From the Department of Molecular Medicine and Surgery
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Improving Assessment of Cardiovascular Arrhythmic Safety of New Pharmacologic Agents

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Karolinska Institutet

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Cover:
Illustration of the relationship between the atrioventricular and ventricular action potentials and the electrocardiogram (ECG). The ECG includes traditionally measured intervals such as the PR, QRS and QT as well as the J-T_{peak} and T_{peak}-T_{end} intervals, which is the main focus of this thesis. See Study III for details.

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Improving Assessment of Cardiovascular Arrhythmic Safety of New Pharmacologic Agents

Thesis for Doctor of Philosophy in Medical Science (PhD)

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Abstract

Fourteen drugs have been removed from the market worldwide due to an increased risk of torsade de pointes (TdP), a potentially fatal ventricular arrhythmia. Almost all of the removed drugs that have been linked to an increased risk for TdP have been shown to block the human ether-à-go-go-related gene (hERG) potassium channel. In addition, block of the hERG potassium channel results in a prolongation of the duration of ventricular repolarization measured as the QT interval on the electrocardiogram (ECG).

Therefore, almost all new drugs must be studied in a thorough QT (TQT) study to determine if they have the potential to prolong the heart rate corrected QT interval (QTc). The TQT study is an expensive study that in addition to including a negative control (placebo), also includes a positive pharmacologic control to ensure assay sensitivity and proper study conduct.

Not all drugs that block the hERG potassium channel and prolong the QTc interval have been linked with a risk for TdP likely due to additional inward current block. For example block of the late sodium (amiodarone, ranolazine) or L-type calcium (verapamil). In addition, not every study is able to detect the QTc prolongation associated with the positive pharmacological control. It is unknown which factors have a greater influence on study quality and the ability to demonstrate assay sensitivity.

TQT studies from the Food and Drug Administration (FDA) ECG-Warehouse were used to investigate factors of assay sensitivity and how they relate to ECG quality metrics, as well as new ECG biomarkers that could complement the QTc interval and increase specificity of the TQT study. In addition, two prospective clinical trials were conducted to evaluate the performance of the new ECG biomarkers. The first clinical trial focuses on comparing selective hERG potassium channel blockers to multichannel blockers. The goal of the second clinical trial is to evaluate

if selective late sodium or L-type calcium channel blockers could reduce drug-induced QTc prolongation. Finally, data from the first clinical trial was used to study dynamic ECG biomarkers.

The retrospective analysis of TQT studies showed that the most influential factors of assay sensitivity is reader variability and stability of heart rate. The latter being driven in part by study conduct. In addition, the retrospective analysis suggested that by breaking down the QTc interval into QRS, J-T_{peak}c and T_{peak}-T_{end} intervals that it is possible to detect the presence of inward current block (late sodium or L-type calcium), that can reduce the risk for TdP.

In two prospective clinical studies the proposed ECG biomarkers were shown to be able to detect the presence of inward current block. Moreover, the second clinical trial showed that a selective late sodium current blocker (mexiletine or lidoacaine) shortens dofetilide-induced QTc prolongation. Lastly, using ECG measurements from periods of postural maneuvers and light exercises it was possible to detect the presence of reverse use dependence and increased instability of the QT interval (dynamic ECG biomarkers) associated with hERG potassium channel block. No changes in the dynamic ECG biomarkers was observed with ranolazine, and only small changes was observed with verapamil.

Overall, the findings in this thesis show that by ensuring consistently measured QT intervals and maximizing heart rate stability the ability to detect the QTc interval prolongation associated with the positive control is improved. Ensuring consistent QT measurements also results in improved quality of QTc measurements, quantified using QTc quality metrics. In addition, the use of the J-T_{peak}c and T_{peak}-T_{end} intervals allows for discrimination between drugs that are selective hERG potassium channel blockers, and are associated with a high risk for TdP, and multichannel blockers with a low risk for TdP.

List of Publications

I. **Johannesen L**, Garnett CE, Malik M. Impact of Electrocardiographic Data Quality on Moxifloxacin Response in Thorough QT/QTc Studies. *Drug Saf* 2014;37(3):183-9

II. **Johannesen L**, Garnett CE, Malik M. Electrocardiographic Data Quality in Thorough QT/QTc Studies. *Drug Saf* 2014;37(3):191-7

III. **Johannesen L**, Vicente J, Gray RA, Galeotti L, Loring Z, Garnett CE, Florian J, Ugander M, Stockbridge N, Strauss DG. Improving the Assessment of Heart Toxicity for All New Drugs Through Translational Regulatory Science. *Clin Pharmacol Ther* 2014;95(4):501-8

IV. **Johannesen L**, Vicente J, Mason JW, Sanabria C, Waite-Labott K, Hong M, Lin J, Guo P, Sørensen JS, Galeotti L, Florian J, Ugander M, Stockbridge N, Strauss DG. Differentiating Drug-Induced Multichannel Block on the Electrocardiogram: Randomized Study of Dofetilide, Quinidine, Ranolazine, and Verapamil. *Clin Pharmacol Ther* 2014;96(5):549-58

V. **Johannesen L**, Vicente J, Mason JW, Erato C, Sanabria C, Waite-Labott K, Hong M, Lin J, Guo P, Mutlib A, Wang J, Crumb WJ, Blinova K, Chan D, Stohlman J, Florian J, Ugander M, Stockbridge N, Strauss DG. Late Sodium Current Block for Drug-Induced QT Syndrome: Results from a Prospective Clinical Trial. *Clin Pharmacol Ther* 2015 (In Press)

VI. **Johannesen L**, Vicente J, Hosseini M, Mason JW, Dang Q, Stockbridge N, Strauss DG, Ugander M. Detection of Drug-Induced Reverse Use Dependence and QT Variability Using Postural Maneuvers. *Manuscript*

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List of Abbreviations

APD action potential duration

bpm beats per minute

CHO chinese hamster ovary

CI confidence interval

CiPA Comprehensive *in vitro* Proarrhythmia Assay

CPMP Committee for Proprietary Medicinal Products

CSRC Cardiac Safety Research Consortium

CYP3A4 cytochrome P450 3A4

ΔΔ baseline, and placebo-adjusted change

EAD early after depolarization

ECG electrocardiogram

FDA Food and Drug Administration

HEK human embryonic kidney

hERG human ether-à-go-go-related gene

HESI Health and Environmental Sciences Institute

$\mathbf{I_{Ca,L}}$ L-type calcium current

ICH International Conference for Harmonisation

I_f pacemaker or funny current

I_{K1} inward rectifier (potassium)

I_{Kr} rapid potassium current

I_{Ks} slow potassium current

I_{Na} sodium current (peak)

$I_{Na,L}$ sodium current (late)

iPSC induced-pluripotent stem cell

IQ International Consortium for Innovation and Quality in Pharmaceutical Development

IRB Institutional Review Board

I_{to} transient outward current (potassium)

