%)
Antidiabetic medications	4 (28%)
Dihydropyridine calcium channel blockers	3 (21%)
β-blockers	2 (14%)
Angiotensin receptor blockers	2 (14%)
Proton-pump inhibitors	2 (14%)
Albuterol	2 (14%)
Ezetimibe	1 (7%)
Levothyroxine	1 (7%)

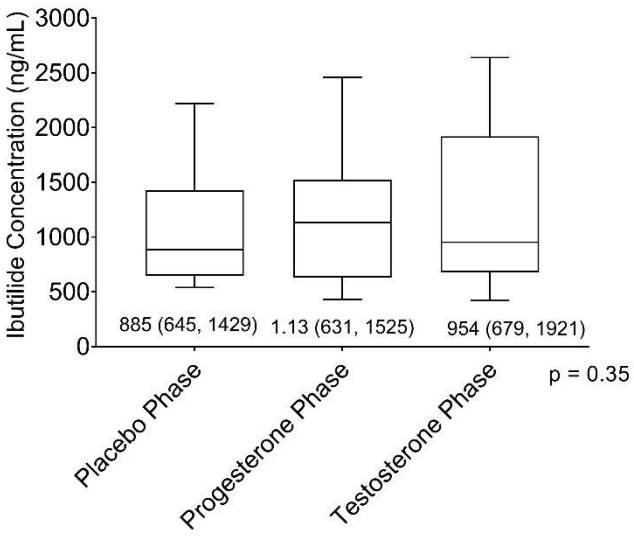

Figure 5 Median (± SD) Maximum Serum Ibutilide Concentrations in Each Phase of the Study.

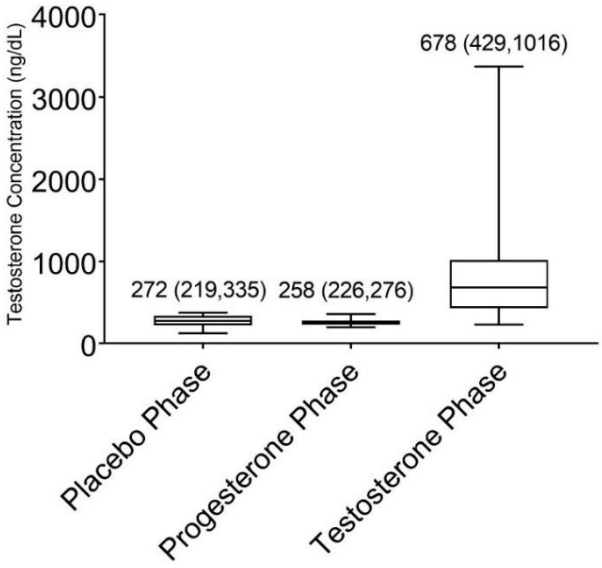

Figure 6 Median (IQR) Serum Testosterone Concentrations After 7 Days of Transdermal Testosterone, Oral Progesterone or Placebo

There was no significant difference in maximum serum ibutilide concentration between the testosterone, progesterone and placebo phases (**Figure 5**). The median serum testosterone concentration was significantly higher during the testosterone phase compared to those during the progesterone and placebo phases (**Figure 6**). The median serum progesterone concentration was significantly higher during the progesterone phase compared to those in the testosterone and placebo phases

Baseline (pre-ibutilide) QT_F and QT_{Fram} intervals

Baseline (pre-ibutilide) QT_F and QT_{Fram} intervals were not significantly different between the testosterone, progesterone or placebo phases (**Table 2**).

QT_F and QT_{Fram} intervals following ibutilide administration

QT_F and QT_{Fram} intervals during the first hour and for 8 hours after the 10-minute ibutilide infusion in the testosterone, progesterone and placebo groups are presented in Figure 6A and 6B. Maximum QT_F and QT_{Fram} intervals were significantly lower during the testosterone phase than during the progesterone phase and placebo phases (**Table 2**, **Figure 5**). There was no significant difference in maximum QT_F and QT_{Fram} intervals between the progesterone and placebo phases (**Table 2**, **Figure 8).**

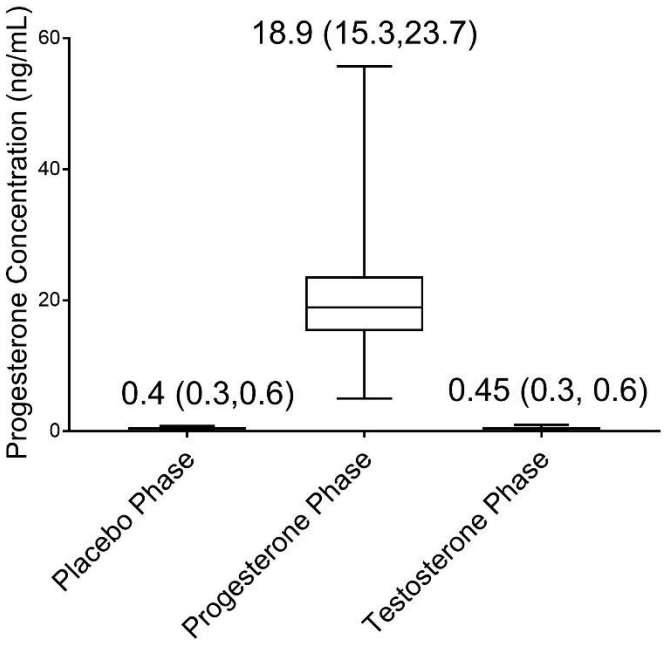

Figure 7 Median (IQR) Serum Progesterone Concentrations After 7 days of Transdermal Testosterone, Oral Progesterone or Placebo

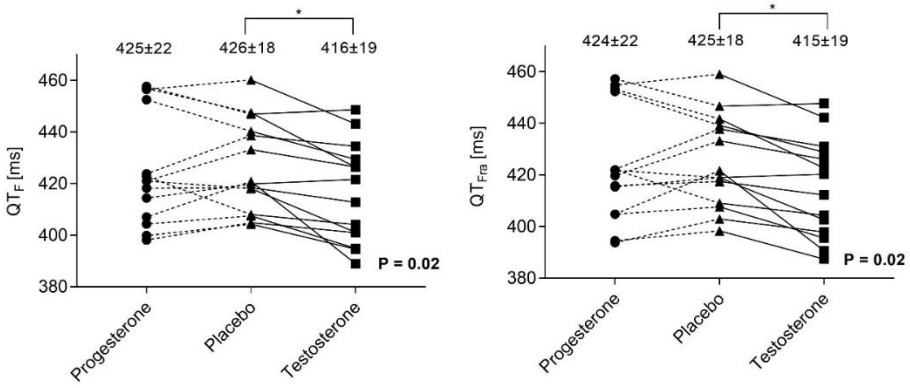

Figure 8 Lead II Maximum QT_F and QT_{Fram} Intervals After Intravenous Ibutilide 0.003 mg/kg During Progesterone, Placebo and Testosterone Phases; * Bonferroni p-value < 0.05 vs placebo

Baseline (pre-ibutilide) QT_F and QT_{Fram} intervals

Baseline (pre-ibutilide) QT_F and QT_{Fram} intervals were not significantly different between the testosterone, progesterone or placebo phases (**Table 2**).

QT_F and QT_{Fram} intervals following ibutilide administration

QT_F and QT_{Fram} intervals during the first hour and for 8 hours after the 10-minute ibutilide infusion in the testosterone, progesterone and placebo groups are presented in Figure 6A and 6B. Maximum QT_F and QT_{Fram} intervals were significantly lower during the testosterone phase than during the progesterone phase and placebo phases (**Table 2**, **Figure 5**). There was no significant difference in maximum QT_F and QT_{Fram} intervals between the progesterone and placebo phases (**Table 2**, **Figure 8**).

Table 2 Lead II QT Interval Response to Intravenous Ibutilide During Testosterone, Progesterone and Placebo Phases (mean ± SD), AUEC = Area under the effect curve; QT_F = Fridericia-corrected QT interval; QT_{Fram} = Framingham-corrected QT interval; SD = Standard deviation, *Bonferroni-adjusted p value < 0.05, Testosterone vs Placebo

	Testosterone	**Progesterone**	**Placebo**	**p**
QT_F				
Baseline (pre-ibutilide) QT_F (ms)	393 ± 19	399 ± 16	399 ± 13	0.09
Maximum % change in QT_F from baseline	5.6 ± 1.8	5.9 ± 2.3	6.1 ± 1.9	0.70
$AUEC_{0\text{-}1.17}$ QT_F (ms·hr)	471 ± 24*	480 ± 24	483 ± 18	<0.001
$AUEC_{0\text{-}8.17}$ QT_F (ms·hr)	3255 ± 173*	3304 ± 145	3335 ± 142	0.002
QT_{Fram}				
Baseline (pre-ibutilide) QT_{Fram} (ms)	392 ± 19	397 ± 12	397 ± 18	0.20
Maximum % change QT_{Fram} from baseline	5.4 ± 1.9	6.0 ± 2.5	6.2 ± 2.0	0.60
$AUEC_{0\text{-}1.17}$ QT_{Fram} (ms·hr)	469 ± 23*	477 ± 25	482 ± 17	0.001
$AUEC_{0\text{-}8.17}$ QT_{Fram} (ms·hr)	3234 ± 160*	3289 ± 146	3327 ± 130	<0.001

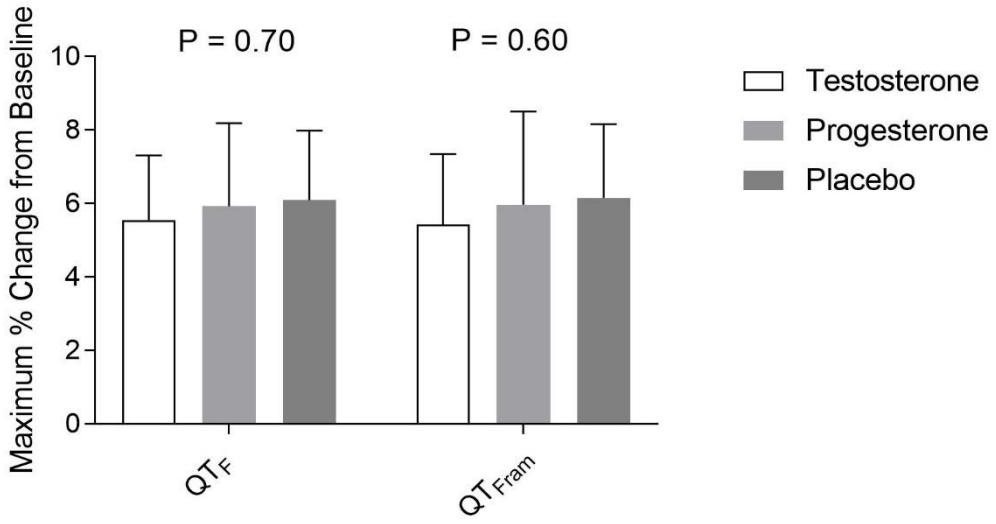

Figure 9 Lead II Maximum % Change from Baseline in QTF and QTFram Intervals After Intravenous Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases

The percent change from baseline to maximum QT_F and QT_{Fram} intervals following ibutilide during the testosterone, progesterone and placebo phases were not significantly different. (**Table 2, Figure 9**).

Area under the QT_F and QT_{Fram} interval versus time curves after initiation of the ibutilide infusion

Compared to placebo, transdermal testosterone significantly decreased the QT_F (**Figure 10**) and QT_{Fram} (**Figure 11**) $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$ (**Table 2**), indicating both a short-term and more prolonged attenuation of drug-induced QT interval lengthening.

In contrast, oral progesterone did not significantly influence QT_F and QT_{Fram} $AUEC_{0\text{-}1.17}$ or $AUEC_{0\text{-}8.17}$ compared to placebo (**Table 2, Figure 11**).

