Novel Approaches for Minimizing Drug-Induced QT Interval Lengthening:

Reducing the Risk of Drug-Induced QT Interval Lengthening in Women

James E Tisdale PharmD, FCCP, FAPhA, FNAP, FAHA, FACC
Professor
Department of Pharmacy Practice
College of Pharmacy
Purdue University
Adjunct Professor
School of Medicine
Indiana University
640 Eskenazi Avenue
Indianapolis, IN 46202
317-880-5418
317-880-0568
jtisdale@purdue.edu

Funding: American Heart Association #18TPA34170523

Co-Investigators:

Heather A W Jaynes, MSN, RN
Research Nurse/Project Coordinator
Department of Pharmacy Practice
College of Pharmacy
Purdue University

Brian Overholser, PharmD, Associate Professor
Department of Pharmacy Practice
College of Pharmacy
Purdue University
Adjunct Associate Professor
School of Medicine
Indiana University

Kevin Sowinski, PharmD, Professor
Department of Pharmacy Practice
College of Pharmacy
Purdue University
Adjunct Professor
School of Medicine

Indiana University

Richard Kovacs MD, Professor
Cardiologist
Krannert Institute of Cardiology
Indiana University School of Medicine
Dr. Kovacs has been a collaborator of Dr. Tisdale's for 16 years. His role on this project is: contributed to hypothesis development; contributed to development of protocol and AHA grant; will contribute to data analysis and interpretation; provides expertise related to ECG interpretation and QT interval measurement and analysis

Elena Tomaselli Muensterman, PharmD
Graduate Student
Department of Pharmacy Practice
College of Pharmacy
Purdue University

Table of Contents:

1.0 Background

Torsades de pointes (TdP) is a life-threatening ventricular arrhythmia associated with heart rate-corrected QT (QTc) interval prolongation, which can be caused by > 150 commonly prescribed drugs. TdP risk is higher in women than men, and is modulated by progesterone, which shortens the QTc interval, and estradiol, which lengthens it. The higher the serum progesterone and the progesterone:estradiol ratio, the lower the risk of QTc interval prolongation and TdP. The TdP risk in women increases with age, likely due to declining postmenopausal serum progesterone concentrations. Methods to reduce TdP risk in older women requiring therapy with QTc interval-prolonging drugs have not been developed. In addition, differential effects of progesterone on drug-induced lengthening of early vs late ventricular repolarization in humans are unknown.

■■■ **Drug-Induced QTc Interval Prolongation and Torsades de Pointes (TdP).** QTc interval prolongation may result in TdP, which is associated with sudden cardiac death.[1] We propose to investigate a novel approach to minimizing drug-induced QTc interval lengthening in postmenopausal women: oral progesterone administration. Over 150 available drugs may cause TdP,[2] including antimicrobials, antidepressants, antipsychotics, antiarrhythmics, methadone, and others. Many QTc interval -prolonging drugs are among the top 200 drugs prescribed in the U.S. and millions of patients are exposed annually to drugs that cause TdP.[3] Methods to reduce the TdP risk in high-risk patients have not been developed; risk reduction strategies are critically needed. The investigation described herein will determine the efficacy of a novel therapeutic approach to attenuate drug-induced QTc lengthening in postmenopausal women.

■■■ **Female Sex as a Risk Factor for QTc Interval Prolongation/TdP**. Women are at higher risk of drug-induced TdP, in part due to testosterone production in males beginning at puberty. However, estrogen lengthens the QTc interval,[4] which is shorter during the luteal phase of the menstrual cycle (when serum progesterone is highest) than in the follicular phase.[5] Progesterone has an androgen structure similar to testosterone and is a testosterone precursor in testicular Leydig cells. In women with long QT syndrome (LQTS), TdP risk is low during pregnancy, but increases post-partum, when serum progesterone declines.[6] Progesterone-induced shortening of ventricular action potential duration (APD) is reversed by progesterone receptor inhibition.[7] In a transgenic rabbit LQTS type 2 model, progesterone reduced the incidence of spontaneous polymorphic ventricular tachycardia (PVT) or sudden cardiac death (SCD) and ventricular early afterdepolarizations.[8] Older age is a risk factor for TdP in women, likely due to the decline in serum progesterone concentrations after menopause. Overall, data indicate that progesterone shortens ventricular repolarization and protects against drug-induced ventricular APD lengthening and arrhythmias.

We conducted a prospective, double-blind "proof-of concept" study, assessing the effect of oral progesterone on drug-induced QT interval lengthening in young women.[10] We randomized n=19 healthy women 21-40 years of age to receive 7 days of therapy with oral progesterone 400 mg once daily or matching placebo. After a washout period, subjects were crossed over to the alternate therapy. Women were studied during the menses phase, to minimize the influence of endogenous sex hormones. Following 7 days of treatment, subjects received intravenous ibutilide 0.003 mg/kg and QTc interval

response was determined. Pre-ibutilide QTc intervals were significantly shorter during the progesterone phase. In addition, both the mean maximum QTc interval and maximum % change in QTc interval from pre-ibutilide value were significantly shorter during the progesterone phase.[10] On the basis of this "proof-of-concept" study, we now propose to investigate the effects of oral progesterone administration on drug-induced QTc interval lengthening in a target population of interest: postmenopausal women, who have low serum progesterone concentrations.

■■■ **Influence of Estradiol on Progesterone-Associated Attenuation of Drug-Induced Lengthening of Ventricular Repolarization**. Estradiol lengthens the QTc interval.[4,11-13] In the transgenic LQTS type 2 model,[8] estradiol increased the incidence of PVT and SCD. Some data suggest that the protective effects of progesterone against drug-induced lengthening of ventricular repolarization may be lessened by estradiol. Our group showed that the effect of progesterone on attenuation of quinidine-associated lengthening of ventricular APD_{90} was diminished by concomitant administration of estradiol in ovariectomized rabbits.[14] In young, healthy women, ibutilide-induced QTc interval prolongation was greatest during menses and ovulation, when the mean serum progesterone:estradiol concentration ratios are low (16 and 31, respectively), and least during the luteal phase, when the ratio is highest (82).[9] This raises the possibility that protective effects of progesterone against drug-induced QTc interval lengthening may be diminished in the presence of higher serum estradiol concentrations.

In contrast, however, patients receiving hormone replacement therapy show a significantly smaller degree of QTc interval prolongation among those taking both estrogen and progesterone compared with those taking estrogen alone, indicating a protective effect of progesterone despite concomitant estrogen therapy.[4-11-13,15] Therefore, whether progesterone administered to premenopausal women remains protective against drug-induced QTc interval lengthening during menstrual cycle phases with higher estradiol concentrations than in the menses phase remains unknown. Before initiating clinical trials of progesterone for attenuation of drug-induced QTc interval lengthening in broader populations of women at risk, it is critical to determine if the protective effects of progesterone persist in premenopausal women with high serum estradiol concentrations. Based on our previous study discussed above,[10] in which oral progesterone 400 mg once daily achieved a mean serum progesterone concentration of 16.2 ± 11.0 ng/mL, we hypothesize that, in premenopausal women in the ovulation or luteal phase, this dose will achieve progesterone:estradiol ratios of roughly 164 and 214, respectively,[9,10] which will be sufficiently high to attenuate drug-induced QTc lengthening.

■■■ **Influence of Progesterone on Early and/or Late Repolarization.** Progesterone enhances I_{Ks} current (influencing late phase repolarization)[7] and decreases $I_{Ca,L}$ density in rabbit cardiomyocytes[16] (early repolarization). Progesterone may also activate the (PI3K/AKT) signaling pathway,[17] inhibiting late sodium current (early repolarization). However, the effects of progesterone on drug-induced lengthening of specific phases of ventricular repolarization in humans are unknown, though this information carries important clinical significance. If progesterone attenuates drug-induced lengthening of both early and late repolarization, then it can be anticipated to attenuate QTc lengthening induced by drugs that lengthen early or late repolarization (or both). However, if progesterone is effective for attenuation of drug-induced lengthening of only early or late repolarization, but not both, then progesterone may be effective for attenuation of QTc lengthening induced by drugs that inhibit only early

repolarization (late sodium activating agents, PI3K inhibitors) or late repolarization (I_{Kr} inhibitors). We will assess progesterone's influence on early and late repolarization by measuring its effect on ibutilide-associated lengthening of J-T_{peak} and T_{peak}-T_{end} in both proposed clinical studies.