MAD multiple ascending dose

MADqt median absolute deviation of QT

MEA microelectrode array

QTVI QT variability index

RIHSC Research Involving Human Subjects Committee

SAD single ascending dose

STVqt short term variability of QT

TdP torsade de pointes

TQT thorough QT

TRiAD triangulation, reverse use-dependence, instability and dispersion

VSD voltage sensitive dye

To my family for their eternal love and support

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Contents

Abstract **v**

List of Publications **vii**

List of Abbreviations **ix**

1 Introduction **1**
1.1 The Cardiac Action Potential 2
1.2 The Waveforms on the Electrocardiogram 2
1.3 Measurement of the Heart Rate Corrected QT Interval 5
1.4 The Discovery of the Long QT Syndrome 7
1.5 Regulatory Response to Discovery of Cardiovascular Effects from Non-Cardiovascular Drugs 9
1.6 Evaluation of Cardiovascular Effects using Preclinical Assays 11
1.7 The Thorough QT Study 13
1.8 Beyond the Thorough QT Study 16

2 Aims **21**

3 Materials and Methods **23**
3.1 Study Populations 23
3.2 Pattern Matching (Studies I and II) 29
3.3 ECG Quality Metrics (Study II) 30
3.4 ECG Simulations (Study III) 30
3.5 Assessment of ECGs (Studies IV-VI) 32
3.6 Holter Analysis (Study VI) 33

3.7 Dynamic Repolarization Markers and Reverse Use Dependence (Study VI) 34
3.8 Statistical Analysis 36

4 Results and Comments **41**
4.1 Evaluation of Factors Influencing the Ability to Detect Effect of Moxifloxacin (Study I) 41
4.2 Factors of ECG Data Quality in Thorough QT Studies (Study II) 44
4.3 Retrospective Analysis of Drug-Induced ECG Patterns (Study III) 48
4.4 Differentiating Selective hERG Potassium Channel Block from Multichannel Block (Study IV) 51
4.5 Late Sodium Current Block for Drug-Induced Long QT (Study V) 54
4.6 Drug-Induced Reverse Use Dependence and QT Variability (Study VI) 58

5 Conclusions **63**

Acknowledgements **65**

References **69**

Studies I–VI **81**

CHAPTER 1

Introduction

The main focus of this thesis is centered around the clinical evaluation of drug-induced effects on cardiac ventricular repolarization. Cardiac ventricular repolarization can be quantified by measuring the QT interval on the electrocardiogram (ECG). More specifically, this thesis focuses on factors of ECG data quality in clinical QT studies or the so-called thorough QT (TQT) studies and the use of new ECG biomarkers to enhance assessment of drug-induced effects.

This introduction starts with a description of the basis of the ECG waveforms and their relationship to the cardiac action potential. Then, the measurement of the QT interval and correction for heart rate effects is described. After describing the ECG waveforms and the QT interval, the history of congenital long QT syndrome is described as well as the acquired or drug-induced long QT syndrome and the regulatory response to the discovery of drug-induced long QT. Following the description of the regulatory response, methods for evaluating cardiac repolarization are described using either preclinical assays or the TQT study will be discussed. Finally, the introduction of this thesis concludes with a discussion of the limitations and shortcomings of the TQT study and recent advances to address these limitations.

1.1 The Cardiac Action Potential

The shape of the ECG waveforms results from the sum of the current flow from all action potentials of the cardiac cells across the heart. Thus, it partly reflects the cellular cardiac action potential and partly the effect of propagating electrical activity in the heart.

The cardiac action potential (Figure 1.1) can be divided into five phases (0 through 4). Phase 0 is the upstroke of the action potential and is primarily driven by the inward sodium ion current (I_{Na}). Phase 1 is the termination of the upstroke and early repolarization as a result of inactivation of the sodium channels and activation of the transient outward potassium ion current (I_{to}). Phase 2, also known as the plateau phase, reflects a balance between the inward L-type calcium ion flow ($I_{Ca,L}$) and outward potassium ion repolarization currents (I_K). A small late sodium current ($I_{Na,L}$) is also present. Phase 3 is the final repolarization phase, which is driven by the rapid (I_{Kr}) and slow (I_{Ks}) potassium currents. The human ether-à-go-go-related gene (hERG) potassium ion channel is one of the main drivers of the I_{Kr} current, which is one of the most critical currents for cardiac safety pharmacology. The channel was first identified in fruit flies, where blockage of the channel resulted in movements that reminded the investigators of the go-go dance.[1] The final phase of the cardiac action potential is phase 4, where the inward rectifier potassium ion current (I_{K1}) is active, which is responsible for restoring the resting potential.

1.2 The Waveforms on the Electrocardiogram

In 1887, Waller recorded the first human ECG.[3] The recording showed two deflections corresponding to two ventricular events. Waller, who was a physiologist, named them V1 and V2, presumably based on the anatomical parts of the heart associated with the events.[4] Later improvements in the electrometers allowed recording of the atrial excitation. While Waller labeled the atrial waveform as A, Einthoven, who was a mathematician, named it as P.[5] Although the reason for selecting P is not clear, it seems that

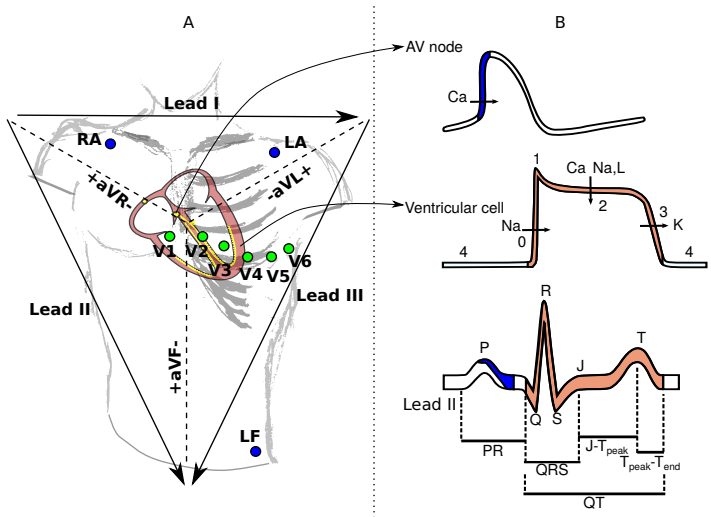

FIGURE 1.1 Overview of the genesis of the electrocardiogram (ECG) (adapted from[2]). The left panel (**A**) shows the relationship between the different leads (I,II, III, aVR, aVL, AVF and V1-6) and electrodes (RA: right arm, LA: left arm, LF: left foot). The triangle with the three limb leads (I, II and III) is also called Einthoven's triangle. The right panel (**B**) shows a ventricular action potential and how it relates to the ECG (lead II). The numbers on the action potential denotes the different phases of the action potential (see text) and the arrows denotes if the currents are inward (pointing inside the action potential) or outward currents. Na: Peak sodium (I_{Na}), Ca: L-type calcium ($I_{Ca,L}$), Na,L: Late sodium ($I_{Na,L}$), K: potassium (I_k).

the mathematician was thinking about Descartes' use of the letter P to designate a point on a curve.[4] The complete understanding on how the predominant ECG events and waveforms came to be named may not be completely certain, but Einthoven's convention,[4] where the ECG consists primarily of five distinct waveforms (P, Q, R, S, and T-waves [Figure 1.1]) has become the dominant naming convention.