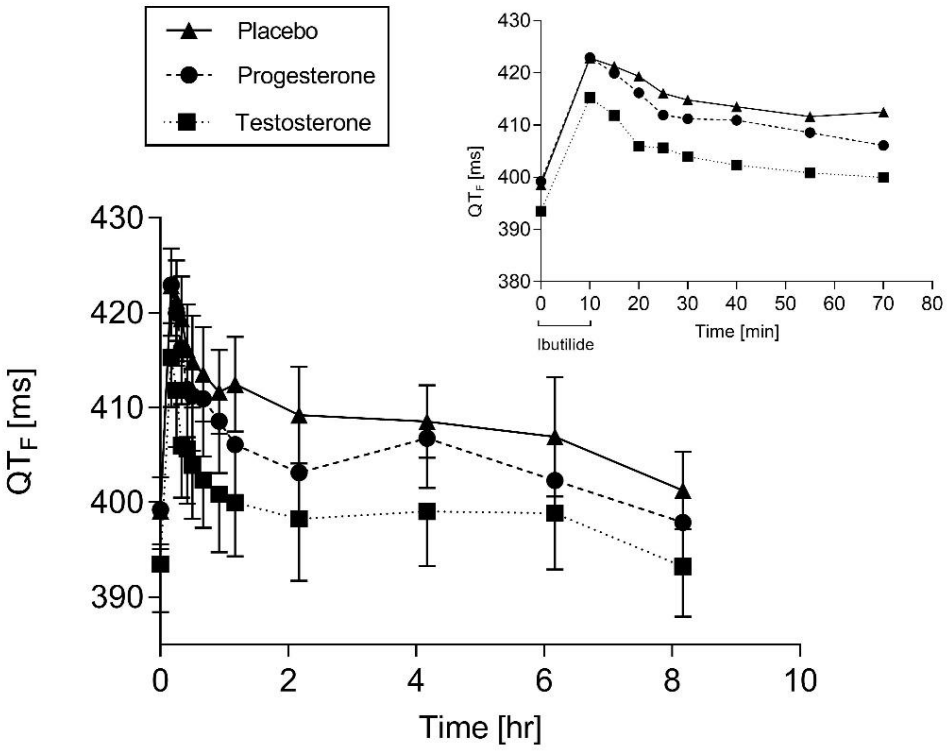

Figure 10 Mean lead II QTF Intervals with SEM During and after a 10-Minute Intravenous Infusion of Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases

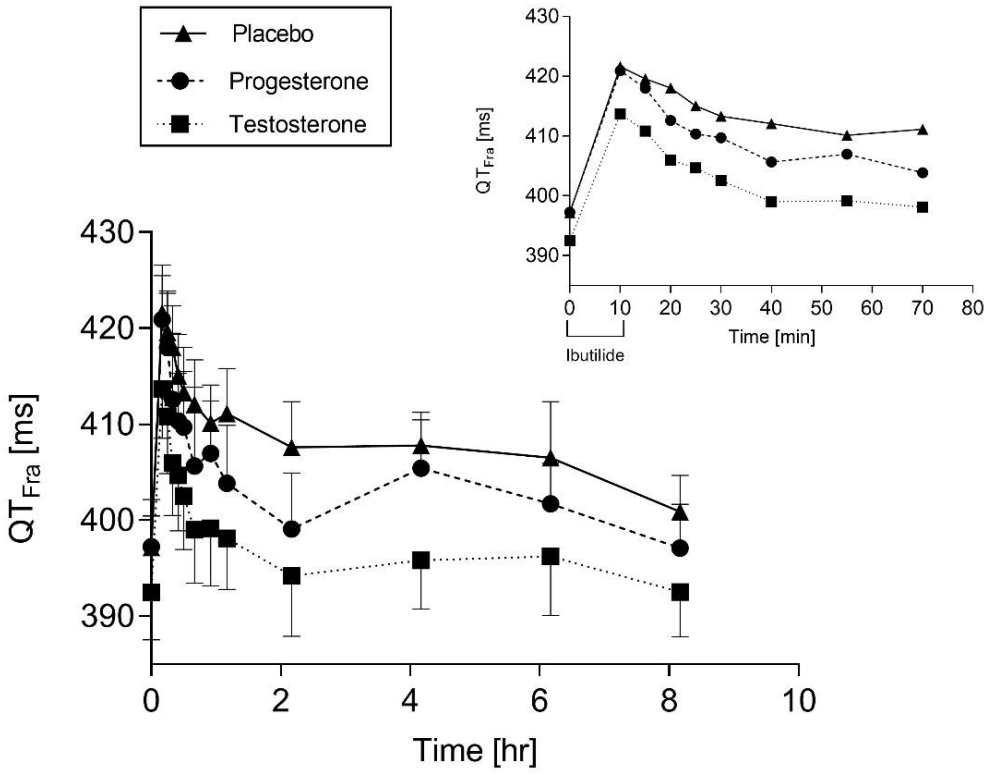

Figure 11 Mean lead II QT_{Fram} Intervals with SEM During and after a 10-Minute Intravenous Ibutilide Infusion during Testosterone, Progesterone and Placebo Phases

Adverse effects

Adverse effects associated with the study interventions were mild and uncommon. One subject complained of fatigue during the progesterone phase (n=1, 7%) and another subject reported a mild skin rash during intervention with placebo transdermal gel (n=1, 7%). There were no adverse effects associated with ibutilide.

Specific Aim 2A: Determine the influence of both oral progesterone and transdermal testosterone administration on drug-induced lengthening of early ventricular repolarization in older men

Baseline (pre-ibutilide) J-Tpeakc intervals

Baseline (pre-ibutilide) J-Tpeakc was significantly lower during the testosterone phase compared with that in the progesterone and placebo phases (**Table 3, Figure 12**). Progesterone did not significantly shorten baseline J-Tpeakc (**Table 3, Figure 12**).

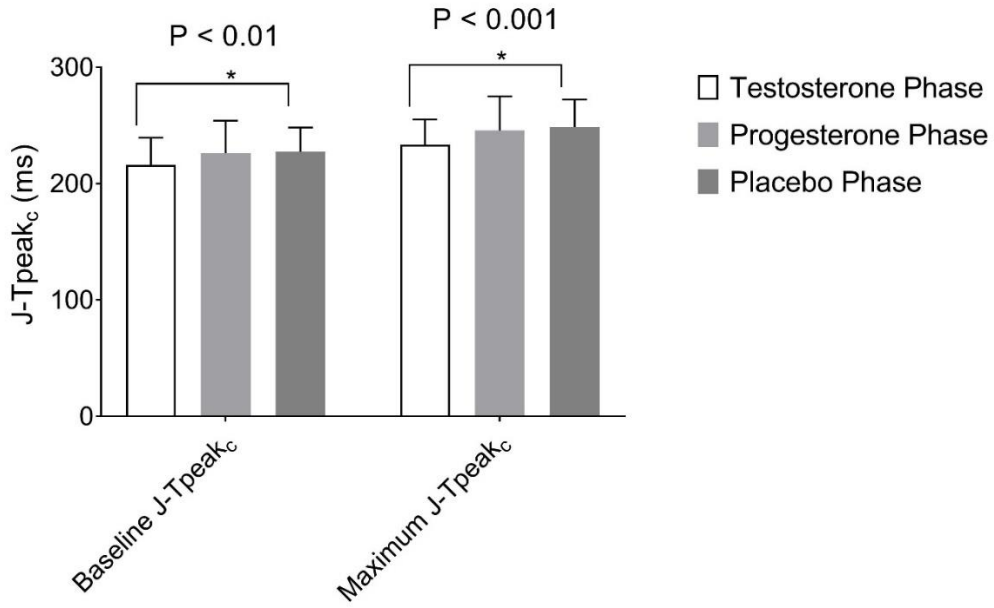

Figure 12 Lead II Baseline (Pre-Ibutilide) and Maximum J-Tpeakc Interval with SD after Intravenous Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases; * Bonferroni post-hoc p-value for the baseline J-Tpeakc between testosterone and placebo = 0.01 and for the maximum J-Tpeakc < 0.001

Effect of transdermal testosterone and oral progesterone on J-Tpeakc intervals following ibutilide administration

J-Tpeakc intervals during the first hour and for 8 hours following the 10-minute ibutilide infusion in the testosterone, progesterone and placebo groups are presented in Figure 5. The mean maximum J-Tpeakc interval was significantly lower during the testosterone phase than during the placebo phase (**Table 3, Figure 12**). There were no significant differences in mean maximum J-Tpeakc intervals between the progesterone and placebo phases (**Table 3, Figure 12**).

Table 3 Lead II J-Tpeakc Response to Intravenous Ibutilide During Testosterone, Progesterone and Placebo Phases (mean ± SD). AUEC = Area under the effect curve; SD = Standard deviation, Bonferroni-adjusted p value < 0.05:
* Testosterone vs Placebo

	Testosterone	**Progesterone**	**Placebo**	**p**
J-Tpeakc				
Baseline (pre-ibutilide) J-Tpeakc (ms)	216 ± 23*	226 ± 28	227 ± 21	0.004
Maximum J-Tpeakc (ms)	233 ± 22*	246 ± 29	249 ± 24	<0.001
Maximum % change in J-Tpeakc from baseline	8 ± 5	9 ± 4	9 ± 5	0.70
$AUEC_{0\text{-}1.17}$ J-Tpeakc (ms·hr)	262 ± 25*	273 ± 32	277 ± 26	<0.001
$AUEC_{0\text{-}8.17}$ J-Tpeakc (ms·hr)	1797 ± 176*	1881 ± 207	1915 ± 191	<0.001

Effect of transdermal testosterone and oral progesterone on area under the J-Tpeakc interval versus time curves after initiation of the ibutilide infusion in older men

Compared to placebo, transdermal testosterone significantly decreased the J-Tpeakc (**Table 3**) and Tpeak-Tend (**Table 3**) $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$, indicating both a short-

term and more prolonged attenuation of both early and late-phase ventricular repolarization. Oral progesterone did not significantly influence J-Tpeakc and Tpeak-Tend $AUEC_{0\text{-}1.17}$ or $AUEC_{0\text{-}8.17}$ compared to placebo (**Table 3, Figure 13**).

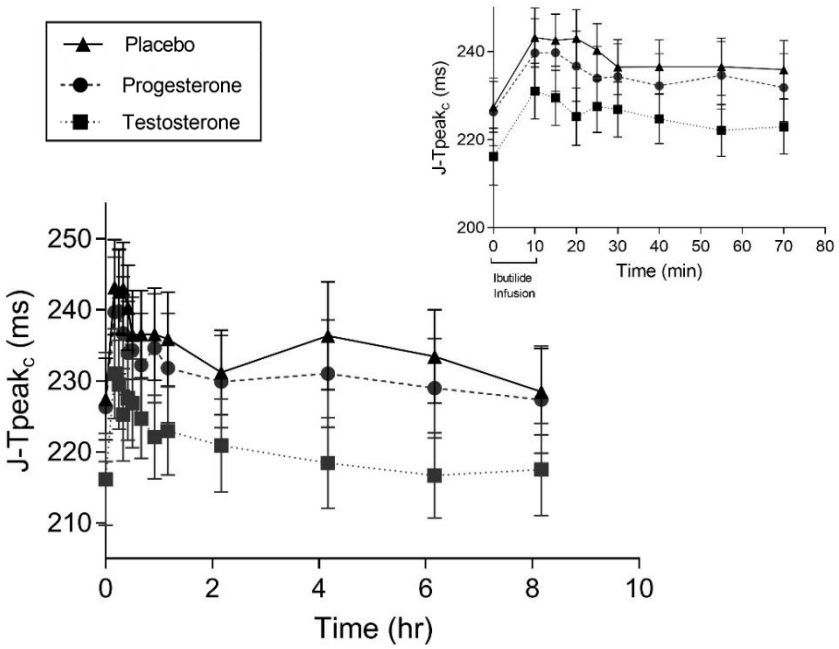

Figure 13 Mean lead II J-Tpeak Intervals with SEM During and After a 10-Minute Intravenous Infusion of Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases

Specific Aim 2B: Determine the influence of both oral progesterone and transdermal testosterone administration on drug-induced lengthening of late ventricular repolarization in older men

Baseline (pre-ibutilide) Tpeak-Tend intervals

Baseline (pre-ibutilide) Tpeak-Tend intervals were not significantly different across the phases of the study. (**Table 4, Figure 14**).

Effect of transdermal testosterone and oral progesterone on Tpeak-Tend intervals following ibutilide administration in older men

Tpeak-Tend intervals during the first hour and for 8 hours following the 10-minute ibutilide infusion in the testosterone, progesterone and placebo groups are presented in **Figure 15**. The mean maximum Tpeak-Tend intervals were significantly lower during the testosterone phase than during the placebo phase (**Table 4, Figure 14**). There were no significant differences in mean maximum Tpeak-Tend intervals between the progesterone and placebo phases (**Table 4**).