■■■ **Feasibility and Potential Clinical Application of Progesterone to Attenuate Drug-Induced QTc Lengthening in High Risk Women.** Upon completion of these investigations, large clinical studies will be conducted to determine feasibility, efficacy and safety of concomitant therapy with oral progesterone for reducing risk of drug-induced QTc prolongation in postmenopausal women with additional TdP risk factors who require short or longer-term therapy with QTc-prolonging drugs. Clinical applications for concomitant therapy with oral progesterone in women with TdP risk factors could include patients requiring: a short course of therapy with QTc interval-prolonging antimicrobial agents, such as fluoroquinolones, macrolides, or antifungal agents; QTc-prolonging antidepressants and/or antipsychotic agents**;** methadone for narcotic addiction or pain control; QTc interval-prolonging anticancer agents**.** Progesterone 400 mg orally daily was associated with no reported adverse effects.[18] In our study,[10] the only adverse effects associated with progesterone with a signficantly greater frequency than placebo were fatigue/malaise, though one subject withdrew due to progesterone-associated vertigo. Therefore, risks associated with oral progesterone appear minimal.

■■■ **Summary**. Women are at increased risk of drug-induced QTc prolongation and TdP. While we have established in our previous study that oral progesterone attenuates drug-induced QTc interval lengthening in young women during menses, when serum estradiol concentrations are at their lowest, it is important to establish that oral progesterone remains effective in premenopausal women with higher serum estradiol concentrations prior to initiating larger clinical studies. In addition, age is a risk factor for drug-induced QTc interval prolongation and TdP in women, likely due to declining serum progesterone concentrations. Prior to initiating larger clinical trials in postmenopausal women, it is important to establish the efficacy of oral progesterone administration in this population. We hypothesize that drug-induced QTc interval lengthening is attenuated by administration of oral progesterone in premenopausal women during menstrual cycle phases with higher serum estradiol concentrations and in postmenopausal women.

Establishing the influence of progesterone administration as a preventive method to reduce the risk of TdP will lead to important advances in proarrhythmia risk reduction. Pre- and postmenopausal women at high risk of drug-induced TdP who require therapy with a QTc interval-prolonging drug could be pre- or concomitantly treated with oral progesterone. This will represent a significant advance in arrhythmia prevention and medication safety.

2.0 Rationale and Specific Aims

Torsades de pointes (TdP) is a life-threatening ventricular arrhythmia associated with corrected QT (QTc) interval prolongation, which can be provoked by commonly prescribed drugs. TdP risk is higher in women and is modulated by the ratio of serum progesterone and estradiol; progesterone shortens the QTc interval, while estradiol

lengthens it. The higher the serum progesterone and the progesterone:estradiol ratio, the lower the risk of QTc interval prolongation and TdP, and vice versa. TdP risk increases with age, likely due to declining postmenopausal serum progesterone. Methods to reduce TdP risk in postmenopausal women requiring therapy with QTc interval-prolonging drugs have not been developed. In addition, the differential effects of progesterone on drug-induced lengthening of early vs late ventricular repolarization in humans are unknown. Evidence by our group, presented in the Background and Significance section and supported by our preliminary data, suggests: 1) Oral progesterone attenuates QTc interval lengthening in young women during the menses phase, when serum estradiol is low, and 2) Progesterone reduces the risk of drug-induced TdP. However, whether oral progesterone remains effective for attenuating drug-induced QTc interval lengthening during menstrual cycle phases with higher serum estradiol concentrations is unknown. The efficacy of oral progesterone for attenuating drug-induced QTc lengthening in older, postmenopausal women is also unknown. Identifying effective means of attenuating drug-induced QTc interval lengthening will improve medication safety in women requiring therapy with QTc prolonging drugs.

Our *long-term goal* is to determine mechanisms by which drugs cause arrhythmias and identify safe and effective methods of prevention and management of drug-induced arrhythmias. The *objective in this application* is to evaluate the efficacy of a novel therapeutic approach to reduce the degree of drug-induced QTc interval lengthening in women. Our *central hypothesis* is that acquired QTc interval lengthening is attenuated by exogenous progesterone administration in women. To test this hypothesis, we propose the following *specific aim*:

Specific Aim 1: Determine the efficacy of oral progesterone as a preventive method to diminish drug-induced QT interval lengthening in postmenopausal women. Hypothesis: Oral progesterone attenuates drug-induced QTc interval response in postmenopausal women. Postmenopausal women will receive progesterone in a prospective, randomized, double-blind, placebo-controlled 2-way crossover study. QTc interval response to ibutilide will be assessed.

Specific Aim 2: Determine the influence of oral progesterone on drug-induced lengthening of early versus late ventricular repolarization in postmenopausal women. Hypothesis: Based on preclinical data reporting effects of progesterone on the slow delayed rectifier potassium (I_{Ks}), and L-type calcium ($I_{Ca,L}$) current, progesterone attenuates drug-induced lengthening of both early (represented by measurement of J-Tpeak) and late (represented by measurement of Tpeak-Tend) ventricular repolarization in postmenopausal women.

Specific Aim 3: Determine the efficacy of oral progesterone to diminish drug-induced QTc interval lengthening in premenopausal women during the ovulation phase of the menstrual cycle, when serum estradiol concentrations are high. Hypothesis: Despite higher serum estradiol, oral progesterone achieves a sufficiently high progesterone: estradiol ratio to attenuate drug-induced QTc interval response in premenopausal women during the ovulation phase.

Specific Aim 4: Determine the influence of oral progesterone on drug-induced lengthening of early versus late ventricular repolarization in premenopausal

women during the ovulation phase of the menstrual cycle, when serum estradiol concentrations are high. Hypothesis: Based on preclinical data reporting effects of progesterone on the slow delayed rectifier potassium (I_{Ks}), and L-type calcium ($I_{Ca,L}$) current, progesterone attenuates drug-induced lengthening of both early (represented by measurement of J-Tpeak) and late (represented by measurement of Tpeak-Tend) ventricular repolarization in premenopausal women during the ovulation phase.

This work will establish the efficacy of progesterone administration for attenuation of drug-induced QTc interval lengthening in older women and in premenopausal women during the ovulation phase, when serum estradiol concentrations are high. Our *expected outcome* is that this novel preventive strategy will be effective, and these data will lead to large clinical trials investigating the efficacy of short- and longer-term administration of oral progesterone for reducing risk of drug-induced QTc interval prolongation in women with multiple TdP risk factors who require therapy with QTc interval-prolonging drugs, such as specific antibiotics, antifungal drugs, anti-depressants, antipsychotics, methadone, cancer chemotherapy and others. Due to catastrophic outcomes associated with TdP, there is a critical need to identify strategies to reduce the risk of drug-induced QTc interval prolongation in high-risk patients. The results of this proposed work will improve patient outcomes and change clinical practice.