Different leads can be thought of as looking at the electrical activity of the heart (generated by the currents described earlier) from different angles. The initial set of leads, leads I-III, were pro-

posed by Einthoven in 1912.[6] Wilson and colleagues investigated in the 1930s how unipolar potentials could be recorded,[7,8] resulting in three additional limb leads V_R,V_L and V_F. In 1942 Goldberger proposed an augmented version of these signals the aVR, aVL and AVF leads, which has 50% larger signal than the additional limb leads.[9,10] Later in 1944, Wilson proposed the use of unipolar leads (V1-6) to study the potentials close to the heart.[11]

The previously described leads together form the traditional 12-lead ECG system consisting of I-III, aVR, aVL, aVF and V1-6. For the purpose of measuring the QT interval either the traditional 12-lead system is used, where the limb leads are on the distal extremities or the Mason-Likar electrode position[12] (Figure 1.1 panel A) is used. The Mason-Likar system has the advantage, over the standard 12-lead system, that the limb leads are placed on the torso (instead of on the distal extremities) making it more suitable for long term recordings.

The polarity, amplitude and morphology of the waveforms depend on which lead is used. The lead depicted in Figure 1.1 panel B is lead II, where the main ECG waveforms are positive. For example, the R-wave is typically large and positive in lead II, because the net current flow during ventricular excitation moves from ventricular base to apex or top to bottom, which is in line with the axis of lead II.

Concerning timing, the P-wave describes atrial depolarization, and the QRS complex describes the ventricular depolarization. Atrial repolarization occurs at the same time as ventricular depolarization (QRS complex), but its waveform is hidden by the ventricular depolarization.

The ST-segment (roughly corresponding to phase 2 of the action potential) and T-wave (roughly corresponding to phase 3 of the action potential) characterize ventricular repolarization. After the T-wave, a six waveform the so-called U-wave is sometimes present, the meaning of which is still debated.[13,14]

1.3 Measurement of the Heart Rate Corrected QT Interval

The QT interval is defined as the interval between the beginning of the QRS complex and the end of the T-wave (Figure 1.2). The measurement of the QT interval can be performed in any lead, but lead II is often used. There is a small time difference between leads, and the amplitude and shape of the waves varies as well. Because of propagation effects, the end of the T-wave occurs later in some leads than others. Therefore, when comparing QT interval measurements within a subject it is crucial to use the same lead or a global measurement.[15] Global measurements of the QT interval are defined as the beginning of the earliest QRS complex to the last T-wave offset across leads.

Depolarization is rapid, so the rate of change in the ECG is high throughout depolarization, making the QRS and QT onset in most cases relatively unambiguous. However, repolarization is a much more protracted process and the T-wave can merge into the isoelectric baseline over 100 ms or more, making the end of the T-wave more arbitrarily defined. In addition, the U-wave, whose origin is unclear,[13,14] is variably included, and multiphasic (*"notched"*) T-waves add further ambiguity. Thus, when looking for untoward effects of drugs, it is as important to maintain consistency in measuring the end of T-waves accurately as it is to have consistency on the lead used. The end of the T-wave is often defined as the point where the steepest descending tangent on the downslope of the T-wave intersects the *"zero-line"*, which is also called the isoelectric line,[16] as shown in Figure 1.2.

The QT interval shortens as the heart rate increases.[17,18] To obtain a heart rate-independent assessment of repolarization, the customary approach is to estimate what the QT interval would be at a heart rate of 60 beats per minute (bpm) or, equivalently, a RR interval of 1000 ms.[19] There are dozens of published formulas for estimating the heart rate-corrected QT (QTc) interval the most common of which is by Bazett:[17] $\text{QTcB}=\frac{QT}{\sqrt{RR}}$. However, it over-corrects the QT at low heart rates and under-corrects at high heart rates.[20] A better formula proposed by Fridericia is

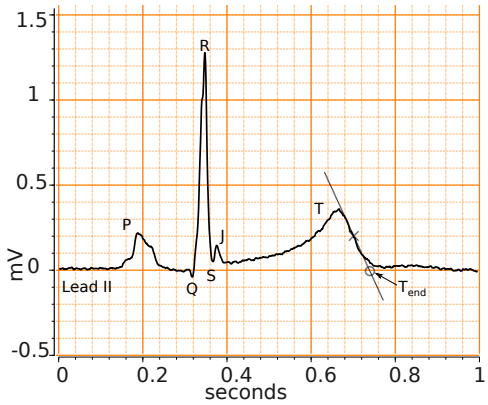

FIGURE 1.2 Example electrocardiogram (ECG) showing the different waveforms and measurement of the QT interval using the tangent methodology.[16]

QTcF=$\frac{QT}{RR^{1/3}}$.[18] Fridericia showed originally that the factor was closer to 0.36, as opposed to 1/3, but elected to use 1/3 due to simplicity, and the approximation is still used in current practice today.

Individuals have quite different QT/RR relationships which are generally consistent over weeks or months.[21] Therefore, the gold standard, which is particularly important when a drug effects the heart rate by more than a few beats per minute (bpm), is individualized correction based upon the observed drug-free QT/RR relationship.

Heart rate dependency for other ECG intervals, such as the PR and QRS intervals[22] and T_{peak}-T_{end}[23] (duration of the descending part of the T-wave) have been detected. However, these dependencies are less important functionally than the one on QT since the heart rate effects on the PR, QRS and T_{peak}-T_{end} intervals are much smaller compared to those of the QT interval. In addition to using individualized QT correction for assessing drug-induced effects, other techniques have also been proposed, that focuses on comparing the QT at equivalent heart rates.[24–26]

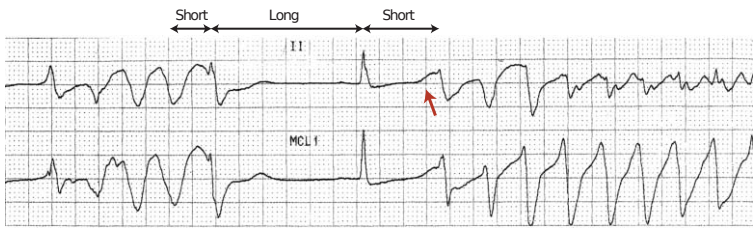

FIGURE 1.3 Example of a case of torsade de pointes (TdP), which is preceded by the typical short-long-short sequence (see text) recorded in lead II (top) and Modified Chest Lead 1 or MCL1 (bottom). The red arrow indicates the premature beat initiating TdP. Reprinted from Roden,[36] with permission from Massachusetts Medical Society.