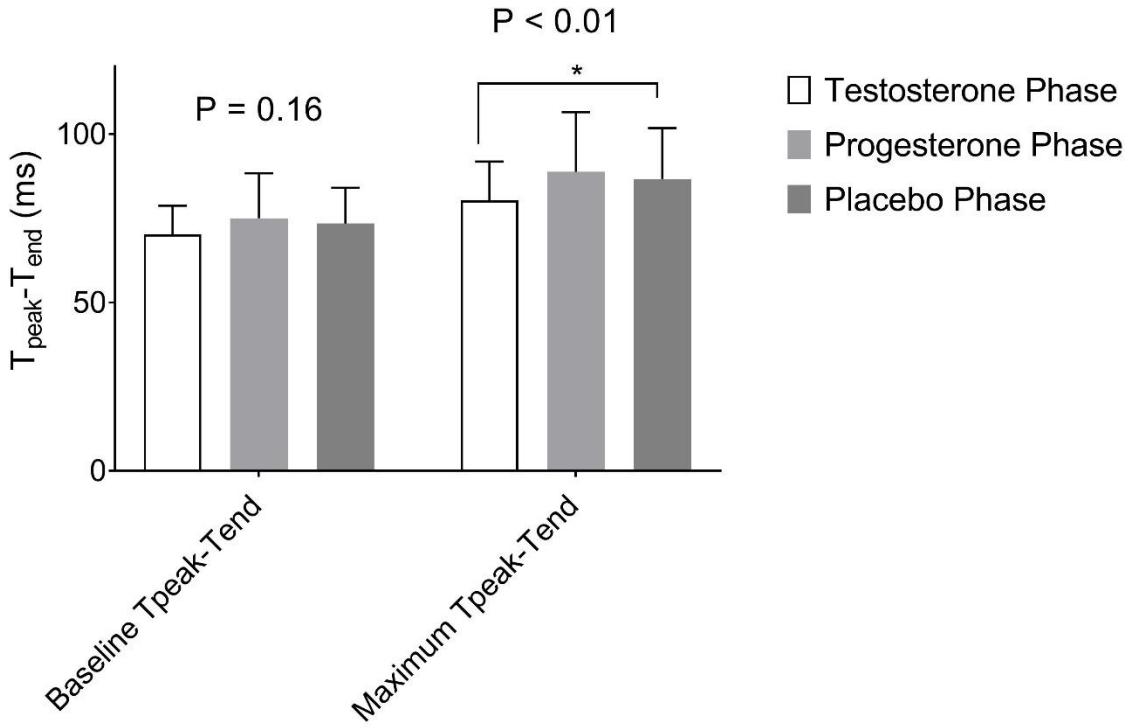

Figure 14 Lead II Baseline (Pre-Ibutilide) and Maximum Tpeak-Tend Intervals with SDAfter Intravenous Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases; * Bonferroni post-hoc p-value between testosterone and placebo, p = 0.05

Effect of transdermal testosterone and oral progesterone on area under the Tpeak-Tend interval versus time curves after initiation of the ibutilide infusion in older men

Compared to placebo, transdermal testosterone significantly decreased the Tpeak-Tend (**Table 4**) $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$, indicating both a short-term and more prolonged attenuation of late-phase ventricular repolarization. Oral progesterone did not significantly influence Tpeak-Tend $AUEC_{0\text{-}1.17}$ or $AUEC_{0\text{-}8.17}$ compared to placebo (**Table 4, Figure 15**).

Table 4 Lead II Tpeak-Tend Response to Intravenous Ibutilide During Testosterone, Progesterone and Placebo Phases (mean ± SD). AUEC = Area under the effect curve; SD = Standard deviation, Bonferroni-adjusted p value < 0.05:
* Testosterone vs Placebo

	Testosterone	**Progesterone**	**Placebo**	**p**
Tpeak-Tend				
Baseline (pre-ibutilide) Tpeak-Tend (ms)	70 ± 8	75 ± 13	73 ± 11	0.16
Maximum Tpeak-Tend (ms)	80 ± 12	89 ± 18	87 ± 15	0.008
Maximum % change Tpeak-Tend from baseline	14 ± 10	18 ± 9	18 ± 11	0.52
$AUEC_{0\text{-}1.17}$ Tpeak-Tend (ms·hr)	86 ± 13*	92 ± 15	93 ± 14	0.001
$AUEC_{0\text{-}8.17}$ Tpeak-Tend (ms·hr)	583 ± 79*	628 ± 95	626 ± 85	0.008

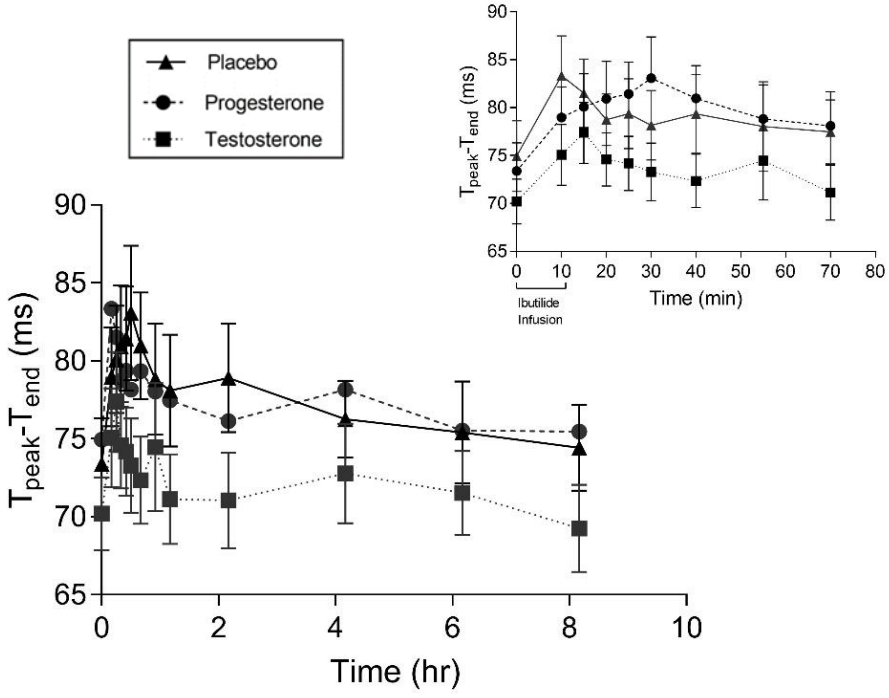

Figure 15 Lead II Tpeak-Tend Interval with SEM During and After a 10-Minute Intravenous Infusion of Ibutilide 0.003 mg/kg During Testosterone, Progesterone and Placebo Phases

Effect of transdermal testosterone and oral progesterone on Tpeak-Tend/QT interval in older men

Compared to placebo, neither transdermal testosterone nor oral progesterone significantly decreased the baseline and Tpeak-Tend/QT interval at the end of the intravenous ibutilide infusion compared to placebo in older men (**Table 5**). Maximum Tpeak-Tend/QT interval is significantly different across the three phases and it is lower in the testosterone phase compared to the placebo phase (**Figure 16**) (LSQ post-hoc test; p = 0.032).

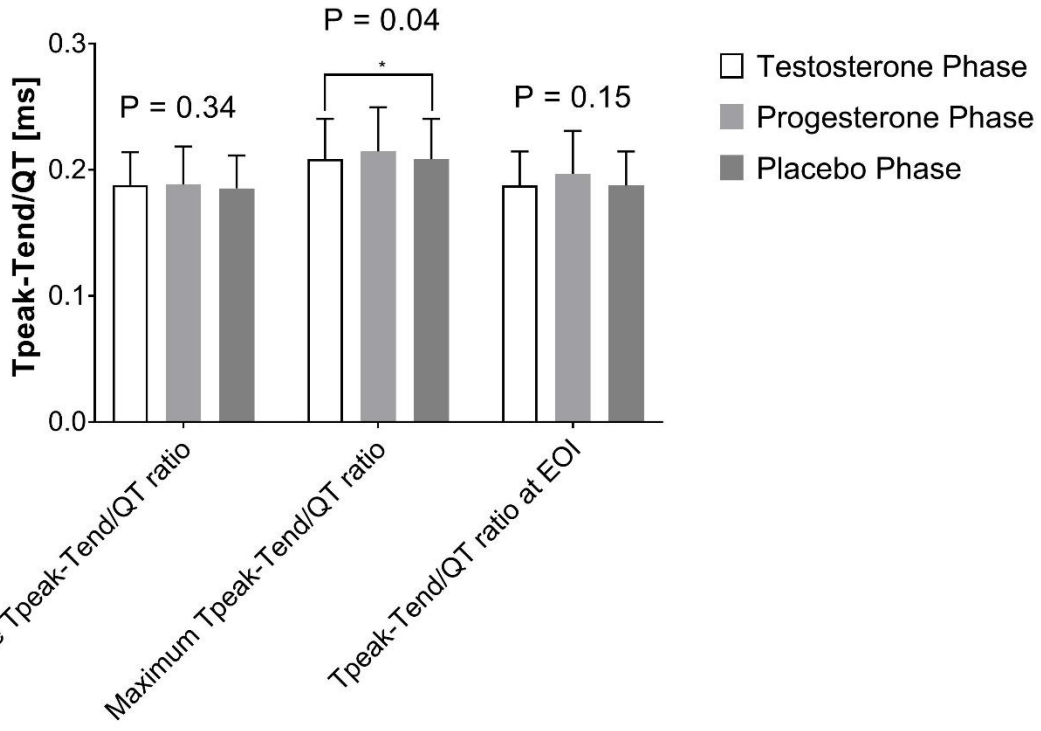

Figure 16 Lead II Tpeak-Tend/QT Interval at Baseline, Maximum and at End of Infusion (EOI) with SD after Intravenous Ibutilide During Testosterone, Progesterone and Placebo Phases; * LSD post-doc test p-value between testosterone and placebo phase <0.05

Table 5 Lead II Tpeak-Tend/QT interval response to Intravenous Ibutilide During Progesterone and Placebo Phases (mean ± SD). Eoi = end of infusion; SD = Standard deviation

	Testosterone		Progesterone	Placebo	P
Baseline Tpeak-Tend/QT ratio	0.18 ± 0.02		0.18 ± 0.03	0.18 ± 0.03	0.34
Maximum Tpeak-Tend/QT ratio	0.19 ± 0.02		0.21 ± 0.03	0.21 ± 0.03	0.04
Tpeak-Tend/QT ratio at eoi	0.18 ± 0.03		0.20 ± 0.03	0.19 ± 0.03	0.15

Specific Aim 2C: Determine the influence of oral progesterone in premenopausal women on drug-induced lengthening of early ventricular repolarization

ECGs from 11 female pre-menopausal subejcts were analyzed for prespecified endpoints.

Baseline (Pre-ibutilide) J-Tpeakc intervals in premenopausal women

Baseline (pre-ibutilide) J-Tpeakc were not significantly different between the placebo and progesterone phase (**Figure 17**, **Table 6**).

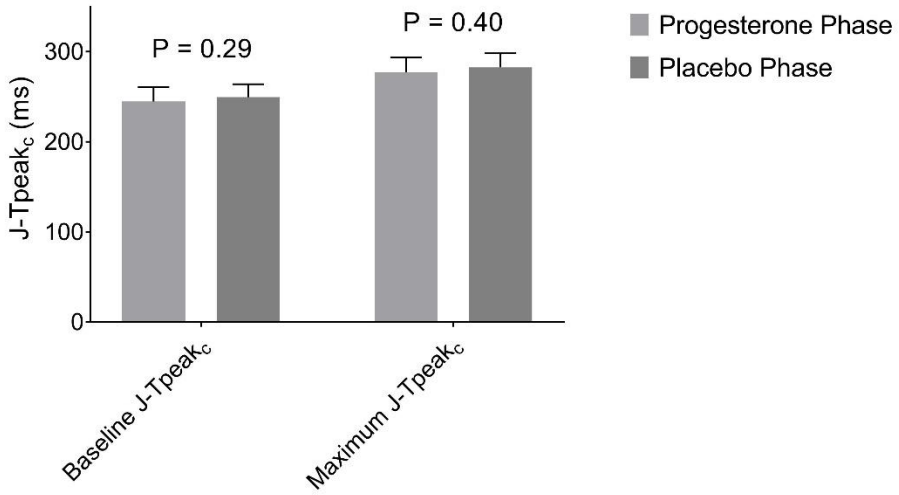

Figure 17 Baseline and Maximum Lead II J-Tpeakc with SD after Intravenous Ibutilide During Progesterone and Placebo Phases

Effect of oral progesterone on J-Tpeakc intervals following ibutilide administration in premenopausal women

Oral progesterone did not significantly attenuate maximum J-Tpeakc interval and maximum % change from baseline compared to placebo (**Figure 17**, **Table 6**). J-Tpeakc intervals during the first hour and for 8 hours following the 10-minute ibutilide infusion in the progesterone and placebo groups are presented in **Figure 18**.