3.0 Inclusion/Exclusion Criteria

Inclusion Criteria:

Specific Aims 1&2: Postmenopausal women, based on the following criteria:

- Age $\geq$ 50 years **AND**
- No menstrual periods for $\geq$ 365 days

Specific Aims 3&4: Premenopausal women, 21-40 years of age

Exclusion Criteria (apply to all specific aims):

- History of breast, uterine or ovarian cancer
- History of hysterectomy and/or ovariectomy
- Weight > 135 kg
- Serum K^+ < 3.6 mEq/L;
- Serum Mg^{2+} < 1.8 mg/dL;
- Hematocrit < 26%;
- Hepatic transaminases > 3x upper limit of normal;
- Baseline Bazett's-corrected QT interval > 450 ms
- Taking hormone replacement therapy
- Diagnosis of heart failure
- Symptoms associated with heart failure:
  - Pitting edema > 2+
  - Crackles or rales on lung auscultation
  - S3 or S4 heart sounds
  - Unable to climb $\geq$ 2 flights of stairs without becoming short of breath
- Current ECG rhythm of atrial fibrillation or other tachyarrhythmia

- Family or personal history of long-QT syndrome or sudden cardiac death not associated with acute myocardial infarction
- Concomitant use of any QTc interval-prolonging drug.
- Permanently paced ventricular rhythm
- Pregnancy
- Using any hormonal contraceptives (oral contraceptives, hormone-secreting intrauterine devices (IUDs), hormonal implants)
- Peanut allergy
- We will exclude subjects who are currently taking medications that induce or are moderate or strong inhibitors CYP 2B6/3A4/3A5 metabolism :

Carbamazepine (tegretol)	Phenytoin	Phenobarbital	Plioglitazone (actos)	Refabutin
Rifampin	St Johns Wort	Troglitazone (rezulin)		

Clopidogrel (Plavix)	Ticlopidine	Voriconazole	HIV antiviral meds	Verapamil
Diltiazem	Suboxone	Aprepitant		

4.0 Enrollment/Randomization

Subject Recruitment:

Forty volunteer subjects (n=20 postmenopausal women, n=20 premenopausal women) will be recruited through 2 methods:

- Potential subjects will be recruited through the use of a recruitment database maintained through the CTSI InResearch Volunteer research database. Potential volunteers who meet inclusion criteria will be contacted via email and will be offered the opportunity to participate in this study.

- Advertisements will be placed in public locations throughout the IUPUI campus. Purdue University Research volunteers website, publications targeted at seniors and on the IU Health website asking for volunteers. Potential subjects will call or Email the PI or research coordinator if interested in participating in the study.

During the initial phone call with the potential subject, the investigator will explain the purpose of the study, study procedures and risks associated with the study. If the potential subject is interested in participating, the investigator will then ask the potential subject specifically about inclusion and exclusion criteria. If the subject meets all inclusion criteria and had no exclusion criteria, the screening visit will be scheduled. A letter will be Emailed or sent via US mail to the subject containing the following information:

- Location of the Clinical Research Center
- Brief explanation of the CRC setting and study activities that will take place there
- What subjects should bring with them (their current medications)
- Directions to IU Health University Hospital and to the CRC and where to park
- That they will be given lunch and that their parking will be reimbursed

- A copy of the informed consent form for them to review

Prior to Visit 1, the screening visit, an investigator will telephone, Email or text the subject (whichever the subject prefers) to remind them of their visit, review the consent form, and answer any questions they may have. This does not constitute the official review ofn the consent form with the subject but provides an opportunity to answer questions that the subject may have prior to them appearing for the screening visit.

5.0 Study Procedures

This study will be performed using a crossover design. Each subject will act as her own control. Subjects will be randomized to the order that they receive either the study medication or the placebo. A random number generator will be used to determine the order of randomization. Subjects will be randomized during their first visit to the Indiana Clinical Research Center (ICRC) after informed consent has been obtained.

This study will be conducted at the ICRC. All subjects will undergo a screening visit (visit 1) that will include obtaining of a 12-lead ECG, performance of a history and physical examination, obtaining height and weight, and bloodwork. Using a two-way crossover design, each subject will be studied **two times**, serving as her own control. During each phase, subjects will receive a single IV dose of ibutilide 0.003 mg/kg, which will be administered by an ICRC nurse.[9,10] Each subject will **take oral progesterone 400 mg orally once daily in the evening**, or use **matching** placebo daily in the evening for 7 days. The crossover order will be randomized. The study design is summarized below in Figure 1:

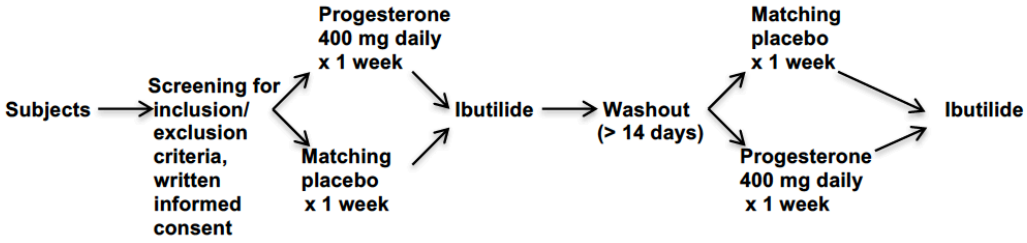

Figure 1. Study design

This study will be conducted with 3 visits: visit one is a screening visit; visits 2 and 3 includes 7 days of medication self-administration followed by a 10 hour ICRC visit. There will be a minimum 14-day washout period between visits 2 and 3. In premenopausal women, during each phase, subjects will be studied during the ovulation phase of the menstrual cycle. The ovulation phase is selected for this study rather than the luteal phase because, while serum estradiol is similar in the two phases, the natural progesterone:estradiol ratio is lower in the ovulation phase.[9] In order to time the administration of ibutilide and measurement of on-drug QT intervals to the ovulation phase, premenopausal women with a typical 28-day menstrual cycle will begin taking their study medication on the 5th day of their menstrual period (see Figure 2). For women who typically have longer menstrual cycles (30-35 days), study drug therapy will be initiated on the 6th or 7th day of their menstrual periods (Figure 2).

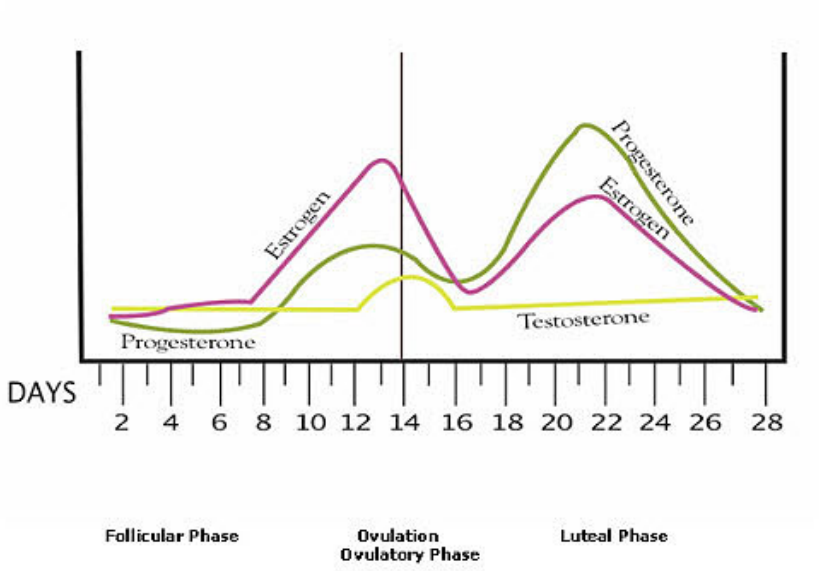

Figure 2. Hormone fluctuation during the menstrual cycle.

Visit 1 (Screening visit):
Subjects will be asked to come to the ICRC at IU Health University Hospital. An investigator will review the procedures and risks of the study in detail and obtain written informed consent. After written informed consent is obtained, subjects will undergo a screening history and physical examination performed by an investigator. Three 12-lead ECGs one minute apart (QTc will be averaged across ECGs to determine eligibility), height, weight and blood work will be obtained at this time to determine serum potassium, magnesium, liver enzymes, hematocrit. Premenopausal women will undergo a urine human chorionic gonadotropin (HCG) test to rule out pregnancy.

Once laboratory results are obtained and the physical is completed and the subject has met inclusion criteria and has no exclusion criteria, the subject will be enrolled in the study. If the subject meets any exclusion criteria, they will not be allowed to proceed in the study. This process will take approximately 1 hour.