The corrected QT interval is usually computed from the immediately preceding RR interval, however adaptation of the QT to heart rate takes approximately 2 to 3 min.[27,28] The lag in adaptation can be accounted for by utilizing the history of RR intervals to correct the QT interval for heart rate.[29] A more common solution is to only perform QT measurements when the heart rate has been stable for several minutes.[30]

1.4 The Discovery of the Long QT Syndrome

In 1957, Jervell and Lange-Nielsen described a rare familial cardiac syndrome that was linked to a prolonged QT interval, deafness and sudden death.[31] Later, Romano, et al., and Ward discovered that there were other types of inherited long QT syndrome that were not associated with deafness.[32,33] Subsequently, Dessertenne discovered that a prolonged QT interval could lead to a cardiac arrhythmia which he called torsade de pointes (TdP). Torsade de pointes is French for *"twisting of the points"* (Figure 1.3).[34,35]

Twisting of the QRS complex around the isoelectric line is one of the characteristics of episodes of TdP.[34] TdP is often initiated by a short-long-short sequence, that is an extra ventricular beat, followed by a compensatory pause, and then an arrhythmia initiating ventricular extra beat (Figure 1.3, red arrow), which occurs

within the previous QT interval.[36,37] TdP is often self-terminating, but in some cases it can degenerate into ventricular fibrillation and cause sudden cardiac death. It is difficult to estimate the incidence of TdP, because it can manifest itself as sudden cardiac death or syncope, which is difficult to diagnose without accompanying ECG documentation.

Several risk factors for TdP have been identified, such as female sex,[38] congenital or drug-induced QT prolongation,[36] hypokalemia,[39,40] or congestive heart failure.[41] It is not known why there is a difference between males and females in terms of risk for TdP. However, it has been shown that when males enter puberty their QTc shortens and increases again as men age.[42] Subsequently, elderly men and women, once more, have similar QTc values.[42] These QTc changes are inversely related to testosterone levels in men.[43] Moreover, differences in body size and pharmacokinetics between genders can result in a greater change in QTc for women compared to men for a fixed dose.[44] Interestingly, some studies have observed a difference in sensitivity to drug-induced QTc prolongation,[45–48] but whether or not a difference between genders exist has not been settled yet.[44,49,50]

Drug-induced TdP was first observed for quinidine in the 1920s and was described as *"quinidine syncope"*.[51] However, the reason for the *"quinidine syncope"* was not discovered until 1964, when Selzer and Wray reported a ventricular arrhythmia associated with *"quinidine syncope"*.[52] It has since been proposed that drug-induced TdP is likely caused by early after depolarizations (EADs), which can arise through blockage of the hERG potassium channel.[53]

Electrophysiological properties similar to those observed with antiarrhythmics have also been observed for drugs where the cardiac electrophysiological effect is not an intended effect of the drug. One such case is the non-sedating antihistamine terfenadine. Between 1983 and 1991, 17 million new prescriptions were made each year, but no concerns about cardiac issues were noticed until 1989. In 1989 it was discovered that excessive concentrations of terfenadine could lead to TdP,[54] and a similar observation in a

non-overdosed patient was made the following year.[55] This led to the Food and Drug Administration (FDA) reviewing reports in its Spontaneous Reporting System pertaining to safety events associated with terfenadine. During this analysis it was discovered that the majority of cases included patients unable to properly metabolize terfenadine because of concomitant use of ketoconazole, a cytochrome P450 3A4 (CYP3A4) inhibitor.[56]

Subsequently, a *"Dear Doctor"* letter was issued in August 1990 to warn physicians about the terfenadine-ketoconazole interaction.[56] Around the same time Woosley and colleagues reviewed 25 cases of TdP reported to the FDA's Spontaneous Reporting System.[57] During the review of the reported cases of TdP, they noted that terfenadine had similar characteristics to quinidine and that in 11 of the cases the patients were taking drugs that inhibit metabolism of terfenadine.[57] They went on to evaluate the effects of terfenadine on the hERG tail current in an *in vitro* assay and confirmed that terfenadine reduces the hERG tail current.[57]

The discovery of terfenadine increasing the risk for development of TdP (with an associated incidence of one per 28,500 to 57,000 prescriptions) led to the withdrawal of terfenadine. The withdrawal came after fexofenadine, the active moiety and metabolite of terfenadine, became available. Subsequently, other drugs (with different structure and therapeutic indications) were removed from the market because of their potential to delay cardiac repolarization and cause TdP (Table 1.1).

1.5 Regulatory Response to Discovery of Cardiovascular Effects from Non-Cardiovascular Drugs

The initial regulatory response to this apparent *"pharmacoepidemic"* included the release of a *"points to consider"* document for assessment of the potential of drugs to cause QTc interval prolongation by the Committee for Proprietary Medicinal Products (CPMP) of the European Union in 1997. The *"points to consider"* document was followed by a Canadian draft in 2001 and the Health Canada/USA draft in 2002. Later, the International Conference

TABLE 1.1 Drugs withdrawn from the market due to risk for torsade de pointes (TdP) (adapted from Stockbridge, et al.[58]).

Drug	Year	Therapeutic class
Prenylamine	1988	Antianginal
Lidoflazine	1989	Antianginal
Terodiline	1991	Antianginal / urinary incontinence
Terfenadine	1998	Antihistamine
Sertindole	1998	Atypical anti-psychotic
Astemizole	1999	Antihistamine
Grepafloxacin	1999	Antibiotic
Cisapride	2000	Gastric prokinetic
Droperidol	2001	Tranquilizer / analgesic
Levacetylmethadol	2001	Methadone substitute
Dofetilide	2004	Atrial fibrillation
Thioridazine	2005	Antipsychotic
Clobutinol	2007	Antitussive
Dextropropxyphene	2009	Opiod analgesic

for Harmonisation (ICH) released two guideline documents: ICH S7B[59] and ICH E14.[60] The ICH S7B describes preclinical testing for effect on ventricular repolarization, and E14 describes clinical testing for effect on QT interval.

The objective of the studies described in the ICH S7B is to identify the potential of a drug and its active metabolites to delay cardiac repolarization in relation to the therapeutic concentration. This can be done by quantifying the drug-induced effect on isolated cardiac ion currents, action potential parameters or *in vivo* using animals.[59]

1.6 Evaluation of Cardiovascular Effects using Preclinical Assays

Drug-induced effects on isolated cardiac ion currents can be evaluated for the hERG potassium current by measuring the reduction of current with increasing drug concentration relative to a control. This can be done using different cell systems such as isolated cardiomyocytes (human or animal), where the activity of the other cardiac ion channels are blocked ionically, pharmacologically or reduced using special voltage clamp protocols.

Alternatively, heterologous expression systems using cloned human ion channels can be used (e.g., expressed in human embryonic kidney (HEK)[61] or chinese hamster ovary (CHO) cells).[59] While single channel recordings provide an evaluation of how a drug interacts with different isolated channels, it can be difficult to translate the findings to the whole heart. Moreover, differences in protocols and testing environment, such as temperature, can influence the results dramatically.[62]

Some of the limitations with the isolated ion channel assay can be addressed by evaluating the drug-induced changes on action potential parameters (e.g., using isolated Purkinje fiber cells from animals). The isolated cells can then be exposed to increasing concentrations of the drug and different parameters such as the maximum slope of the upstroke (V_{max}) or the action potential duration (APD) (APD from start until x% repolarization, e.g. APD_{60}, APD_{90}, etc.) can be measured. The action potential parameter studies can help understand why a drug that reduces the hERG tail current does not prolong the QTc interval.[63] An example of a drug that blocks the hERG potassium channel but does not prolong action potential duration or QTc is verapamil. This occurs because verapamil also blocks the L-type calcium channel, which counteracts the effects of hERG potassium channel block.[64]

However, as with the isolated ion channel assay, there are limitations that are important to remember. One of those limitations is that autonomic nervous system effects are not captured with isolated cells. In addition, effects on specialized pacemaker cells,

Purkinje fibers, and gap junctions for intercellular communication are nor present. Thus, an isolated cell might differ from the effect on the whole heart.