Effect of oral progesterone on area under the J-Tpeakc and Tpeak-Tend interval versus time curves after initiation of the ibutilide infusion in premenopausal women

Compared to placebo, oral progesterone did not significantly decrease the J-Tpeakc (**Table 6, Figure 18**) $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$.

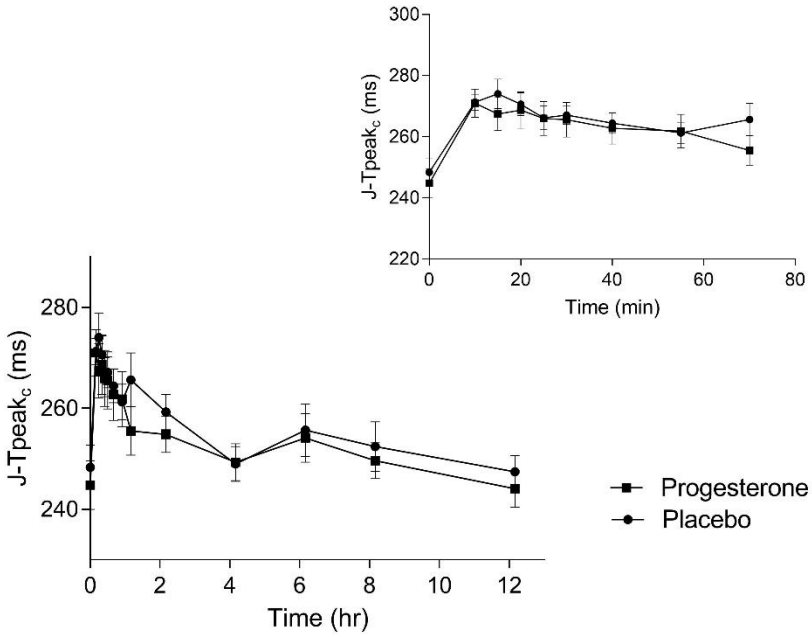

Figure 18 Lead II Tpeak-Tend Interval Response to Intravenous Ibutilide During Progesterone and Placebo Phases in Premenopausal women

Table 6 Lead II J-Tpeakc Response to Intravenous Ibutilide During Progesterone and Placebo Phases (mean ± SD). AUEC = Area under the effect curve; SD = Standard deviation

	Progesterone	**Placebo**	**P**
J-Tpeakc			
Baseline (pre-ibutilide) J-Tpeakc (ms)	245 ± 16	249 ± 14	0.296
Maximum J-Tpeakc (ms)	277 ± 16	282 ± 16	0.402
Maximum % change in J-Tpeakc from baseline	13 ± 4	13 ± 5	0.975
$AUEC_{0\text{-}1.17}$ J-Tpeakc (ms·hr)	307 ± 18	311 ± 11	0.441
$AUEC_{0\text{-}8.17}$ J-Tpeakc (ms·hr)	3085 ± 152	3094 ± 120	0.785

Specific Aim 2D: Determine the influence of oral progesterone in premenopausal women on drug-induced lengthening of late ventricular repolarization

Baseline (pre-ibutilide) Tpeak-Tend intervals in premenopausal women

Baseline (pre-ibutilide) Tpeak-Tend were not significantly different between the placebo and progesterone phase (**Figure 19**, **Table 7**).

Table 7 Lead II Tpeak-Tend Response to Intravenous Ibutilide During Progesterone and Placebo Phases (mean ± SD). AUEC = Area under the effect curve; SD = Standard deviation

	Progesterone	**Placebo**	**P**
Tpeak-Tend			
Baseline (pre-ibutilide) Tpeak-Tend (ms)	81 ± 50	83 ± 50	0.301
Maximum Tpeak-Tend (ms)	94 ± 56	95 ± 59	0.838
Maximum % change Tpeak-Tend from baseline	17 ± 8	15 ± 12	0.680
$AUEC_{0\text{-}1.17}$ Tpeak-Tend (ms·hr)	102 ± 65	103 ± 67	0.950
$AUEC_{0\text{-}8.17}$ Tpeak-Tend (ms·hr)	1025 ± 642	1026 ± 656	0.960

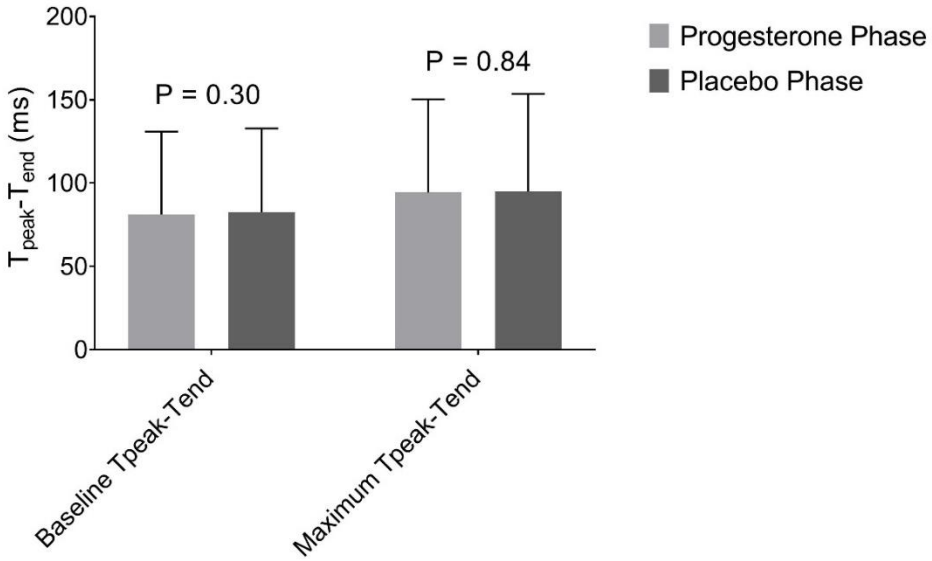

Figure 19 Baseline and Maximum Lead II Tpeak-Tend with SD after Intravenous Ibutilide During Progesterone and Placebo Phases

Effect of oral progesterone on Tpeak-Tend intervals following ibutilide administration in premenopausal women

Oral progesterone did not significantly attenuate maximum Tpeak-Tend interval and maximum % change from baseline compared to placebo (**Figure 19**, **Table 7**). Tpeak-Tend intervals during the first hour and for 8 hours following the 10-minute ibutilide infusion in the progesterone and placebo groups are presented in **Figure 20**.

Effect of oral progesterone on area under the Tpeak-Tend interval versus time curves after initiation of the ibutilide infusion in premenopausal women

Compared to placebo, oral progesterone did not significantly decrease the Tpeak-Tend (**Table 7, Figure 20**) $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$.

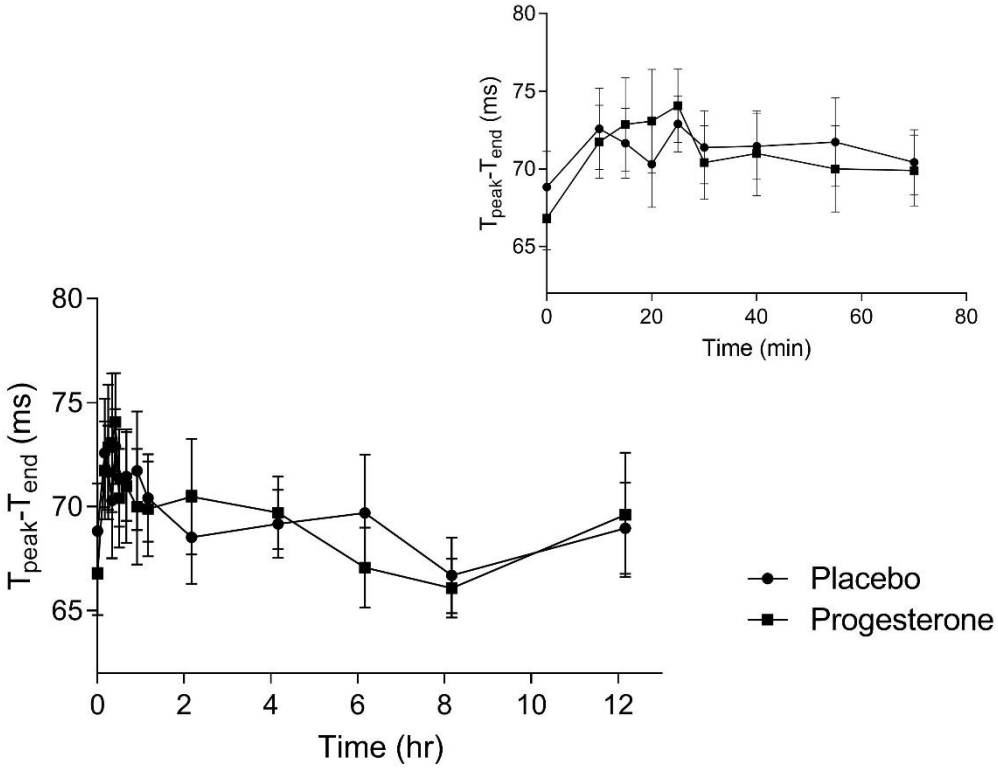

Figure 20 Lead II Tpeak-Tend Interval Response to Intravenous Ibutilide During Progesterone and Placebo Phases in premenopausal women

Specific Aim 3: Identify predictors of response to progesterone-mediated attenuation of drug-induced QT interval lengthening in premenopausal women

Study subjects and demographic characteristics

Data from the 15 subjects who completed all phases of the study were utilized for the PKPD model development [81]. Subjects were predominantly Caucasian (60%, n=9), with fewer African American (33%, n=5) and Middle Eastern (7%, n=1) subjects. Mean age was 29 ± 5 years and mean weight was 81 ± 22 kg. Median (± IQR range) progesterone, estradiol plasma concentration and P:E values are listed in **Table 8**. Mean (±SD) dose of ibutilide administered was 0.24 mg (±0.06).

Table 8 Subjects' Serum Progesterone and Estradiol Concentrations and Ratio of Serum Progesterone:Estradiol during the Progesterone and Placebo Phases of the study (median ± IQR range)

Serum concentrations and ratio	Progesterone Phase	Placebo Phase
Progesterone concentration (ng/mL)	14.9 (9.0, 24.5)	0.8 (0.5, 1.7)
Estradiol concentration (pg/mL)	70.0 (54.7, 117.5)	60.0 (45.7, 86.5)
P:E	129 (86, 284)	14 (8, 39)

P:E = Ratio of molar concentrations of progesterone:estradiol

Base structural model: A 2-compartment model with first-order elimination from the central compartment

A 2-compartment model with first-order elimination from the central compartment was chosen as the base structural PK model (subroutine ADVAN3 TRANS4 in NONMEM® version 7.2.1). Individual PK model fits (representative subjects are shown in **Figure 21**).