Immediately following the screening history and physical examination, subjects will be randomly assigned to two groups:

Group A: (n= 20 subjects)
Each subject will take two placebo capsules once daily in the evening for 7 days.

Group B: (n= 20 subjects)
Each subject will take oral progesterone 400 mg orally once daily (two x 200 mg capsules) in the evening for 7 days.

Subjects will be sent home with one week's supply of the study medication to which they are initially randomized and a medication diary with which to keep track of when they take the medication, any adverse effects they may experience, and any changes in their own

concurrent medications. They will receive written and verbal instructions about the study medication and dates will be established for Visit 2.

Visit 2:
Prior to the visit subjects will be NPO and remain NPO until 4 hours after the end of the ibutilide dosing. Subjects may drink non-caloric drinks during this period such as coffee, tea, diet soda, water ect. If the serum potassium concentration is low as defined previously in the protocol, then the subject may have orange juice or a banana as previously defined in the protocol.[26,27,28]

Two indwelling intravenous catheters will be placed, one in each arm. Blood will be drawn to determine serum progesterone, estradiol, and pre-ibutilide potassium and ibutilide concentrations. Blood for a serum ibutilide concentration will be obtained prior to administration of ibutilide (and therefore will have no detectable ibutilide) will be obtained because sometimes the assay lab uses a "blank" serum sample in validation of the assay. If any subjects are found to have hypokalemia as defined in the exclusion criteria:

- They will be offered a glass of orange juice and/or a banana. After consuming the potassium-containing food, another blood sample for serum potassium concentration will be obtained and sent to the laboratory OR
- The subject's visit will be rescheduled

If the subject chooses to drink a glass of orange juice and/or eat a banana, and the follow-up serum potassium concentration is still < 3.6 mEq/L, the subject's visit will be rescheduled, or the subject can withdraw from the study, whichever of those options they prefer

Pre-menopausal women will also undergo a urine HCG test to rule out pregnancy prior to ibutilide administration. Pre-menopausal women with a positive HCG urine test will be withdrawn from the study.

All subjects will receive a single intravenous (IV) dose of ibutilide 0.003 mg/kg, which will be administered by an ICRC nurse. The ibutilide dose will be calculated based on the subject's weight during their screening visit. However, subjects will be weighed upon arrival at the CRC; if their weight is > 10% different than the weight recorded during their screening visit, the weight recorded upon arrival for this visit will be used to calculate the ibutilide dose. An MD (Dr. Munro Peacock or his designee) will be notified regarding the scheduled time of ibutilide administration.

Continuous ECG monitoring will be initiated prior to ibutilide administration and will continue until the post-ibutilide Bazett's-corrected QT interval is < 450 ms, which generally occurs within 2 hours following administration of this low, subtherapeutic dose of ibutilide. Three 12 lead ECGs approximately one minute apart (three ECGs within a total of 5 minutes) will be selected for QT interval measurement before ibutilide administration and after ibutilide at: at 5 minutes during infusion, end of infusion, 5, 10, 15, 20, 30 and 45 minutes and 1, 2, 4, 6, and 8 hours. Blood (6 mL) for determination of ibutilide concentrations will be obtained from the indwelling catheter in the arm contralateral to that into which ibutilide was infused at approximately the following times (within 5 minutes of the times listed) 5 minutes during infusion, the end of infusion, and 5, 10, 15, 20, 30 & 45 minutes, and 1, 2, 4, 6, and 8 hours following end of infusion. An 8-hour data

collection and monitoring period is sufficient, as QT_c intervals return to baseline within 2-6 hours.[9,10]

At the end of this visit, and after a minimum 14-day washout period, subjects will take another of the study drugs as follows:

Group A: (n= 20 subjects)
Each subject will take oral progesterone 400 mg orally once daily (two x 200 mg capsules) in the evening for 7 days.

Group B: (n= 20 subjects)
Each subject will take two placebo capsules once daily in the evening for 7 days.

Subjects will be sent home with one week's supply of the study medication to which they are randomized for this phase and a medication diary with which to keep track of when they take the medication, any adverse effects they may experience, and any changes in their own concurrent medications. They will receive written and verbal instructions about the study medication and dates will be established for Visit 3.

At 3-7 days following the study visit, subjects will be called/texted or emailed based on preference to assess for any potential adverse events related to study medications or procedures.

Visit 3:
Prior to the visit subjects will be NPO and remain NPO until 4 hours after the end of the ibutilide dosing. Subjects may drink non-caloric drinks during this period such as coffee, tea, diet soda, water ect. If the serum potassium concentration is low as defined previously in the protocol, then the subject may have orange juice or a banana as previously defined in the protocol.[26,27,28]

Two indwelling intravenous catheters will be placed, one in each arm. Blood will be drawn to determine serum progesterone, estradiol, and pre-ibutilide potassium and ibutilide concentrations. Blood for a serum ibutilide concentration will be obtained prior to administration of ibutilide (and therefore will have no detectable ibutilide) will be obtained because sometimes the assay lab uses a "blank" serum sample in validation of the assay. If any subjects are found to have hypokalemia as defined in the exclusion criteria:

- They will be offered a glass of orange juice and/or a banana. After consuming the potassium-containing food, another blood sample for serum potassium concentration will be obtained and sent to the laboratory OR
- The subject's visit will be rescheduled

If the subject chooses to drink a glass of orange juice and/or eat a banana, and the follow-up serum potassium concentration is still < 3.6 mEq/L, the subject's visit will be rescheduled, or the subject can withdraw from the study, whichever of those options they prefer

Pre-menopausal women will also undergo a urine HCG test to rule out pregnancy prior to ibutilide administration. Pre-menopausal women with a positive HCG urine test will be withdrawn from the study.

All subjects will receive a single intravenous (IV) dose of ibutilide 0.003 mg/kg, which will be administered by an ICRC nurse. The ibutilide dose will be calculated based on the subject's weight during their screening visit. However, subjects will be weighed upon arrival at the CRC; if their weight is > 10% different than the weight recorded during their screening visit, the weight recorded upon arrival for this visit will be used to calculate the ibutilide dose. An MD (Dr. Munro Peacock or his designee) will be notified regarding the scheduled time of ibutilide administration.

Continuous ECG monitoring will be initiated 30 minutes prior to ibutilide and will continue until the post-ibutilide Bazett's-corrected QT interval is < 450 ms, which generally occurs within 2 hours following administration of this low, subtherapeutic dose of ibutilide. Three 12 lead ECGs approximately one minute apart (three ECGs within a total of 5 minutes) will be selected for QT interval measurement before (time 0) and after ibutilide at: at 5 minutes during infusion, end of infusion, 5, 10, 15, 20, 30 and 45 minutes and 1, 2, 4, 6, and 8 hours. Blood (6 mL) for determination of ibutilide concentrations will be obtained from the indwelling catheter in the arm contralateral to that into which ibutilide infused was approximately the following times (within 5 minutes of the times listed) 5 minutes during infusion, at the end of infusion, and 5, 10, 15, 20, 30 & 45 minutes, and 1, 2, 4, 6, and 8 hours following end of infusion. An 8-hour data collection and monitoring period is sufficient, as QT_c intervals return to baseline within 2-6 hours.[9,10]

In addition, blood will be drawn to determine serum progesterone, estradiol, and potassium concentrations at the same time as the time 0 ibutilide concentration is drawn. If the subjects is found to have hypokalemia as defined in the exclusion criteria, then they will be withdrawn from the study.

At 3-7 days following the study visit, subjects will be called/texted or emailed based on preference to assess for any potential adverse events related to study medications or procedures.

Following visit 3, subjects' participation in the study will be complete.