Other types of cardiac preparations that can be used for investigating proarrhythmic effects include the ventricular wedge model,[65] which has been used to describe increased transmural dispersion, a phenomenon proposed as being proarrhythmic. Although the wedge model does provide useful insights into the mechanism of the drug-induced effect, it is not the same as a whole heart.[66]

Finally, the ICH S7B document describes the use of *in vivo* studies in animals to capture the drug-induced effects on the cardiovascular system. This can provide insights into how the drug works on a complete system, unlike the two previously described assays. Although an integrated analysis of the studies described in the ICH S7B provides insight into the potential of a drug to delay cardiac repolarization, there are still gaps in terms of how to translate the findings to humans. One limitation is that the expression of cardiac ion channels differs between humans and animals, a limitation that also holds true for studying cardiomyocytes isolated from animals.[67] However, the results of the S7B studies can provide insights into the mechanism and help better understand the findings in humans.

Some of the limitations described above can be addressed by studying induced-pluripotent stem cell (iPSC) derived cardiomyocyte, a new and emerging platform for studying effects of drugs on cardiac ion channels. The expression of cardiac ion channels in iPSC derived cardiomyocytes is largely similar to adult cardiomyoctes.[68] However, there is a lower expression of the inward rectifier (I_{K1}) and the presence of pacemaker currents (I_f) resulting in spontaneous beating.[68] While the resulting spontaneous beating makes it easier to study them, as it removes the need for pacing, it means that the cells are less adult like which could impact translation of results. Interestingly, the iPSC derived cardiomyocyte cells carry the genetic markup of the donor as has been shown by studies of iPSC cells made from patients with congenital long QT syn-

drome.[69] Typical assays for studying the drug-induced effects on iPSC derived cardiomyocytes are voltage sensitive dye (VSD)[70], microelectrode array (MEA)[71] and impedance.[72] These different assays have their advantages and disadvantages. Specifically, the VSD allows for recording action potentials, but at cost of using a dye. In contrast, the MEA and impedance systems do not require the use of a dye, but do not provide a direct measurement of the membrane potential but rather an aggregate signal. Overall, the iPSC derived cardiomyocyte model is a new technology that allows for studying drug-induced effects on cardiac ion channels, which seem to complement the current ICH S7B paradigm.

1.7 The Thorough QT Study

The ICH E14 describes the so-called TQT study. The goal of this study is to determine if the study drug or any of its active metabolites has the potential to delay cardiac depolarization or to prolong the QTc interval.[60] This study is mandatory for almost all new drugs and approved drugs for which a higher dose will be used for a new indication. An exception to this rule is biopharmaceuticals (biologics), as these can be exempt because of their inability to interact with the hERG channel because of their size.[73] To date, more than 250 TQT study reports have been submitted to the FDA for review,[58] and the results from some of them have been published.[74]

Study design

TQT studies are typically conducted using healthy subjects, as the goal of the study is to determine if the study drug delays cardiac repolarization, and this is generally not thought to be markedly different in the patient population. Studies in healthy volunteers can be smaller than studies in patients because there is less variability introduced by coexisting morbidities and administration of concomitant medications. Including male and female subjects into the TQT study is recommended but not mandatory.[60]

Not all drugs are appropriate for evaluation in healthy subject volunteers (e.g., cytotoxic oncologic drugs), and for these drugs a QT study would need to be conducted using actual patients, which may not include a placebo or positive control.[60,75] In such settings, however, there is also a higher tolerance for risk and an inability to detect small effects, due to the lack of a positive control, is acceptable.

TQT studies employ either a parallel or cross-over design.[60] A cross-over design is often preferable because of its efficiency: each subject serves as his or her own control, allowing for a smaller sample size and a shorter study duration. For some drugs, notably when the half-life is long or the drug requires titration, a cross-over design is not feasible, and a parallel design must be used.

Cross-over studies typically include a pre-dose baseline assessment, and it is important that subjects are randomized 1:1 to treatment order, i.e., treatment A followed by B or vice-versa.[76] The parallel study requires a time-matched baseline day for each subject, to correct for diurnal variability in the QTc,[77] which is not necessary in the cross-over design, as the subject serves as their own control.[78]

The doses of the drug explored in the TQT study should cover at least the worst case exposure scenario, after accounting for extrinsic (e.g., food) and intrinsic factors (e.g., renal or hepatic impairment). The worst case exposure of both the study drug and any active metabolites or metabolites associated with electrophysiological effects must be addressed by the study.[60]

ICH E14 considers a prolongation of QTc of 5 ms to be potentially clinically relevant. In practice, that is assessed by assuring that the upper bound of the effect is less than 10 ms. This is only about 1 % of the duration of the normal QTc. Because such an effect might be excluded with a study that was insensitive to change, all TQT studies should include a positive pharmacologic control. The pharmacologic control is typically the fluoroquinolone moxifloxacin, which has a well-characterized QTc prolonging effect[79] of approximately 10 to 12 ms for a single oral dose of 400 mg.

The goal of including the positive control is to establish that the

study is capable of detecting small changes in the QTc interval. This is typically achieved by showing that increased QTc with moxifloxacin follows the expected time course of moxifloxacin and that the lower single-sided 95 % confidence interval of the mean increase in QTc for moxifloxacin is greater than 5 ms.[60,79] The administration of the positive control can be open-label, as long as the ECG interpretation is blinded and as long as the study protocol is the same for all treatment arms.[74,80]

The QTc is measured on ECGs recorded at several time points after dosing. The ECGs are obtained prior to drawing the blood sample, to avoid autonomic effects associated with blood draw, and after the subject has been supine for 5 min to reduce effects from changing heart rates.[27,28]

The sampling schedule should allow for capturing the QTc effect around maximum plasma levels of both the study drug and major metabolites. In addition, the ECG sampling should allow for capturing the time course of the effects of the positive pharmacologic control.[60,81]

Typically, three ECGs, each 10 s in duration, are recorded at each nominal time point and the average QTc measurement for each time point is used in the analysis to reduce variability.[82] Initially, these studies were conducted with a cart with an ECG recorder, for which a 10 s record is the industry standard. However, by employing a continuous recording methodology one can decrease the variability in the QTc measurement by extracting ECGs with minimal noise in segments of stable heart rates.[30] This approach is now more common than are discrete short recordings. Continuous recordings also permit post hoc decisions to supplement originally planned data with additional time points.