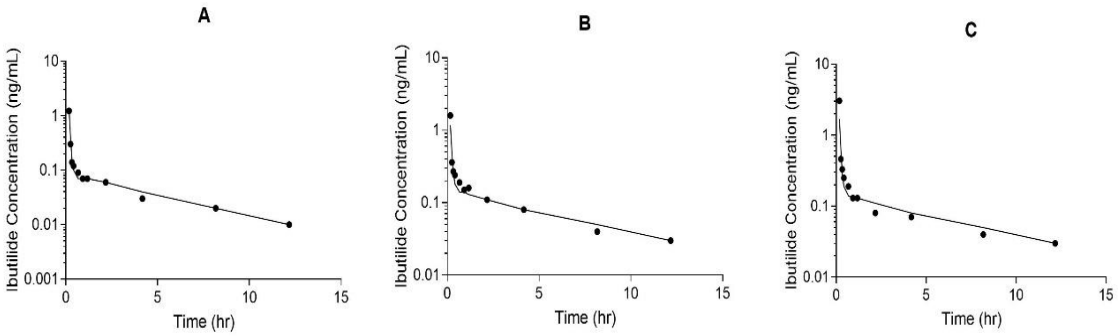

Figure 21 Individual pharmacokinetic and pharmacodynamic model fit obtained in three representative subjects. A, represents the best fitting concentration vs. time profile from subject No. 3. B, represents an average fitting concentration vs. time profile from subject No. 1. C, represents the worst concentration vs. time profile from subject No. 2; Solid circles represent observed data points; solid lines represent the fitted line to the data sets.

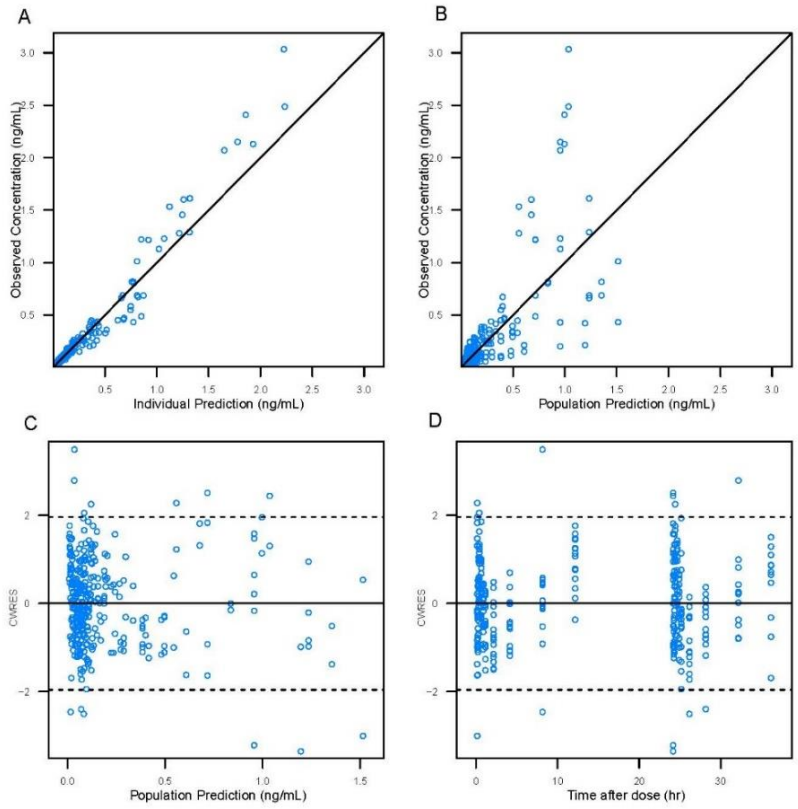

Figure 22 Goodness of Fit Plots of the final 2-compartment pharmacokinetic model. (A) Individual predicted ibutilide concentration versus observed concentration. (B) Population predicted ibutilide concentration versus observed concentration. (C) Conditional weighted residuals (CWRES) versus population predicted concentration. (D) Conditional weighted residuals versus time. The black solid line in (A) and (B) represent the line of identity, whereas in (C) and (D) it represents the position where conditional weighted residual equals 0.

A 2-compartment model with first-order elimination from the central compartment was chosen as the base structural PK model (subroutine ADVAN3 TRANS4 in NONMEM version 7.2.1). While we observed a reduced OFV in the 3-compartment model compared to the 2-compartment (Δ27), there were no differences in the GOF plots or individual concentration time profiles between the 2- and 3-compartment, suggesting that the decrease in OFV was only due to high number of parameters explaining residual variability. Furthermore, the 3-compartment model did not estimate both the two peripheral volumes of distribution and distribution clearance as accurately as the 2-comparment which was reflected by higher shrinkage. Compared to a 3-compartment model, the PK estimates of the 2-compartment model were also more aligned with those from previously published literature [114, 115]. Residual variability was best described by a proportional residual error model. Inter-occasion variability was added on ibutilide systemic clearance (CL_S) as it decreased the inter-patient variability associated with this parameter as well as significantly decreased OFV. None of the covariates tested were found to have a significant impact on any of the PK parameters. The GOF diagnostic plots indicated no model misspecification (**Figure 22**). The VPCs showed adequate model predictive performance as all observed data points were included in the 95% CI of the model predictions (**Figure 23**). Final population PK parameter estimates for both the fixed-effect and random-effect parameters were in close agreement with the corresponding median estimates derived from the bootstrap indicating model robustness and precision of final parameter estimates (**Table 9**).

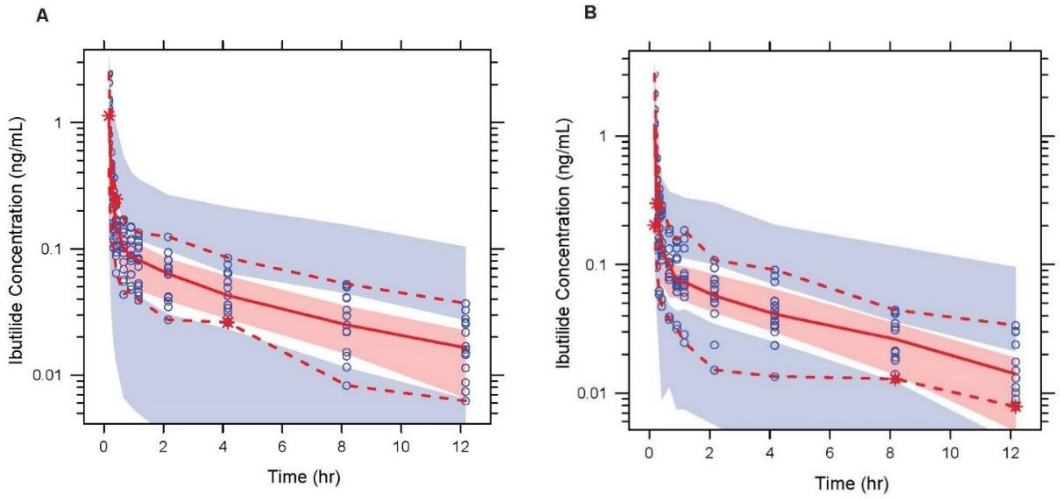

Figure 23 Visual Predictive Checks of final Pharmacokinetic Model During the Placebo (A) and Progesterone Phase (B). Visual predictive checks of final pharmacokinetic model during the placebo (A) and progesterone phase (B). The x-axis represents the time (hr) and the y-axis represents the ibutilide serum concentration (ng/mL). The solid red line represents the median observed plasma concentration (nanogram per liter; prediction-corrected plasma concentration in the pcVPC to the right), and the semitransparent red field represents a simulation-based 95% confidence interval for the median. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semitransparent blue fields. The observed plasma concentrations (prediction corrected in the pcVPC) are represented by blue circles.

Table 9 Final Model and Bootstrap Parameter Estimate for the Population Pharmacokinetic Model of Ibutilide.

Parameter	**Population Estimates (RSE%)**	**Bootstrap Estimates (95 CI%) ***
CL_s, L/hr	307 (14)	311 (235, 400)
V_c, L	109 (23)	113 (66, 168)
CL_d, L/hr	1040 (21)	1075 (709, 1534)
V_p, L	1550 (14)	1584 (1145, 2122)
IOV on CL_s	0.09 (46)	0.09 (0.02, 0.8)
ω CL_s (%)	48.1 (34)	45.7 (25, 60)
ω V_c (%)	68.4 (31)	64.7 (17, 83)
ω CL_d (%)	66.8 (30)	63.5 (26, 80)
ω V_p (%)	54.4 (30)	51.9 (30, 65)
σ PROP (%)	4 (12)	4 (3.2, 5.4)

CL_s = systemic clearance, V_c = central compartment volume of distribution, CL_d = distribution clearance, V_p = peripheral compartment volume of distribution, IOV = inter-occasion variability, ω = inter-individual variability (omega), σ = residual variability (sigma), PROP = proportional, RSE = residual standard error, CI = confidence interval.
*Based on percentiles

An Emax model with intercept (α) as base pharmacodynamic model

The relationship between serum ibutilide concentrations and baseline-corrected QT_F intervals (**Figure 24**) was best described by an E_{max} model with an intercept term (α), which was introduced to enhance model stability by allowing prediction of negative baseline-corrected QT_F interval values as seen in observed data (**Figure 25 vs 26**).

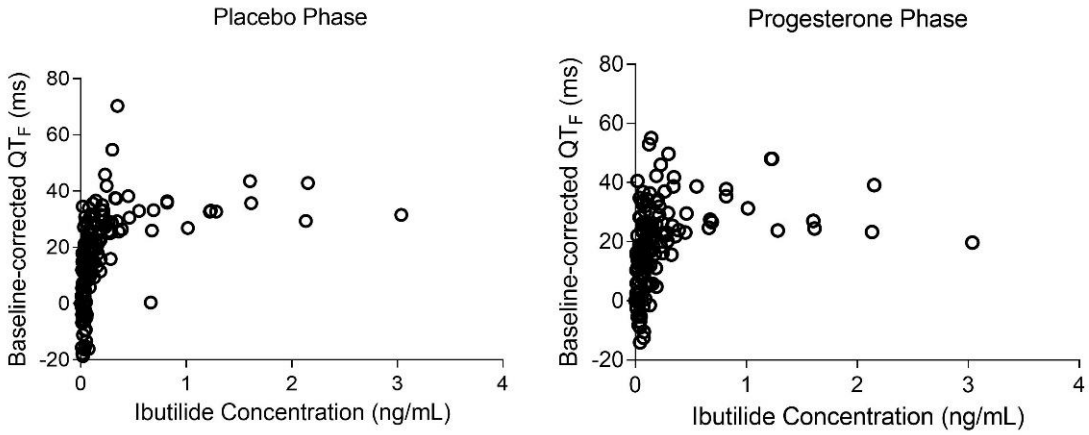

Figure 24 Relationship between serum ibutilide serum concentrations and baseline-corrected QT_F intervals during the progesterone and placebo phases.

Table 10 Final Model and Bootstrap Parameter Estimate for the Population Pharmacokinetic/Pharmacodynamic Model;

Parameter	**Population Estimates (RSE%)**	**Bootstrap Estimates (95 CI%) ***
E_{max} (ms)	69.2 (20)	68.7 (55, 87)
EC_{50} (ng/mL)	0.06 (44)	0.06 (0.03, 0.1)
α (ms)	-24 (27)	-24 (-34, -15)
P:E on E_{max}	-2.5 (33)	-2.5 (-4.1, -0.68)
ω E_{max}	30.0 % (85)	28.5 % (14, 43)
ω EC_{50}	45.6 % (84)	38.9 % (0.004, 94)
σ ADD (ms)	12.2 (16)	11.9 (10, 14)

E_{max} = maximum effect of ibutilide on baseline-corrected QT_F interval length, EC_{50} = serum ibutilide concentration required to achieve 50% of the maximum effect on baseline-corrected QT_F, α = intercept, ω = inter-individual variability (omega), σ = residual variability (sigma), ADD = additive, RSE = residual standard error, CI = confidence interval.
* Based on Percentiles.