Serum ibutilide concentrations will be performed in the CPAC Laboratory[9,20] and serum estradiol and progesterone concentrations will be performed in the IU Health Pathology Laboratory.[21] In each of the two groups of subjects (postmenopausal women and premenopausal women), peak serum ibutilide concentrations and area under the serum ibutilide concentration:time curves from 0-1 hours (AUC_{0-1}) be compared across the groups to assure no differences that could influence QT intervals. QT intervals will be measured by an investigator with vast training and experience in QT interval measurement, and who will be blinded to subjects' assigned treatment groups. Area under the effect (AUEC; effect = QT interval) vs time curves from 0-1 hour following ibutilide administration ($AUEC_{0-1.17}$, accounting for the 10-minute ibutilide infusion) and from 0-8 hours following ibutilide ($AUEC_{0-8.17}$), indices of QTc interval exposure, will be calculated using the linear trapezoidal rule.

In summary, this study will take a total of 15 days to complete, excluding the washout periods. The total study period will take place over a minimum of 15 days. Each subject will need to complete 3 visits, and take a medication for 7 days prior to visits 2 and 3. The first visits involves no drug administration, but simply involves a 1 hour stay in the ICRC and includes a screening visit. Then subjects will begin self-administration of study medications for 7 days. Visit 2 begins the day after the 7th day of medication administration. During visit 2, the subjects will undergo an approximate 10 hour stay in the ICRC. After a minimum of a 14 day washout period, subjects will self-administer study medications for 7 days. Visit 3 begins on the day after the 7th day of medication administration. During visit 3, the subjects will undergo an approximate 10 hour stay in the ICRC.

Genetic testing:
Subjects have the option of consenting to a single 10ml blood draw at the time of another scheduled blood draw in the study. This sample with be used for genetic testing. Subjects will be genotyped for polymorphisms for an array of relevant genes, including those that encode for cytochrome P (CYP) 450 3A5, which is a major contributor to progesterone metabolism;3 other CYP enzymes contributing to progesterone metabolism, including 2C19, 2C9, and 3A4;4 progesterone receptors;5 estrogen receptors; potassium current, including hERG and KCNQ1; and calcium current.

Process of obtaining written informed consent:

We will either provide prospective subjects with the informed consent document during recruitment or we will send it via Email or regular mail, and we will establish a time to review the informed consent document with the subject. Written informed consent will be obtained at the beginning of Visit #1, immediately prior to the baseline assessment, in a private room at the CRC by the PI or co-investigator prior to the screening exam and blood work.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others;

Ibutilide is an antiarrhythmic drug used to terminate atrial fibrillation and flutter.[22] Ibutilide prolongs QTc interval dose-dependently, with a rapid onset and return to baseline in 2-6 hours .[9,10] Serum ibutilide concentrations decline rapidly, and there are no active metabolites. Ibutilide prolongs QT interval via inhibition of I_{Kr} and activation of slow inward sodium current.[23,24] The proposed ibutilide dose (0.003 mg/kg) is approximately 20% of the lowest therapeutic dose (1.0 mg), and approximately 10% of the highest therapeutic dose (2.0 mg). Ibutilide has been administered safely at this dose healthy volunteers (n=58) with no episodes of TdP (Table).[9] Further, we recently completed a study in premenopausal female volunteers during which n=15 subjects safely received this ibutilide dose, each on two separate occasions.[10] In addition, substantially higher doses of ibutilide (0.7 – 1.0 mg) have been administered to healthy volunteers (n=253), with no episodes of TdP (Table).[18]

The risks associated with this investigation will be minimized by the administration of a subtherapeutic dose of ibutilide (0.003 mg/kg, approximately 20% of the lowest therapeutic dose, approximately 10% of the highest therapeutic dose). In addition, risks will be minimized, in large part, by the exclusion criteria employed, which will allow us to exclude patients that are at risk for experiencing excessive QT interval prolongation associated with a subtherapeutic dose of ibutilide. Subjects with specific risk factors for TdP will be excluded – these risk factors include hypokalemia, hypomagnesemia, pretreatment QTc interval > 450 ms, liver disease, NYHA class IV HF, LVEF < 20%, history of TdP, and taking other QT interval-prolonging drugs. During the day on which ibutilide will be administered, patients will be maintained on continuous ECG telemetry monitors until the post-ibutilide Bazett's-corrected QT interval is < 450 ms, which generally occurs within 2 hours following administration of this low, subtherapeutic dose of ibutilide. Ibutilide will be administered in the ICRC which is housed within University Hospital and has full medical services if needed in the case of an emergency. There is a cardiac arrest (code) cart in the CRC that includes magnesium sulfate. A physician investigator (or his designee) will be notified regarding the planned time of administration of ibutilide administration. Subjects will not be discharged from the ICRC if they have a QTc interval of > 450ms after treatment. They will be monitored until the QTc interval is below 450ms. This study has a DSMB to periodically monitor the safety and any adverse events which may occur.

In all of the published literature, the overall incidence of TdP associated with ibutilide is 2.0%. TdP has never been reported at the dose that we propose to administer in this study. The lowest dose of ibutilide that has ever been associated with TdP in the published literature is 0.5 mg, and there has only been one reported case at this dose. That is more than double the dose that we propose to administer. In order to achieve this dose in our study, a patient would have to weight 166 kg (based on our dose of 0.003 mg/kg). No subject in this study will receive a dose as high as 0.5 mg, because we have an upper weight exclusion of 135 kg. Therefore, this risk of TdP in our volunteer subjects, at a low dose of 0.003 mg/kg (roughly 20% of the lowest clinical dose), is extremely low, and again, TdP has never been reported at this low dose.

Oral progesterone 400 mg once daily is the dose that was effective for attenuating drug-associated QTcI interval response in premenopausal women. Oral progesterone 400 mg daily has been administered to pre-menopausal women with minimal adverse events including abdominal cramping, nausea, vomiting, mood disturbances and menstrual spotting or bleeding following withdrawl of the medication. Subjects will be monitored via phone calls throughout the dosing period and advised to discontinue the medication if adverse events become intolerable.

Risks related to the blood draws and intravenous catheter placement will be minimized through the use of experienced staff placing the intravenous catheter. Subjects will be evaluated for anemia prior to inclusion in order to minimize the risk of anemia due to blood draws.

Safety Monitoring

Any serious or unexpected adverse event will be reported to the Research and Sponsored Programs Committee on the IUPUI campus within 3 working days of notification of the event. A written report of the adverse event will also be submitted. Any Serious Adverse Event (SAE) that is observed during the study or within 30 days after administration of ibutilide will be recorded and reported to the SAE contact investigator. The SAE contact investigator will serve as the chair for the Data and Safety Monitoring Board (DSMB).

A Data Safety & Monitoring Board (DSMB) has been established for this study. The DSMB will be chaired by Sara Quinney, PharmD, PhD, Assistant Professor of Obstetrics & Gynecology, School of Medicine, Indiana University. The other members of the DSMB are:

- Noll Campbell, PharmD, Assistant Professor, College of Pharmacy, Purdue University, and Faculty Associate, Center on Aging and Life Course. Dr. Campbell has substantial experience in clinical research studies, and was recently awarded an NIH R01 as principal investigator for a large clinical investigation
- John Hertig, PharmD, Associate Professor, College of Pharmacy and Health Sciences, Butler University. Dr. Hertig previously was Associate Director of the Center for Medication Safety Advancement at Purdue University, and has extensive experience in assessing patient safety.

. The designated DSMB will be responsible for notifying the Sponsored Programs Committee in the time specific time allotted from above.

Study/Data Monitoring

The following documents will be on file in the primary investigators office before patient enrollment:

Copy of the IU IRB/Ethics Committee approval of the protocol and consent;
Copy of the IRB approved informed consent;

A pre-study start meeting will take place to ensure that investigators and other essential personnel are aware of the protocol requirements for ibutilide administration and data collection.