The ICH E14 document requires two types of analysis of the data from TQT studies. The first analysis is a central tendency analysis (or analysis of time-dependent changes in baseline, and placebo-corrected change ($\Delta\Delta$)). The other analysis is an outlier analysis, which is a summary table of the number of ECGs that exceed some pre-specified thresholds for change in QTc as well as absolute QTc values.[60] In addition, to evaluate the drug-

induced changes for the central tendency, the central tendency analysis is also performed for the pharmacological positive control as described earlier. Finally, a concentration dependent analysis is often conducted to evaluate relationship between drug exposure and changes in the QTc interval.[83] These analyses typically utilize linear mixed effects modeling to quantify the relationship between plasma concentrations of the parent drug or metabolite, and the baseline, and placebo-adjusted change ($\Delta\Delta$) in the QTc interval.[83]

1.8 Beyond the Thorough QT Study

The TQT study has been successful in the sense that no drugs have been removed after reaching the market because of risk of TdP after the introduction of the TQT.[58] However, the TQT study is a relatively expensive study, which can cost 2 to 4 million US dollars.[84] As such, there is an incentive to optimize the study. Moreover, the focus on the QT interval and the hERG potassium current might have led to abandonment of useful drugs.[58] Different potential approaches for optimizing the TQT study or improved methods to evaluate cardiac safety have been proposed, including exploring the potential of doing early QT assessments,[85] and evaluating new biomarkers of TdP risk that might be more predictive than the QTc interval.[86–88] Finally, there are efforts to enhance preclinical evaluations, and potentially eliminate the need for the TQT.[89]

Novel study ideas

As mentioned previously, the TQT study includes a pharmacological positive control, which is typically moxifloxacin. Besides the potential ethical issue of administering a non-necessary drug, the inclusion of a positive pharmacologic control is also inefficient and not always possible. In single ascending dose (SAD) and multiple ascending dose (MAD) studies, the inclusion of a positive control may not be feasible. Therefore, different approaches to demonstrate assay sensitivity have been explored, including food

effects,[90] consistency of QTc measurements[91] and postural maneuvers[92]. Detecting the changes during meal intake or postural maneuvers may not guarantee that the study has sensitivity for all the tested data because of their brevity. Studying consistency of the QTc measurements and the ability to detect circadian QTc patterns might be better approaches as they allow for demonstrating appropriate measurement over a longer time span, but so far there has only been limited experience.[91] This thesis focuses specifically on evaluation of factors of ECG data quality, and how they relate to the ability to demonstrate assay sensitivity in TQT studies.

QT assessment early in drug development

Recently, it has been proposed that instead of conducting a TQT study, it could be sufficient to perform a concentration dependent analysis using data from SAD and MAD studies. In the current paradigm, the TQT study is powered to detect a small difference in the mean baseline, and placebo-adjusted ($\Delta\Delta$) change in QTc. This type of analysis is not appropriate in early clinical studies, because of the small number of subjects per cohort, typically six on active drug and two on placebo. The early clinical studies have, however, one distinct advantage over the TQT study, which is that the dose in those studies will often exceed the worst-case dose studied in the TQT study.[93] The issue with reduced power can be solved by using a concentration dependent analysis, which will have sufficient power to detect a change in the QTc interval around the threshold of regulatory concern ($\approx$5 ms),[85] instead of doing an analysis of effect by time, as in the TQT study.

A study was started in early 2014 to evaluate whether a SAD or MAD study could detect QTc changes around the threshold of regulatory concern.[94] The study was a parallel study of six drugs, five of which at the studied doses is expected to result in a prolongation of the QTc interval around the threshold of regulatory concern (10 ms), while the last drug has no effect on the QTc interval.[95] It was a successful study that showed it is possible to

detect the QTc prolongation of each of the five positive drugs, but not the negative drug. The findings support that a concentration dependent analysis can be used to detect QTc prolongation around the threshold of regulatory concern in small sample sizes.

Novel ECG biomarkers

The two previously mentioned approaches deal with optimizing the QTc evaluation and have the potential to reduce cost, however they do not address the issue that QTc prolongation is a sensitive but not specific biomarker of TdP risk. Commonly cited examples of false positives include verapamil and ranolazine, both of which are potent hERG potassium channel blockers with minimal effects on the QTc interval and no associated risk for TdP. The low TdP risk of these drugs supports that additional block of inward currents such as the calcium (verapamil) or late sodium current (ranolazine), reduces the TdP risk associated with hERG potassium channel block. It has been shown that blockage of these currents prevents the occurrence of EADs,[96,97] which likely causes TdP. In addition, amiodarone is a drug with strong hERG potassium channel block and substantial QTc prolongation (≈60 ms prolongation),[98] but is associated with low TdP risk.[99] Like verapamil and ranolazine, amiodarone blocks multiple inward currents (L-type calcium and late sodium), which can counteract the adverse effects of hERG potassium channel block.

There are several research efforts to develop better biomarkers for TdP risk. While some of these markers assess the concordance between ventricular depolarization and repolarization,[88,100] others assess ventricular repolarization changes reflected in varying T-wave morphology, such as T-wave notching, asymmetry and flatness.[86,87] Most new ECG biomarkers are typically evaluated using data from the same study,[101] that is the study of 160 and 320 mg of oral d,l sotalol, thus limiting the ability for generalization.[102] That being said, the results are promising, and hopefully future research will further elucidate usefulness of novel ECG parameters and their ability to differentiate between selective hERG

potassium channel blocking drugs and hERG potassium channel blocking drugs with additional inward current block which likely have a lower risk for TdP. This thesis focuses specifically on assessment of J-T_{peak} and T_{peak}-T_{end} for this purpose.

The biomarkers described in the previous paragraphs are all generally assessed at stable heart rates to reduce the effects of hysteresis (see section 1.3). Evaluation of the effects only at stable heart rates and removal of heart rate effects by applying heart rate correction factors reduces the variability of the measurements and allows for studying the drug-induced effects independent of heart rate. However, by doing so, potentially valuable information about proarrhythmic potential is removed. Specifically, evaluation of the beat-to-beat variability of the QT and the QT/RR relationship can be viewed as clinical surrogates of action potential instability and reverse use dependence (preferential prolongation of QT at lower rates) respectively. Both of which are thought to provide additional information about proarrhythmic potential in addition to QTc. These strategies are part of the triangulation, reverse use-dependence, instability and dispersion (TRiAD) concept proposed by Shah and Hondeghem.[103] This thesis investigates the clinical assessment of reverse use dependence and QT instability.