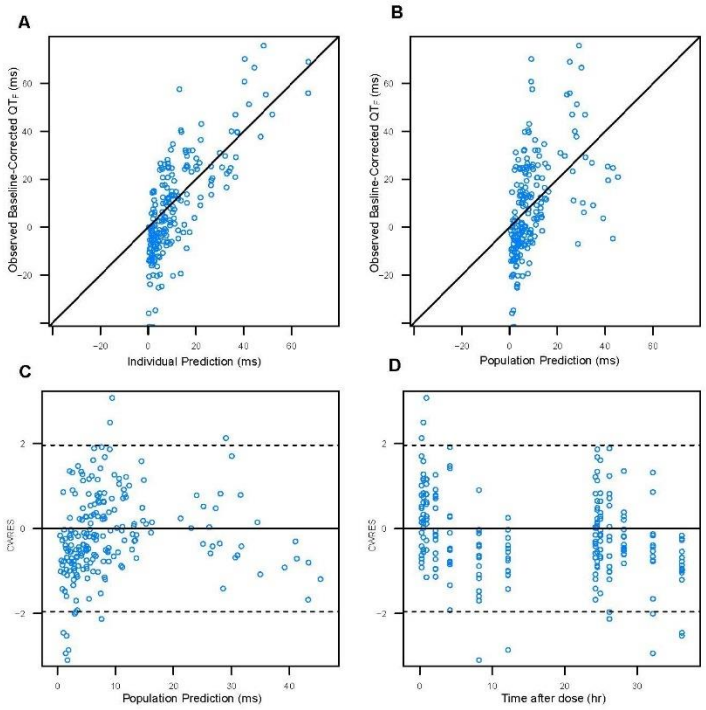

Figure 25 Goodness of Fit Plots of the pharmacodynamic Emax model without intercept term. (A) Individual predicted concentration versus observed baseline-corrected QTF. (B) Population predicted baseline-corrected QTF versus observed baseline-corrected QTF. (C) Conditional weighted residuals versus population predicted concentration. (D) Conditional weighted residuals versus time. The black solid line in (A) and (B) represent the line of identity, whereas in (C) and (D) it represents the position where conditional weighted residual equals 0.

Addition of a hypothetical effect compartment into the model did not result in significant reduction in OFV or in any improvement in GOF plots. The additive error model best described the observed data and resulted in adequate model performance based on GOF plots (**Figure 26**).

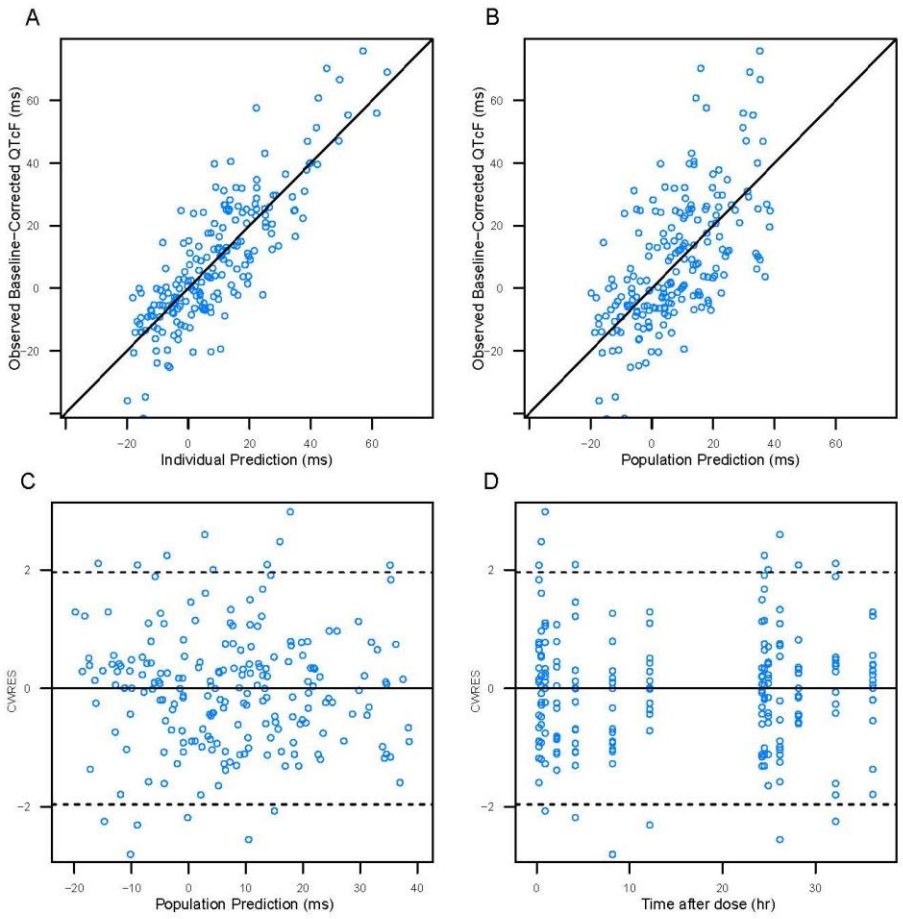

Figure 26 Goodness of Fit Plots of the final pharmacodynamic Emax model with intercept term. (A) Individual predicted concentration versus observed baseline-corrected QTF. (B) Population predicted baseline-corrected QTF versus observed baseline-corrected QTF. (C) Conditional weighted residuals versus population predicted concentration. (D) Conditional weighted residuals versus time. The black solid line in (A) and (B) represent the line of identity, whereas in (C) and (D) it represents the position where conditional weighted residual equals 0.
CWRES = Conditional weighted residuals

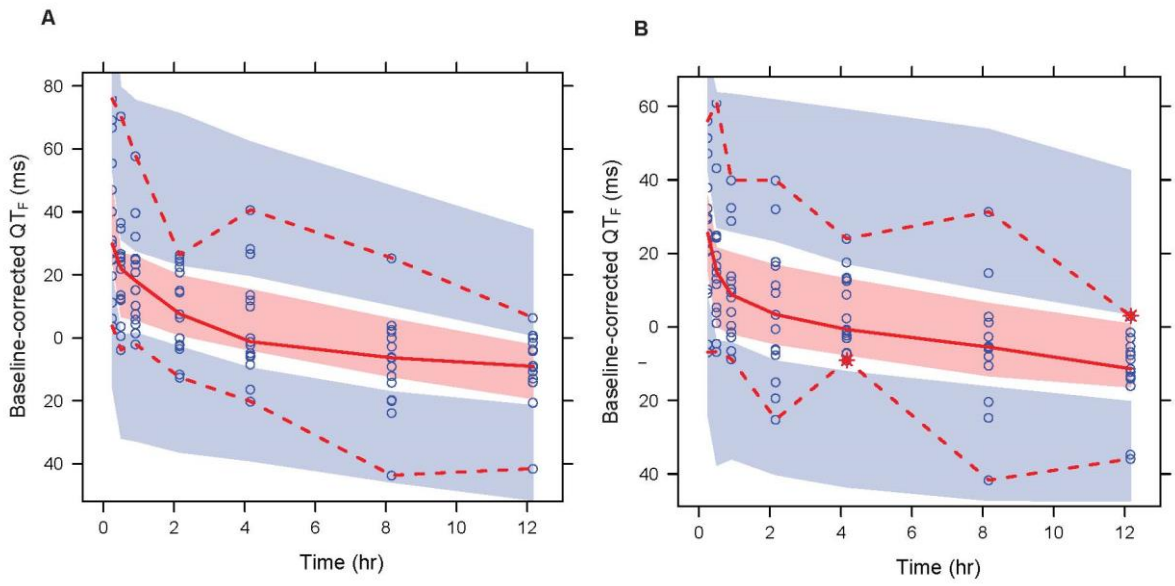

Figure 27 Visual Predictive Checks of final Pharmacodynamic Model During the Placebo (A) and Progesterone Phase (B). Visual predictive checks of the final pharmacokinetic/pharmacodynamic model during the placebo (A) and progesterone phase (B). The x-axis represents the time (hr) and the y-axis represents the baseline-corrected QTF (ms). The solid red line represents the median observed plasma concentration (nanogram per liter; prediction-corrected plasma concentration in the pcVPC to the right), and the semitransparent red field represents a simulation-based 95% confidence interval for the median. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are shown as semitransparent blue fields. The observed plasma concentrations (prediction corrected in the pcVPC) are represented by blue circles.

Progesterone-to-estradiol ratio as A predictor of maximum progesterone-mediated attenuation of drug-induced QT_F lengthening

Of the covariates tested on the PD parameter estimates, P:E was the only significant covariate influencing E_{max} (ΔOFV -10.745, $p<0.01$), EC_{50} (ΔOFV -3.984, $p<0.05$), and INT (ΔOFV -6.014, $p<0.05$). A forward stepwise inclusion approach led to a final model with P:E as a covariate influencing both E_{max} and EC_{50} but not INT. However, after performing nonparametric bootstrapping, it was observed that the 90% CI of the P:E effect on EC_{50} term included zero; therefore P:E was removed as a covariate related to EC_{50} from the model. The final PK/PD model therefore included only P:E as a covariate for E_{max}, which significantly reduced the OFV (ΔOFV -10.745, $P<0.01$) The final population PK:PD fixed- and random-effect parameters were compared to the median parameter estimates obtained from the 500 non-parametric estimates (**Table 10**). A strong level of agreement between NONMEM and bootstrap estimates ensured robustness and stability of the final model. VPCs showed that the observed data were well-described by the final PKPD model with no model misspecifications in both phases (**Figure 27 A & B**).

Monte-carlo simulations of P:E on drug-induced QT_F lengthening

Monte Carlo simulations revealed that in the simulated subjects centered around the placebo phase P:E median, the maximum ibutilide induced-baseline-corrected QT_F lengthening was on average 40.8 ms and 29.5 ms in the simulated subjects centered around the progesterone phase P:E. A P:E ratio of 45, which represent the median ratio observed in the study subjects across both phases, resulted in a maximum ibutilide induced-baseline-corrected QT_F lengthening of 37.8 ms. The maximum ibutilide induced-

baseline-corrected QT_F lengthening for the simulated subjects representing the 75^{th} and 99^{th} percentile P:E observed during the progesterone phase was 25.5 ms and 18.68 ms, respectively (**Figure 28**). The ibutilide induced-baseline-corrected QT_F interval lengthening returned to baseline within the first hour after ibutilide administration in the simulated population centered around the median P:E of the progesterone phase.

Such return to baseline was much slower among the simulated population centered around both P:E of 15 and 45, requiring 4 hours or longer (**Figure 29**).

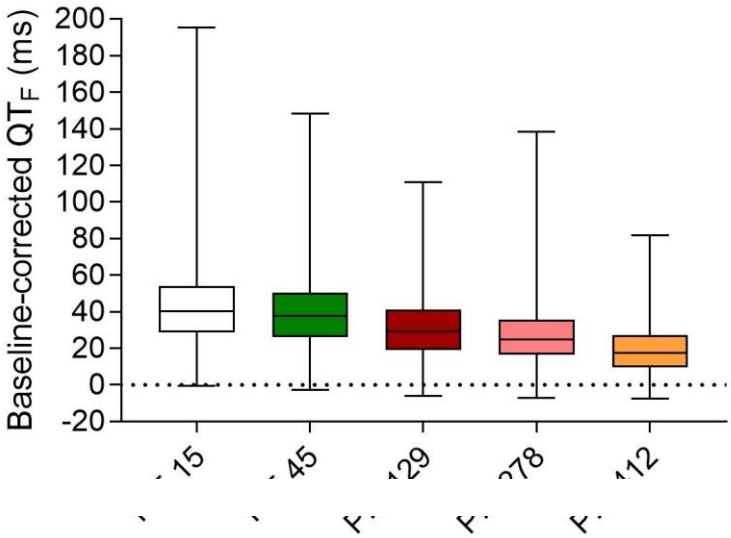

Figure 28 Visual Representation of Simulation Results for the Effect of Progesterone-To-Estradiol Ratios (P:E) on maximum baseline-corrected QT_F Interval Lengthening