The DSMB will meet after 1/4 (n=10) of the study subjects have completed the study, after ½ of the study subjects have completed the study (n=20), and after ¾ (n=30) of the study subjects have completed the study. The DSMB will monitor subject recruitment, accrual, retention, adverse event data, results of related studies that may impact subject safety, and procedures designed to protect the privacy of subjects. The DSMB will analyze and interpret data submitted by the principal investigator. In the unlikely event that a subject experiences an ibutilide-associated arrhythmia, the DSMB will determine whether the study should continue or should be suspended.

Data from the study will be monitored continuously throughout the study and will be reviewed at each DSMB meeting.

Definitions (ICH Guideline on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and FDA Regulation 21 CFR 312.32, Final Rule October 7,1997)

- **Adverse Event**: Any untoward event, which occurs regardless of its causality, including and side effect, injury, toxicity or sensitivity reaction during testing of protocol treatments (whether or not considered drug-related), will be designated as an adverse clinical event.
- **Serious Adverse Event**: An event that is fatal, life-threatening, or leads to persistent or significant disability; one that requires or prolongs hospitalization; or one that results in a congenital anomaly, or significant medical event.
- **Unexpected Adverse Event**: Any adverse event not identified in nature, severity, or frequency in current package label for ibutilide or product information.
- Adverse events will be defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. (NCI definition). The level of severity of an adverse event will be based on a grading scale which is adopted from the NCI Common Terminology Criteria for Adverse Events: Grade 1, Mild AE; Grade 2, Moderate AE; Grade 3, Severe AE; Grade 4, Life threatening or disabling AE; Grade 5, death related to AE. For prolonged QTc interval Grade 1, QTc>0.48-0.50 seconds; Grade 2, QTc >0.50 seconds and/or increase in QTc $\geq$0.06 seconds above baseline; Grade 3, QTc >0.55 seconds; Grade 4, QTc >0.60 seconds and/or life-threatening signs or symptoms (e.g., arrhythmia, CHF, hypotension, shock, syncope) and/or *torsades de pointes*; Grade 5, Death.

Ethical Aspects of the Proposed Research

The minimum possible number of subjects is proposed, based on sample size calculations. Progesterone 400 mg orally daily was associated with no reported adverse effects.[18] In our study,[10] adverse effects associated with progesterone were primarily fatigue/malaise. Therefore, risks associated with oral progesterone appear minimal. Subjects will be carefully screened and a through physical examination will be performed prior to initiation of the protocol. Extensive exclusion criteria have been included to minimize the risk of adverse effects to subjects enrolled in the study.

Safety measures have been put into place to minimize the potential risks in this study, and the results of this study may provide benefit to society by enhancing patient safety.

DSMB Safety Monitoring

A Data Safety & Monitoring Committee (DSMB) has been established for this study. The DSMB will monitor subject recruitment, accrual, retention, adverse event data, results of related studies that may impact subject safety, and procedures designed to protect the privacy of subjects. The DSMB will analyze and interpret data submitted by

the principal investigator. In the unlikely event that a subject experiences an ibutilide-associated arrhythmia, the DSMB will determine whether the study should continue or should be suspended. During the anticipated two and a half-year duration of the study, the DSMB will meet after 1/4 (n=10) of the study subjects have completed the study, after ½ of the study subjects have completed the study (n=20), and after ¾ (n=30) of the study subjects have completed the study.

The IRB will receive information regarding frequency and dates of monitoring, summary of cumulative adverse events, assessment of external factors, including scientific reports, therapeutic developments, & results of related studies that could impact subject safety, summaries of subject privacy and research data confidentiality outcomes, and any changes to the risk: benefit ratio.

7.0 Study Withdrawal/Discontinuation

Subjects may withdraw from the study at anytime during the study. If a subject wishes to withdraw from the study, he/she may call the principal investigator and withdraw himself/herself from the study. The subject will receive compensation for their time up to the period of withdrawal.

The principal investigator may withdraw a subject from the study for the following reasons: a change in the subject' medical condition resulting in an increased risk profile, change in any inclusion or exclusion criteria for the subject, or subject unwilling or unable to complete part of the protocol as stated. If the PI determines that a subject needs to be withdrawn from the study, he will contact the subject by phone and also in writing. The subject will receive compensation for any part of the study that he/she has completed.

8.0 Statistical Considerations

Statistical analysis will be performed separately for the two subject populations:

- Postmenopausal women (progesterone vs placebo)
- Premenopausal women (progesterone vs placebo)

QT intervals will be corrected for heart rate using two correction methods, the Fridericia (QT_F) and Framingham (QT_{Fra}) methods.

Study Endpoints: Comparison of progesterone and placebo phases for: 1) Baseline (pre-ibutilide) maximum QT_F & QT_{Fra} 2) Ibutilide effect on maximum QT_F & QT_{Fra}, 3) Ibutilide effect on maximum % change in QT_F & QT_{Fra}, and 4) Ibutilide effect on $AUEC_{0\text{-}1.17}$ and $AUEC_{0\text{-}8.17}$. Endpoints will be correlated with progesterone concentrations. 5)

Statistical analyses: Means (standard deviations) or medians (interquartile range) will be presented for continuous variables. Distribution normality will be assessed using the Kolmogorov-Smirnov test. Paired t-test (if normality holds) or signed-rank rest (if normality does not hold) will be used to compare outcome measures between the two treatment phases; 95% confidence intervals will be calculated. SAS 9.4 (Cary, NC) will be

used. Correlations between the primary endpoints and serum progesterone concentrations will be performed using Pearson's correlation coefficient.

Anticipated Outcomes: During the progesterone phase, the following outcome measures will be significantly lower than vs placebo: 1) Pre-ibutilide QT_F and QT_{Fra}; 2) Post-ibutilide maximum QT_F and QT_{Fra}; 3) AUEC 1 hour and 8 hours after ibutilide; 4) Post-ibutilide % change in maximum QT_F and QT_{Fra}, 5) J-T_{peak} and T_{peak}-T_{end}.

Sample Size Calculation: In this 2-way crossover study, at a two-sided α level of 0.05 and a power of 0.80, a sample size of 20 subjects in the postmenopausal population and n=20 subjects in the premenopausal population will be sufficient to detect a difference in maximum QT_F and QT_{Fra} of 15 ms, assuming a QT_F and QT_{Fra} interval prolongation of 24±12 ms associated with ibutilide in the absence of progesterone.

9.0 Privacy/Confidentiality Issues

All ECGs will be collected using a standard ECG machine, on which the ECG data are collected on paper. This information will not contain the patients' names, just an unique identifier. Blood samples will be collected and maintained in a locked laboratory freezer, they will not contain the patients' names, just an unique identifier. Medical information, such as laboratory and echocardiogram information will be stored on a password protected computer database containing only an unique identifier. All information linking the patient's name and health information to the unique identifier will be stored in a locked cabinet in the study coordinator's office. All this information will not be stored electronically. Information on the password protected computer database will only be accessed by the investigators. All information with unique identifiers will be locked in the study coordinator's office or laboratory, or on a password protected computer. To assure study coordinator's office is physically secure, it is located on the 3th floor of the FTB Faculty Office Building at Eskenazi Medical Center, which requires authorized entry. Furthermore, the study coordinator's office remains locked and any information that can be used to identify patients will be kept in a locked cabinet and not stored electronically. All study documentation will be stored for a minimum of seven years after completion of the study. All paper documentation will be shredded and electronic files deleted.

There will be paper copies of spreadsheet for the purpose of statistical analysis, pharmacokinetic and pharmacodynamic analysis shared with the co-investigators involved in the analysis, however, the unique identifier will be the only information that is available on the spreadsheets. The information linking the unique identifier to the patient will be kelp in a locked cabinet in the study coordinator's office.

Safeguards used to protect the confidentiality and security of health information

Any information that is gathered about these patients will be kept confidential and any information that is generated on a patient will does not ultimately participate in the study will be destroyed immediately.