Comprehensive preclinical assessment

Besides ongoing research to improve how changes in cardiac repolarization are evaluated clinically, there is another area of active research, in the preclinical space. A think tank sponsored by the Cardiac Safety Research Consortium (CSRC), Health and Environmental Sciences Institute (HESI) and the FDA met at the FDA headquarters in July 2013, to discuss this new initiative which is also called the Comprehensive *in vitro* Proarrhythmia Assay (CiPA).[89]

Briefly, the goal of CiPA is to combine a more comprehensive *in vitro* assessment, that is the functional effects on multiple human cardiac ion currents (I_{Kr}, I_{Na}, $I_{Na,L}$, $I_{Ca,L}$, I_{K1}, I_{to} and I_{Ks}) and use the data from the *in vitro* experiments to drive *in sil-*

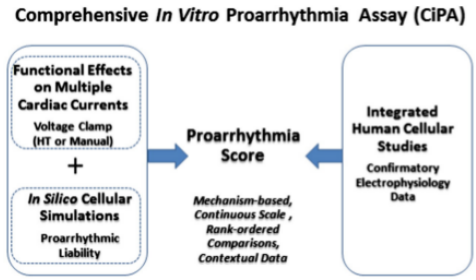

FIGURE 1.4 Overview of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) paradigm. Reprinted from Sager, et al.,[89] with permission from Elsevier.

ico reconstructions of the entire cardiac action potential to assess proarrhythmic risk (Figure 1.4).

It is envisioned that *in vitro* data on effects of drugs in isolated human cardiac myocytes can be used to confirm the adequacy of the voltage clamp data and *in silico* reconstructions, but such model systems probably are not mature enough to substitute for them at present.

Future of proarrhythmic risk assessment

The current cardiac safety paradigm as described in the ICH E14 guidance has been successful in the sense that the number of reported TdP events for non-antiarrhythmic drugs has stabilized. Besides the immediate obvious cost of the study design, the introduction of the ICH E14 and ICH S7B guidances and the associated focus on hERG potassium channel block and QTc prolongation may have led to improper discontinuation of beneficial drugs.[58] In particular, beneficial drugs such as amiodarone and verapamil might not have been developed in today's cardiac safety assessment paradigm. Thus, there is a need to improve cardiac safety assessment. This can hopefully be achieved by a combination of enhanced assessment of drug-induced effects in early clinical studies to confirm findings from enhanced preclinical assays as proposed by CiPA.

CHAPTER 2

Aims

The overall purpose of this thesis is to develop methods to discern cardiac ion channel effects from electrocardiogram (ECG) recordings and evaluate these methods using data from two prospective clinical trials. In addition, results from *in vitro* experiments (patch clamp using over-expression systems) and computer simulations are presented.

The first part of the thesis (Studies I and II) focuses on evaluation of factors associated with ECG data quality. The second part (Studies III - VI) focuses on the relationship between different ECG biomarkers and specific cardiac ion channel effects.

The specific aim for each study included in this thesis is outlined below:

I. To test the hypothesis that adjustment of the QT interval measurements based on ECG waveform similarity improves the ability to detect the QTc prolongation associated with moxifloxacin. In addition, this study evaluated if heart rate stability predict the ability to detect the QTc prolongation associated with moxifloxacin.

II. To evaluate QTc quality metrics and how they relate to different factors that could influence study quality, including QT measurement methodology, recorder type and reader variability.

III. To test the hypothesis that an integrated analysis of ECG intervals can identify drugs that selectively block the L-type calcium channel (PR interval), peak sodium current (QRS duration) or the human ether-à-go-go-related gene (hERG) potassium channel (T_{peak}-T_{end} interval).

IV. To test the hypothesis that selective hERG potassium channel blocking drugs prolong both J-T_{peak}c and T_{peak}-T_{end}, and that drugs with additional inward current block (L-type calcium or late sodium) preferentially prolong T_{peak}-T_{end}.

V. To test the hypothesis that co-administration of a selective late sodium current blocker reduces QTc interval prolongation associated with hERG potassium channel block, and that the QTc interval shortening due to late sodium current block is primarily driven by shortening of the J-T_{peak}c interval.

VI. To evaluate if it is possible to detect reverse use dependence and QT interval instability associated with hERG potassium channel block using postural maneuvers.

CHAPTER 3

Materials and Methods

3.1 Study Populations

Retrospective analysis of Thorough QT Studies (Studies I-III)

Studies I-III are based on retrospective analyses of digital electrocardiograms (ECGs) from the Food and Drug Administration (FDA) ECGWarehouse (`http://www.ecgwarehouse.com`), which currently contains data from over 250 conducted thorough QT (TQT) studies.[58] Studies in the ECGWarehouse were performed with informed consent and the respective protocols were reviewed by the local Institutional Review Board (IRB). In addition, the retrospective analyses described below were approved by the FDA Research Involving Human Subjects Committee (RIHSC).

TQT studies from the ECGWarehouse submitted to the FDA between January 2006 and December 2012 were considered for inclusion in Studies I and II (N = 238). From this set TQT studies that were completed before 2005 (n = 14), inappropriate design (n = 21) or that did not include digital ECGs (n = 5) were excluded.

This resulted in a set of 179 TQT studies considered for inclusion in Studies I and II. From this set 19, TQT studies failed to demonstrate assay sensitivity and were included in the first part

of the Study I, and 58 TQT studies included pharmacokinetic analysis of moxifloxacin and were thus included in the second part of Study I. In addition, 20 studies with multiple drug-free baseline days were identified for inclusion in Study II.

Finally, TQT studies that included digital ECGs and had a mean baseline, and placebo-adjusted ($\Delta\Delta$) change in the QTc interval >10 ms (n = 17) from the ECGWarehouse were included in Study III. In addition, a set of random QTc studies that did not meet this criteria (n = 17) were also included in Study III.

Prospective clinical trials (Studies IV-VI)

Studies IV through VI focuses on data from two FDA sponsored clinical trials conducted at a phase I clinical research unit (Spaulding Clinical Research, West Bend, WI, USA). The two prospective clinical trials were reviewed by the FDA RIHSC as well as the local IRB and all subjects gave written informed consent.

The two prospective trials included 22 healthy subjects each, and similar inclusion and exclusion criteria as for TQT studies were used.[60] Briefly, the inclusion and exclusion criteria included: no family history of congenital long QT syndrome as well as no history of heart disease or unexplained episodes of syncope, be 18 to 35 years of age at the start of the study, weigh at least 50 kg and have a body mass index between 18 to 27 kg/m^2. In addition to the typical exclusion criteria employed by TQT studies, subjects with >10 ectopic beats during a 3 h ECG recording at screening were also excluded.

ECGs in both prospective clinical studies were recorded using continuous 24-hour 12-lead Holter recorders (Surveyor, Mortara Instrument, Milwaukee, WI, USA) using the Mason-Likar configuration.[12] The ECGs were recorded at 500 Hz with an amplitude resolution of 2.5 µV. In both trials, triplicate 10 s ECGs were extracted from the Holter recordings during supine rest with the most stable heart rate and highest signal quality.[30] ECGs from both studies were analyzed in a semi-automatic fashion using previously developed software (see section 3.5).[104,105]

In both studies post-dose blood samples samples were drawn at the end of each nominal time point to enable pharmacokinetic analysis. The blood samples were analyzed using a validated liquid chromatography with tandem mass spectroscopy method by Frontage Laboratories (Exton, Philadelphia, PA, USA).

Prospective clinical trial 1 (Studies IV and VI)

The first prospective clinical trial (SCR-002) was a five-way crossover study separated by a seven-day washout period. The primary endpoint of the study was to evaluate the ECG signature of drugs with well known electrophysiological effects. Specifically, it was hypothesized that human ether-à-go-go-related gene (hERG) potassium channel block prolongs both the heart rate corrected J-T_{peak} (J-T_{peak}c) and the T_{peak}-T_{end} intervals. In addition, it was hypothesized that addition of inward current block would primarily shorten J-T_{peak}c.