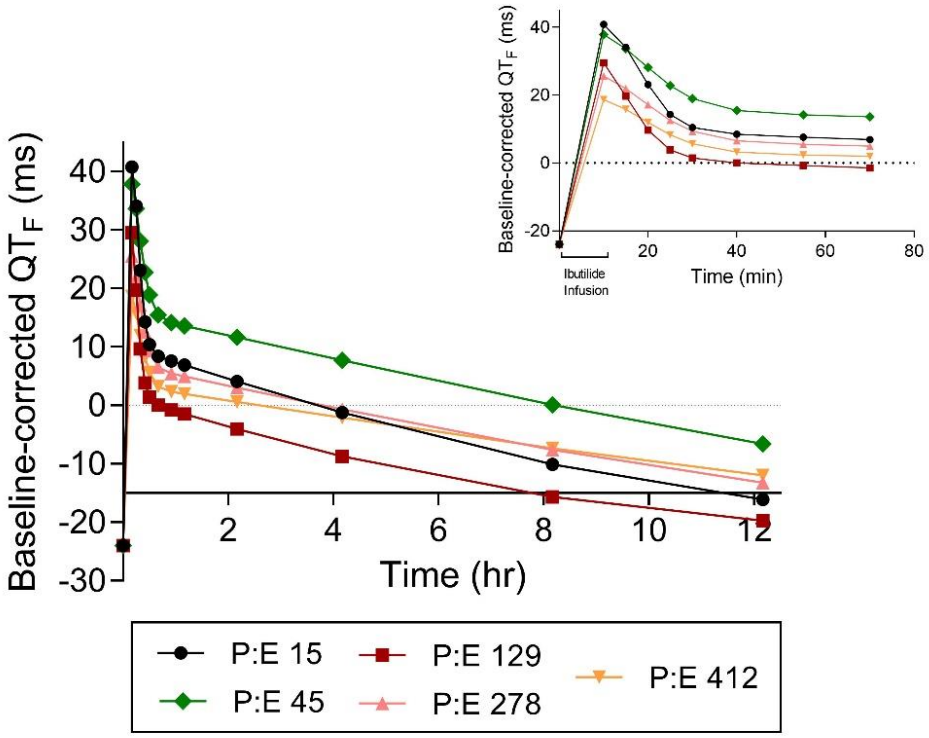

Figure 29 Visual Representation of Simulation Results for the Effect of Progesterone-To-Estradiol Ratios (P:E) on the baseline-corrected QT_F Interval Lengthening post ibutilide infusion up to 12 hours. Effects of different P/E on simulated baseline-corrected QTF interval lengthening post ibutilide infusion (10 min) from zero to 12 hours post-infusion in the bottom left graph and from zero to 1 hour post-infusion in the inset in the top left corner. Each line represents the mean baseline-corrected QTF interval lengthening for four different simulated populations of 1000 virtual subjects each based on the median P:E of the placebo (15) and progesterone phase (129), the median of the entire study population (45) and the 75th (278) and 95th percentile P:E (412) in the progesterone phase.

DISCUSSION

Aim 1: Transdermal testosterone attenuates drug-induced QT interval lengthening in older men. In contrast, oral progesterone does not attenuate drug-induced QT interval lengthening in older men.

This is the first study to investigate the efficacy of transdermal testosterone and oral progesterone on drug-induced QT interval lengthening in older men. We found that transdermal testosterone 100 mg daily administered for 7 days significantly attenuated QT_F and QT_{Fram} interval response to low-dose ibutilide. In contrast, oral progesterone 400 mg once daily, which resulted in serum progesterone concentrations at the high end of the range of those observed in women during the luteal phase of the menstrual cycle, did not attenuate drug-induced QT interval lengthening in older men. Though these data were obtained from a relatively small sample size, these findings suggest that transdermal testosterone could be effective for reducing the risk of drug-induced QT interval prolongation in older men requiring therapy with QT interval-prolonging drugs. The results of our study provide support for further investigation of the effectiveness of transdermal testosterone as a novel therapeutic approach to attenuate drug-induced QT interval lengthening in older high-risk male patients.

Previous studies suggest that higher testosterone concentrations are associated with shorter QT intervals, and that testosterone administration or supplementation may shorten ventricular repolarization in specific populations.[120] QT intervals in boys and girls are similar until puberty, after which QT and J-Tpeak intervals diverge, becoming shorter in males, likely due to increased testosterone production.[55] Administration of testosterone or DHT shortens the QT interval and attenuates drug-induced lengthening of ventricular repolarization in vivo.[70, 121,

[122] Epidemiologic data and research in hypogonadic men demonstrate an inverse relationship between serum testosterone concentrations and QT intervals.[63] In a prospective analysis of the European pharmacovigilance database, 41 individual cases of men with drug-induced QT interval prolongation were identified. Of these, 15 were cases of drug-induced TdP which were considered to be attributable to androgen deprivation therapy. Among these, 7 were male subjects with hypogonadism, in the majority of whom correction of low serum testosterone concentrations either spontaneously or via testosterone administration resulted in normalization of QTc intervals and no subsequent episodes of TdP.[123] Our study is the first to show that administration of transdermal testosterone to older men with age-related declining serum testosterone concentrations attenuates drug-induced QT interval lengthening by minimizing drug-induced lengthening of both early and late ventricular repolarization.

Previous studies have suggested that progesterone may also be protective against lengthening of ventricular repolarization and/or drug-induced arrhythmias. Progesterone shortens ventricular APD in guinea pig ventricular myocytes, an effect that was reversed by the progesterone receptor inhibitor mifepristone.[82] QTc intervals are shorter during the luteal phase of the menstrual cycle, when serum progesterone concentrations are highest, compared to those during the follicular phase.[48] In women with congenital long QT syndrome, the risk of cardiac events is low during pregnancy, when serum progesterone concentrations are high, but increases postpartum, when serum progesterone concentrations decline.[124] In healthy female volunteers studied during different phases of the menstrual cycle, drug-induced QT interval lengthening was inversely correlated with serum progesterone concentration.[50] Progesterone and DHT protected against sudden cardiac death in a transgenic rabbit model of long QT syndrome type 2.[125] We conducted a proof-of-concept study in which found that oral progesterone administration

attenuated drug-induced QT interval lengthening in a group of young healthy women, studies during the menses phase of the cycle to minimize the effects of endogenous sex hormones.[81] However, in the present study, oral progesterone administration was not effective at attenuating drug-induced QT interval lengthening in older men, despite the fact that we achieved high serum progesterone concentrations. While progesterone receptors may be present in female hearts,[126] it is not clear whether male hearts have appreciable progesterone receptors. In a study of tissue samples obtained from non-human male primates and men, genomic progesterone receptors were expressed in multiple tissues, including prostate, mammary gland, and pituitary.[127] However, progesterone receptors were not expressed in cardiac tissue.[127] Therefore, the lack of response to progesterone in older men in this study may be due to an absence or paucity of cardiac progesterone receptors.

Ibutilide lengthens QT interval via modulation of late ventricular repolarization via I_{Kr} inhibition[104] as well as influencing early repolarization via augmentation of slow inward sodium current[24], facilitating assessment of the influence of sex hormones on drug-induced lengthening of both early and late ventricular repolarization. We administered a mean ibutilide dose of 0.27 ± 0.05 mg which represents 27% of the lowest therapeutic dose (1 mg). This subtherapeutic dose (0.003mg/kg) has been shown previously to induce a clinically relevant lengthening, but not prolongation to > 500 ms, of the QTc interval in healthy volunteers.[50, 81]

The results of this study support further investigation of the efficacy and safety of transdermal testosterone for reducing the risk of drug-induced QT prolongation for older men with one or more additional risk factors for QT prolongation who require therapy with a QT interval-prolonging drug. In our study, short-term administration of transdermal testosterone 100 mg daily was safe and well-tolerated. The feasibility and safety of longer-term administration of

transdermal testosterone requires additional study. Longer-term (> 18 months) testosterone treatment has been associated in some studies with adverse cardiovascular effects, including an increased risk of all-cause mortality, myocardial infarction and stroke.[128] Conversely, however, other studies have found that men with lower serum testosterone are at higher risk of metabolic syndrome and other cardiovascular risk factors.[129] A meta-analysis of randomized controlled trials found no increase in the endpoint of cardiovascular death, non-fatal myocardial infarction, and stroke associated with testosterone therapy compared to placebo.[130] The European Medicines Agency's Pharmacovigilance Risk Assessment Committee concluded that the benefits of testosterone therapy outweigh risks in appropriate patients.[131] A post-marketing study required by the FDA is currently being conducted to investigate the long-term safety of transdermal testosterone administration. Until completion of study, long-term administration of transdermal testosterone as a tool to attenuate drug-induced QT interval prolongation should not be attempted. However, short-term administration of testosterone in older high-risk male subjects who are prescribed azithromycin or needs to be initiated on a dofetilide or sotalol treatment may be feasible and should further studied. When studying the latest, it is essential to determine if testosterone may negatively affect the antiarrhythmic effects of sotalol and dofetilide given its influence on cardiac ion channels associated with their pharmacological effect.

Aim 2: Testosterone shortens ventricular repolarization in men by attenuating drug-induced lengthening of both early and late ventricular repolarization. Oral progesterone attenuates neither J-Tpeakc (early repolarization) not Tpeak-Tend (late repolarization) in both older men and premenopausal women.

In our investigation, the influence of transdermal testosterone on drug-induced QT interval lengthening was attributable to attenuation of both drug-induced lengthening of J-Tpeakc and Tpeak-Tend. Since transdermal testosterone attenuated drug-induced lengthening of both early and late phase repolarization, testosterone may be effective for attenuation of lengthening of QT interval induced by inhibitors of I_{Kr} as well as drugs that modulate early phase repolarization. Progesterone did not attenuate lengthening of either early or late repolarization in older men. This result is not surprising given that this sex hormone did not attenuate drug-induced lengthening of QT interval either in this study population. Reasons for lack of efficacy may be the absence or paucity of progesterone receptors in the male cardiomyocytes.[127]

It has been commonly believed that drugs that prolong the QT interval do so primarily via I_{Kr} inhibition and associated effects on late phase ventricular repolarization. However, recent evidence suggests that some QT interval-prolonging drugs may modulate early repolarization as well, through effects on late sodium or other currents.[104] In our investigation, the influence of transdermal testosterone on drug-induced QT interval lengthening was attributable to attenuation of both drug-induced J-Tpeakc and Tpeak-Tend. These ECG biomarkers have been proposed to differentiate between predominant inhibition of hERG and hERG inhibition combined with inward current block, making it possible to use these measures to differentiate multichannel effects of a drug.[5] In the presence of hERG inhibition, changes in J-Tpeakc interval may represent the effect of a QT interval-prolonging drug on early repolarization due to modulation

of the inward calcium and/or late sodium currents, while changes in Tpeak-Tend interval may represent late repolarization via modulation of the outward currents I_{Ks} and I_{Kr}.[5] Furthermore, Tpeak-Tend has been used as a measure of transmural dispersion of repolarization and was recently shown to be a useful biomarker to stratify the risk of arrhythmic and mortality outcomes for a variety of diseases.[132] Our data also showed that testosterone significantly attenuated Tpeak-Tend/QT compared to placebo. The Tpeak-Tend/QT interval is a novel biomarker that has been suggested to be a novel marker of arrhythmogenesis.[97] Higher Tpeak-Tend/QT predisposes subjects to subjects to ventricular tachycardia/fibrillation (VT/VF).[96] Based on these preliminary data, we hypothesize that transdermal testosterone administration may attenuate the risk of older men on QT prolonging drugs to develop ventricular arrhythmia and/or fibrillation but further study is needed to determine whether transdermal testosterone administration may attenuate the risk of ventricular arrhythmias in older men.

Testosterone has been shown to influence L-type calcium currents and I_{Ks}. Testosterone shortened ventricular action potential duration (APD) in isolated guinea pig ventricular myocytes via nongenomic enhancement of the slow component of the delayed rectifier potassium current (I_{Ks}) and inhibition of L-type calcium current.[78] The effects of testosterone were reversed by a nitric oxide scavenger and a nitric oxide synthase 3 (NOS3) inhibitor, suggesting that testosterone's ion current effects are modulated by nitric oxide released from NOS3. Testosterone's effects were also reversed by inhibitors of testosterone receptors, c-Src, phosphatidylinositol 3-kinase (PI3K), and Akt. Therefore, the influence of testosterone on early and late ventricular repolarization may be a result of effects on L-type calcium current and I_{Ks}, respectively. In view of data suggesting that testosterone's effects may be reversed by PI3K inhibition, whether testosterone may also influence late sodium current requires further study[82].

A study published by Tisdale et al established that oral progesterone attenuates drug-induced QT prolongation in premenopausal women when administered during the menses phases of the cycle.[81] By using electrocardiographic data from this study, we sought to determine wherever this attenuation of drug-induced QT lengthening is a consequence of attenuation of early repolarization (J-Tpeakc), late repolarization (Tpeak-Tend) or both. We were only able to measure these ECG biomarkers in 9 of 15 women who completed the clinical study due to missing or deteriorated ECGs. Based on our results, oral progesterone attenuated either early nor late ventricular repolarization. We suspect that the lack of significance in these results may be due to the too small sample size and the possible the lack of some of strong responders' ECGs among the available data. Furthermore, Tisdale et al' clinical study was not powered to detected significant differences in J-Tpeakc or Tpeak-Tend, but only a difference is QT interval length of 8 ms.[81] Further research should seek to determine the effects of oral progesterone on early vs late ventricular repolarization to optimize the clinical utilization of this sex hormone as a tool to decrease the risk of drug-induced QT prolongation in women at high-risk.