All ECGs will be collected using digital ECG recordings. Information entered will not contain the patients' names, just a unique identifier. Blood samples will be collected and maintained in a locked laboratory freezer, they will not contain the patients' names, just a unique identifier. Medical information, such as laboratory information will be stored on a password protected computer database containing only a unique identifier. All information linking the patient's name and health information to the unique identifier will be stored in a locked cabinet in Dr. Tisdale's office. All this information will not be stored electronically. Information on the password protected computer database will only be accessed by the investigators. All information with unique identifiers will be locked in the study coordinator's office or laboratory, or on a password protected computer. To assure the study coordinator's office is physically secure, it is located on the 3th floor of the FTB Faculty Office Building at Eskenazi Medical Center, which requires authorized entry. Furthermore, the study coordinator's office remains locked and any information that can be used to identify patients will be kept in a locked cabinet and not stored electronically. All study documentation will be stored for a minimum of seven years after completion of the study. All paper documentation will be shredded and electronic files deleted.

There will be paper copies of spreadsheet for the purpose of statistical analysis and will be shared with the co-investigators involved in the analysis, however, the unique identifier will be the only information that is available on the spreadsheets. The information linking the unique identifier to the patient will be kelp in a locked cabinet in the study coordinator's office.

Discuss the methods for ensuring participant privacy, and the methods for protecting privacy and confidentiality.

10.0 Follow-up and Record Retention

The estimated time frame for completion of this study is 24 months. All identifiable data (informed consents) will be stored in a locked filing cabinet in the PI or study coordinator's office behind a locked door with limited access. All electronic data will be stored on a password protected computer in the same location. Data will be stored a minimum of 7 years per Indiana State Law. All ECGs, clinical assessment data, laboratory data and blood samples will be de-identified at the time of data collection. List the duration of the study. List the duration of record retention and the method for destruction or the possibility of indefinite archiving of information.

References

1. Trinkley KE, Page II RL, Lien H, Yamanouye K, Tisdale JE. QT interval prolongation and the risk of torsades de pointes: essentials for clinicians. Curr Med Res Opin 2013;29:1729-1736. https://www.tandfonline.com/doi/full/10.1185/03007995.2013.840568

2. Woosley RL, Romero KA. *QTdrugs Lists* [database]. Oro Valley (AZ): AZCERT, Inc; [accessed June 22, 2018]. Available from: https://www.crediblemeds.org/index.php/login/dlcheck

3. Curtis LH, Østbye T, Sendersky V, Hutchison S, Allen LaPointe NM, Al-Khatib SM, Yasuda SU, Dans PE, Wright A, Califf RM, Woosley RL, Schulman KA. Prescription of QT-prolonging drugs in a cohort of about 5 million outpatients. Am J Med 2003;114:135-141. https://www.sciencedirect.com/science/article/pii/S0002934302014559?via%3Dihub

4. Carnethon MR, Anthony MS, Cascio WE, Folsom AR, Rautaharju PM, Liao D, Evans GW, Heiss G. A prospective evaluation of the risk of QT prolongation with hormone replacement therapy: the atherosclerosis risk in communities study. Ann Epidemiol 2003;13:530-536. https://www.sciencedirect.com/science/article/pii/S1047279703000504?via%3Dihub

5. Nakagawa M, Ooie T, Takahashi N, Taniguchi Y, Anan F, Yonemochi H, Saikawa T. Influence of menstrual cycle on QT interval dynamics. Pacing Clin Electrophysiol 2006;29(6):607-613. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1540-8159.2006.00407.x

6. Seth R, Moss AJ, McNitt S, Zareba W, Andrews ML, Oi M, Robinson JL, Goldenberg I, Ackerman MJ, Benhorin J, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, et al. Long QT syndrome and pregnancy. J Am Coll Cardiol 2007;49:1092-1098. https://www.sciencedirect.com/science/article/pii/S0735109706031184?via%3Dihub

7. Nakamura H, Kurokawa J, Bai C-X, Asada K, Xu J, Oren RV, Zhu ZI, Clancy CE, Isobe M, Furukawa T. Progesterone regulates cardiac repolarization through a nongenomic pathway. An in vitro patch-clamp and computational modeling study. Circulation 2007;116:2913-2922. http://circ.ahajournals.org/content/116/25/2913.long

8. Odening KE, Choi B-R, Liu GX, Hartmann K, Ziv O, Chaves L, Schofield L, Centracchio J, Zehender M, Peng X, Brunner M, Koren G. Estradiol promotes sudden cardiac death in transgenic long QT type 2 rabbits while progesterone is protective. Heart Rhythm 2012;9:823-832. https://www.sciencedirect.com/science/article/pii/S1547527112000100?via%3Dihub

9. Rodriguez I, Kilborn MJ, Liu X-K, Pezzullo JC, Woosley RL. Drug-induced QT prolongation in women during the menstrual cycle. JAMA 2001;285:1322-1326.

https://jamanetwork.com/journals/jama/fullarticle/193628

10. Tisdale JE, Jaynes HA, Overholser BR, Sowinski KM, Flockhart DA, Kovacs RJ. Influence of oral progesterone administration on drug-induced QT interval lengthening. A randomized, placebo-controlled crossover trial. JACC Clin Electrophysiol 2016;2:765-774. http://www.electrophysiology.onlinejacc.org/content/2/7/765?sso=1&sso_redirect_count=2&access_token=xUvnCF5lfwfMz1xSIvjEypWI2ZDd2oB4VF9RjH597HV58GNZ0HHrZjAjs1jvq8UHGFrV0uB3AGPOESBTzgvff7JVXuCRaiauMxyv3rTK8qJiIwqy9ecw6bEZ

11. Gökçe M, Karahan B, Yilmaz R, Örem C, Erdöl C, Özdemir S. Long term effects of hormone replacement therapy on heart rate variability, QT interval, QT dispersion and frequencies of arrhythmia. Int J Cardiol 2005;99:373-379.

12. Kadish AH, Greenland P, Limacher MC, Frishman WH, Daugherty SA, Schwartz JB. Estrogen and progestin use and the QT interval in postmenopausal women. Ann Noninvas Electrocardiol 2004;9:366-374.

13. Salem J-E, Alexandre J, Bachelot A, Funck-Brentano C. Infulence of steroid hormones on ventricular repolarization. Pharmacol Ther 2016;167:38-47.

14. Tisdale JE, Overholser BR, Wroblewski HA, Sowinski KM. The influence of progesterone alone and in combination with estradiol on ventricular action potential duration and triangulation in response to potassium channel inhibition. J Cardiovasc Electrophysiol 2011;22(3):325-331.

15. Haseroth K, Seyffart K, Wehling M, Christ M. Effects of progestin-estrogen replacement therapy on QT-dispersion in postmenopausal women. Int J Cardiol 2000;75(2-3):161-165.

16. Farkas AS, Makra P, Csik N, Orosz S, Shattock MJ, Fûlòp F, Forster T, Csanády M, Papp JG, Varró A, Farkas A. The role of the Na+/Ca2+ exchanger, INa and ICaL in the genesis of dofetilide-induced torsades de pointes in isolated, AV-blocked rabbit hearts. Br J Pharmacol 2009;156(6):920-932. https://bpspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1476-5381.2008.00096.x

17. Li X, Zhang J, Zhu X, Wang P, Wang X, Li D. Progesterone reduces inflammation and apoptosis in neonatal rats with hypoxic ischemic brain damage through the PI3K/Akt pathway. Int J Clin Exp Med 2015;8:8197-8203. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4509338/

18. Sofuoglu M, Mouratidis M, Mooney M. Progesterone improves cognitive performance and attenuates smoking urges in abstinent smokers. Psychoneuroendocrinol 2011;36(1):123-32. https://www.ncbi.nlm.nih.gov/pubmed/?term=Progesterone+improves+cognitive+performance+and+attenuates+smoking+urges+in+abstinent+smokers.

19. Harlow SD, Gass M, Hall JE, et al. Executive summary of the stages of reproductive

aging workshop +10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab 2012;97:1159-1168.