Aim 3: Progesterone-to-estradiol ratio is a predictor of progesterone-mediated-attenuation of drug-induced QT interval lengthening in premenopausal women

A population PK/PD model was developed to identify predictors of progesterone-mediated attenuation of drug-induced QT lengthening in a population of premenopausal women. Study subjects were administered oral progesterone or placebo during the menses phase of the menstrual cycle. The data on which this model is based on were collected in a randomized, double-blind clinical trial.[81] This clinical study established that oral progesterone 400 mg administered daily for 1 week significantly attenuates drug-induced QT interval lengthening in

young healthy women. While progesterone was effective in this study, a large degree of inter-subject variability in response to progesterone mediated-attenuation in drug-induced QT interval lengthening was observed. We originally hypothesized that the clinical variables weight, age, serum progesterone concentration, serum estradiol concentration, and the ratio of serum progesterone:estradiol concentrations explain inter-subject variability in progesterone-mediated attenuation of drug-induced QT interval lengthening in premenopausal women. However, the only covariate that was significant in our final PK/PD model was the ratio of serum concentrations of progesterone-to-estradiol, not progesterone concentration. This finding proposes P:E as a new covariate that could to be utilized to assess the efficacy of oral progesterone in attenuating drug-induced QT interval prolongation. Based on this hypothesis, we performed simulations to determine how different P:E would influence maximum drug-induced QTc interval lengthening. The simulations revealed that patients with higher P:E observed had the greater degree of progesterone-mediated attenuation of drug-induced QT_F lengthening. The maximum baseline corrected QT_F lengthening observed in the progesterone phase simulated population was on average 11 ms shorter compared to the QT_F lengthening in placebo phase simulated population. We considered this difference to be clinically significant based on the consideration that, per FDA guidance, a drug causes clinically relevant QT interval prolongation when the baseline-and placebo-corrected Fridericia-corrected QTc interval is prolonged by ≥ 5 ms. [4, 119]

Other investigators proposed that the P:E has may be a predictor of the risk of drug-induced QTc interval prolongation in women.[53, 120] Rodriguez et al conducted a study to determine the extent of drug-induced QTc interval lengthening in women during different phases of the menstrual cycle and in men. They established that drug-induced QTc lengthening is

significantly shorter during the luteal phase compared to the other phases of the menstrual cycle and that the extent of drug-induced QTc lengthening in the luteal phase is comparable to the drug-induced QTc lengthening observed in men.[50] Rodriguez et al also established that both progesterone concentration (r = -0.40) as well as P:E but not estradiol are inversely correlated (r = -0.41) with drug-induced QTc interval prolongation.[50] Other clinical studies have shown that the QTc interval as well as the extent of drug-induced QTc interval lengthening in women is not determined by individual sex hormones concentrations, but rather it is under the influence of both progesterone and estradiol which exhibit opposing effects on ventricular repolarization.[4, 51]. In a retrospective case-control study, Haseroth et al. showed that the QTc interval is increased in women exposed to estrogen replacement therapy compared to both untreated controls and to women who were exposed to progestin-estrogen therapy.[51] Carnethon et al. demonstrated that in women who are exposed to estrogen therapy, QTc is moderately but significantly prolonged and that the risk of QTc interval prolongation is double compared to never-users.[53] Progesterone's protective effects against drug-induced QTc prolongation have been associated with its inhibitory effects on L-type Ca2+ current during early repolarization and with its activating effects on the slow-delayed rectifier potassium (I_{Ks}) current in the presence of hERG block during late ventricular repolarization.[63, 82] Progesterone was also shown to inhibit I_{Kr} at high concentration, however this should not play a role in our study since none of the subjects achieved such high concentration after progesterone administration.[79] Estradiol on the other hand was determined to have inhibitory effects on the I_{Ks} current, a critical current that ensures proper late ventricular repolarization in presence of hERG blockade.[120] Estradiol was also shown to promote sudden cardiac death in a transgenic rabbit model of LQTS type 2, whereas both progesterone and dihydrotestosterone protected against sudden cardiac death.[125]

Based on the described preclinical and clinical data showing that progesterone and estradiol may have opposite effects on QT interval length, it seems clinically plausible that different P:E may be associated with varying degrees of response to oral progesterone as a tool to attenuate drug-induced QT interval lengthening. Our study was not designed to identify the specific P:E threshold above which efficacy occurs. A larger clinical study should be conducted to confirm what the clinically relevant threshold is and to determine what is the minimum efficacious dose of progesterone needed to achieve such threshold. A shortcoming of our research was the lack of information on the potential presence of CYP3A5 genetic polymorphisms in our study subjects. Since CYP3A5 is one of the two main metabolizing enzymes of progesterone together with CYP3A4, adding such pharmacogenetic data to the model may have helped explained more of residual variability as well as better variability in response to oral progesterone.[133] Our PK/PD model determined that the intercept baseline-corrected QT value to be – 24 ms. While from a physiological standpoint this value does not seem not realistic, one must keep in mind that this represents the simulated difference in baseline-corrected QT_F length between the pre-ibutilide QT_F value in the placebo compared to the progesterone phase. In the clinical trial published by Tisdale et al, the pre-ibutilide QT_F length was significantly shorter in the progesterone phase compared to the placebo phase; this may explain why the intercept value of our model is negative. Finally, the sample size employed in our study was small and limited the predictive power of the non-parametric bootstrapping, VPCs and simulations.

SUMMARY & CONCLUSIONS

With this series of investigations, we sought to explore 1) the effects of testosterone and progesterone on attenuation of drug-induced lengthening of the QT, J-Tpeak and Tpeak-Tend intervals in older men and in healthy premenopausal women, and 2) determine sources of inter-subject variability in progesterone-mediated attenuation of drug-induced QT interval lengthening among young premenopausal women. The overarching concept behind this research is that administration of the sex hormones testosterone and progesterone as drugs to patients who are at high risk of QT interval prolongation, but who require therapy with QT interval-prolonging drugs, may diminish the degree of drug-induced QT interval lengthening and improve medication safety. Determination of the specific phases of repolarization influenced by these sex hormones provides clinically valuable information regarding the utilization of these agents for attenuation of drug-induced QTc interval prolongation in high-risk populations; i.e., whether progesterone and testosterone may attenuate QTc interval prolongation induced by drugs that primarily lengthen early-phase repolarization, late-phase repolarization, or both. While we have determined that oral progesterone is efficacious at attenuating drug-induced QT interval lengthening in premenopausal women during the menses phase of the menstrual cycle, we observed a great degree of inter-subject variability in response. Identifying sources of inter-subject variability in progesterone-mediated attenuation of drug-induced QT interval lengthening is therefore important to optimize future clinical utilization of oral progesterone as a novel therapy to attenuate drug-induced QT interval lengthening in high-risk female patients.

In Aim 1, we investigated the effects of oral progesterone and transdermal testosterone on drug-induced QT lengthening in a population of older men by conducting a randomized, double-blind, placebo-controlled study in fourteen male subjects aged > 65. Study subject was

randomized to one of three phases, during which they were exposed to either oral progesterone, transdermal testosterone or double-placebo. There were no significant differences in peak serum ibutilide concentrations across the three study phases. Serum testosterone and progesterone concentrations were significantly higher during the transdermal testosterone and oral placebo phases, respectively. Baseline pre-ibutilide QTc was not significantly difference between testosterone, progesterone and placebo phases. Maximum post-ibutilide QTc interval during the testosterone phase was shorter than during progesterone or placebo. Area under the effect (QTc):time curve for 1 hour ($AUEC_{0\text{-}1.17)}$ and $AUEC_{0\text{-}8.17}$ following the 10-minute ibutilide infusion were lower during the testosterone phase than during progesterone or placebo, suggesting that testosterone exhibit both a short as well as longer term protective effect against lengthening of ventricular repolarization. Oral progesterone did not attenuate drug-induced QT interval lengthening in older, possibly due to the lack of progesterone receptors in male cardiomyocytes. Adverse effects included a mild skin rash on the placebo gel application site (n=1, 7%). Our data also showed that testosterone significantly attenuated Tpeak-Tend/QT compared to placebo, a biomarker that has been suggested to be a novel marker of arrhythmogenesis.[127] Our findings indicate that testosterone attenuates drug-induced QT interval lengthening and may reduce the risk of ventricular arrhymias in older men, while progesterone does not.

In Aim 2, we investigated the influence of oral progesterone and transdermal testosterone on early vs late ventricular repolarization in older men and premenopausal women. For this aim, we utilized ECG biomarkers J-Tpeakc and Tpeak-Tend to assess exogenous effects of sex hormones on early vs late ventricular repolarization. ECG data collected in the older men and premenopausal women study was used for this analysis. Baseline (pre-ibutilide) J-Tpeakc was

significantly lower during the testosterone phase compared with that in the placebo phase, suggesting that testosterone shortens early late repolarization by inhibiting L-type calcium and/or sodium currents in the absence of a QT prolonging drug. However, baseline Tpeak-Tend intervals in the testosterone and placebo phases were not significantly different, implying that the protective effects of testosterone on late repolarization are mediated by I_{Ks} which in enhanced as compensatory mechanism when I_{Kr} current is also impaired. The mean maximum J-Tpeakc and Tpeak-Tend intervals were significantly lower during the testosterone phase than during the placebo phase. Compared to placebo, transdermal testosterone significantly decreased the J-Tpeakc and Tpeak-Tend $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$, indicating both a short-term and more prolonged attenuation of both early and late-phase ventricular repolarization. Oral progesterone did not significantly attenuate early or late ventricular repolarization in either the older men study subjects nor in the premenopausal women study subjects. This finding indicates that transdermal testosterone but not oral progesterone is effective at attenuating drug-induced lengthening of both early as well late ventricular repolarization. The efficacy and safety of transdermal testosterone as a tool to attenuate lengthening of the QT interval induced by drugs that prolong early repolarization, late repolarization or both should be investigated in a larger clinical study with other QT prolonging drugs that lengthen the QT interval more moderately than ibutilide. Furthermore, the effect of testosterone on late sodium current should be investigated in ventricular myocytes as well as in an animal model to determine if this current is also modulated by testosterone and this modulation occurs via PI3K/Akt pathway. With this aim, we also showed demostrated that testosterone significantly attenuated Tpeak-Tend/QT compared to placebo, a biomarker that reflects arrhythmogenesis.

In Aim 3, we investigated what covariates influence drug-induced QT interval response to progesterone in premenopausal women. For this aim, we utilized a modeling approach. A pharmacokinetic/pharmacodynamic (PK/PD) model describing the relationship between QT intervals and serum ibutilide concentrations was developed based on the data collected from the premenopausal women study. A two-compartment model with elimination from the central compartment best described ibutilide concentration-time profiles. An E_{max} model with an intercept term best described the ibutilide concentration-QT_F relationship. The PK/PD model identified the ratio of serum progesterone:estradiol concentrations (P:E) as a predictor of progesterone-mediated attenuation of drug-induced QT interval lengthening and that P:E $\geq$ 129 is associated with clinically relevant attenuation of drug-induced QT interval lengthening. This finding suggests that P:E may be utilized in future clinical trials as surrogate measure of response to progesterone-mediated attenuation of drug-induced QT interval lengthening. Further research is needed to confirm that 129 is the proper threshold associated with clinically relevant attenuation of drug-induced QT lengthening. Finally, future studies should determine which is the minimally effective dose of oral progesterone needed to ensure efficacy.

In conclusion, these results demonstrate that transdermal testosterone attenuated lengthening of drug-induced QT interval length by shortening both early as well as late ventricular repolarization in a population of older men and may have the ponetial to reduce the arrhythmogenicity associated with administration of a QT prolonging drug in older men as well as. Oral progesterone was determined to induce clinically relevant attenuation of drug-induced QT interval lengthening in women with a P:E > 129. Overall, these studies allow optimization of the use of sex hormones for the prevention of drug-induced QTc interval prolongation and TdP for future studies and clinical utilization.

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