20. Tisdale JE, Overholser BR, Sowinski KM, Wroblewski HA, Amankwa K, Borzak S, Kingery JR, Coram R, Zipes DP, Flockhart DA, Kovacs RJ. Pharmacokinetics of ibutilide in patients with heart failure due to left ventricular systolic dysfunction. Pharmacotherapy 2008;28:1461-1470. https://www.ncbi.nlm.nih.gov/pubmed/19025427

21. Ren S, Wang X, Lin Z, Ying X, Chen G, Lin JM. Development of a high-throughput, indirect antibody immobilization format chemiluminescence immunoassay (CLEIA) for the determination of progesterone in human serum. Luminescence 2008;23:175-181. https://onlinelibrary.wiley.com/doi/abs/10.1002/bio.1031

22. Nair M, George LK, Koshy SK. Safety and efficacy of ibutilide in cardioversion of atrial flutter and fibrillation. *J Am Board Fam Med* 2011;24:86-92. https://www.ncbi.nlm.nih.gov/pubmed/21209348

23. Yang T, Snydres DJ, Roden DM. Ibutilide, a methanesulfonanilide antiarrhythmic, is a potent blocker of the rapidly activating delayed rectifier K+ current (IKr) in AT-1 cells: concentration-, time-, voltage-, and use-dependent effects. *Circulation* 1995;91:1799-1806. http://circ.ahajournals.org/content/91/6/1799.long

24. Lee KS. Ibutilide, a new compound with potent class III antiarrhythmic activity, activates a slow inward Na+ current in guinea pig ventricular cells. *J Pharmacol Exp Ther* 1992;262:99-108. http://jpet.aspetjournals.org/content/262/1/99.long

25. Kannankeril PJ, Norris KJ, Carter S, Roden DM. Factors affecting the degree of QT prolongation with drug challenge in a large cohort of normal volunteers. *Heart Rhythm* 2011;8:1530-1534. https://www.sciencedirect.com/science/article/pii/S1547527111003420?via%3Dihub

26. Taubel J, Wong AH, Naseem A, Ferber G, Camm AJ. Shortening of the QT interval after food can be used to demonstrate assay sensitivity in thorough QT studies. *J Clin Pharmacol* 2012;52:1558-65. doi: 10.1177/0091270011419851.

27. Mohamed MF, Zeng J, Jiang P, Hosmane B, Othman AA. Use of early clinical trial data to support thorough QT study waiver for upadacitinib and utility of food effect to demonstrate ECG assay sensitivity. *Clin Pharmacol Ther* 2018;103:836-842. doi: 10.1002/cpt.804.

28. Durak A, Olgar Y, Tuncay E, et al. Onset of decreased heart work is correlated with increased heart rate and shortened QT interval in high-carbohydrate fed

overweight rats. Can J Physiol Pharmacol 2017;95:1335-42. doi: 10.1139/cjpp-2017-0054.

Appendix. **Incidence of Ibutilide-Associated Torsades de Pointes in Published Literature**

Paper	n	Population	**Ibutilide 0.003 mg/kg** Dose for our study – 0.21 mg in a 70 kg person	Ibutilide 0.25 mg	Ibutilide 0.5 mg	Ibutilide 0.005 mg/kg	Ibutilide 0.7 mg	Ibutilide 1.0 mg x 1	Ibutilide 1.0 mg x 1 then 0.5 mg x 1	Ibutilide 0.025 mg/kg	Ibutilide 1.0 mg x 2	Total incidence, all doses combined
Guo, J Am Coll Cardiol 1996	n=8 ibutilide two x 1 mg n=4 ibutilide 1 x 1.0 mg then 1 x 0.5 mg n=13 ibutilide 1.0 mg n=10 placebo	Spontaneous sustained a.flutter	--	--	--			0/13	0/4		0/8	
Stambler Am J Cardiol 1996	N=18 ibutilide N= 11 placebo	Patients with atrial flutter				0/2	0/1	0/2	0/4	0/3	0/3	
Ellenbogen J Am Coll Cardiol 1996	N=157, dose-ranging N=41 placebo	Patients with AF or atrial flutter	--		1/42 (2.4%) (n=1)¶		2/39 (5.1%)§	1/39 (2.6)%		1/39 (2.6%)		
Stambler Circulation 1996	N=79, ibutilide 1.0 mg then 0.5 mg N=82, ibutilide 1.0 mg then 1.0 mg N=81, placebo	Patients with AF or a. flutter	--	--	--		--	11/161 (6.8%)	2/144 (1.4%)		2/144 (1.3%)	

Vanderlugt Circulation 1999	2.0 mg – 70 1.0 mg – 73 0.5 mg – 75 Placebo - 84	Post-CABG AF conversion	--	0	0			2/70 (2.9%)*			2/70 (2.9%)*	
Stambler Am J Cardiol 1997	Ibutilide 1 mg - 12 Ibutilide 2 mg - 12 Placebo - 12	Patients with or without HF	--	--	--						0/12 – no HF 1/12 (8.5%) – with HF	
Oral NEJM 1999	N = 64, Ibutilide N=36, placebo	Patients with AF undergoing DCC	--	--	--		--	2/64 (3.1%)‡	--		--	
Glatter Circulation 2001	n=70	Elective conversion of AF – also on amio	--	--	--			--			1/70 (1.4%)	
Rodriguez JAMA 2001	n=58	Young healthy volunteers (38 men, 20 women) – received 3 doses in crossover fashion	0/58	--	--			--			--	
Gowda Am J Ther 2003	n=52 – two x 1 mg n =6 – 1 x 1 mg	Patients with new-onset AF or a.flutter	--	--	--			0/6			4/52 (7.7%)	
Giudici, J Cardiovasc Nurs 2008	n=238, retrospective study		--	--	--			4/238 (1.7%)			--	
Kannankeril, Heart Rhythm 2011	N=253 Ibutilide 10 mcg/kg up to total dose of 1 mg	Healthy volunteers aged 18-40	--	--	0		--	0/253			--	

Tisdale, J Clin Pharmacol 2012	N=15	Patients with AF N=6 had decreased LVEF	--	--	--			0/6			--	
Tisdale JE et al, JACC Clin EP 2016;2:765-774	N=19, randomized crossover (n=31 doses of ibutilide 0.003 mg/kg)	Healthy premenopausal female volunteers during menses phase	0/31									
Muenstermann Tomaselli E, Tisdale JE, et al. Circulation 2018 (anstract: in press)	n=14, randomized 3-way crossover (n=42 doses of ibutilide 0.003 mg/kg)	Men ≥ 65 years of age	0/42									
Tisdale JE, et al. Circulation 2018 (abstract; in press)	n=20, randomized parallel (n=20 doses of ibutilide 0.003 mg/kg)	n=10 subjects with HFpEF n=10 matched control subjects	0/20									
Total in all studies			0/151 0% incidence at dose used in our studies	0/252	1/42	0/2	2/40	20/852 (2.3%)	2/152 (1.3%)	1/42 (2.4%)	10/371 (2.7%)	36/1,904 (1.9%)

*patients with torsades had reduced LVEF
¶This patient had NYHA class II HF with decreased LVEF
§These 2 patients had NYHA class III HF with LVEF 45% and NYHA class II HF with LVEF 30%

‡both patients had LVEF < 20%
Other data:
Gowda, et al - Am J Ther 2002 – 4 cases of ibutilide-induced TdP – two doses of 1.0 mg (n=3), one dose of 1.0 mg (n=1)

Investigators' Experience Administering Intravenous (IV) Ibutilide 0.003 mg/kg

In our studies to date, we have administered n=118 doses of IV ibutilide 0.003 mg/kg to study volunteers. This has resulted in:

- n=0 occurrences of torsades de pointes
- n= 0 occurrences of ventricular tachyarrhythmias of any kind
- n=2 (1.7%) instances of transient QTc interval > 500 ms. In both cases, the QTc interval declined to < 500 ms within a couple of minutes, and rapidly declined to < 450 ms shortly thereafter