The study included 22 subjects, five replacements, and 20 subjects were expected to complete. Based on sample-size calculations proposed by Zhang and Machado[106] the study was powered to detect a change in 10 ms, which based on the retrospective study of 34 drugs (Study III) was expected to be appropriate.

During each visit, the subjects would check-in the day before dosing, and were fasting overnight. The study included dofetilide, quinidine, ranolazine, verapamil and placebo. The drugs were selected because they all have well characterized electrophysiogical effects, and represent a group of drugs with high risk for torsade de pointes (TdP) (500 µg dofetilide, 400 mg quinidine) and low risk for TdP (120 mg verapamil, 1500 mg ranolazine). The selected doses were clinically used doses with the exception of ranolazine, which was studied at 1500 mg. The reason for the higher dose of ranolazine was to achieve plasma concentrations more similar to steady-state levels.[107] The selected drugs allow for studying the effects of selective hERG potassium channel block (dofetilide) in contrast to hERG potassium channel blockers that also block inward currents such as the L-type calcium (quinidine, verapamil)

or late sodium current (ranolazine).

The drugs were administered in the morning of the treatment day. Before drug administration and at 15 time points after dose (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 14 and 24 h post-dose) the subjects were resting in a supine position for 10 min, during which ECGs were extracted as describe above.

In addition, at the pre-dose time point and at a six post-dose time points (1, 2, 3, 4, 6 and 7 h) the subjects performed a series of postural maneuvers: unsupported sitting and standing (5 min each at pre-dose, 2, 4 and 6 h post-dose) or unsupported sitting and leg raises (5 min each at 1, 3 and 7 h post-dose). The postural maneuvers were performed to increase heart rate and allow for analysis of rate dependent effects (Study VI).

At all post-dose time points after the postural maneuvers or leg raises for the extended time points or the supine rest for the other time points a blood sample was drawn for pharmacokinetic analysis.

Prospective clinical trial 2 (Study V)

The second prospective trial (SCR-003, Balanced Ion Channel Trial) was also a five-way cross-over study separated by a seven-day washout. The primary end-point of the second clinical trial was to test whether or not selective block of the late sodium current by (mexiletine or lidocaine) could shorten QTc prolongation resulting from block of the hERG potassium channel by dofetilide. It was hypothesized that the shortening would be primarily due to shortening of the J-T_{peak}c interval. The secondary endpoint was to evaluate whether or not L-type calcium channel block by diltiazem could shorten drug-induced block of the hERG potassium channel by moxifloxacin.

Prior studies suggested that administration of a late sodium current blocker such as mexiletine[108] and lidocaine[109] would be expected to shorten the QTc interval by 20 to 40 ms. Based on this, sample size calculations similar to the first study were carried out resulting in an enrollment of 22 subjects, with five potential

replacements, expecting 20 subjects to complete.

During each visit, doses were administered in the morning, afternoon and in the evening. Each dosing period (morning, afternoon and evening) started with an oral dosing of dofetilide, mexiletine or placebo. After administration of the oral dose, an intravenous dose of: lidocaine, moxifloxacin, diltiazem or placebo was administered over 60 min (loading dose) followed by a 30 min maintenance dose (Figure 3.1).

The choice of multiple doses throughout the day was to allow for studying selective late sodium current block in the morning as well as increasing doses of both late sodium current and hERG potassium channel block throughout the day. In addition, this design allowed for studying low and high doses of moxifloxacin as well as moxifloxacin combined with diltiazem.

The specific doses were either selected based on our prior study (dofetilide), to match highest studied doses (moxifloxacin), label (diltiazem) or previous studies (mexiletine and lidocaine). In the case of mexiletine a study that showed shortening of quinidine-induced QTc prolongation,[108] and for lidocaine a study showing shortening of QTc in patients with congenital long QT type 3 (sodium channel defect) and healthy controls.[109] All the intravenous doses were modified to allow for a 60 min loading dose and 30 min maintenance dose.

In contrast to the first clinical trial, oral dosing was performed after food administration in the second clinical trial. The reason for administering the doses under fed conditions was to minimize gastrointestinal tract related adverse events following oral dosing of one of the drugs (mexiletine).

ECGs were extracted at the pre-dose time point as well as at four time points for each dosing interval (30, 60, 90, 120 min post start of intravenous loading dose). Blood samples were drawn at the end of the supine rest associated with each ECG extraction.

Similar to the first clinical trial, the subjects underwent a set of postural maneuvers (5 min unsupported sitting, and 5 min standing) following the supine rest during each dosing period, but before collection of blood samples.

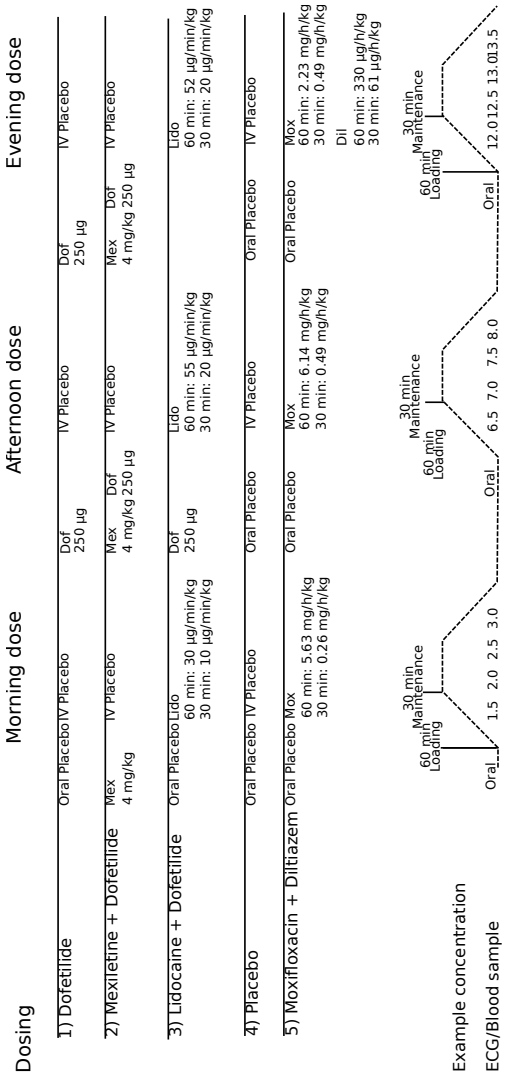

FIGURE 3.1 Overview of dosing for the second prospective trial for each of the five treatment arms: dofetilide alone, dofetilide + mexiletine, dofetilide + lidocaine, placebo or moxifloxacin + diltiazem. IV: Intravenous, Dof: Dofetilide, Mex: Mexiletine, Lido: Lidocaine, Mox: Moxifloxacin, Dil: Diltiazem. Reprinted from Johannesen, et al.,[110] with permission from John Wiley and Sons.

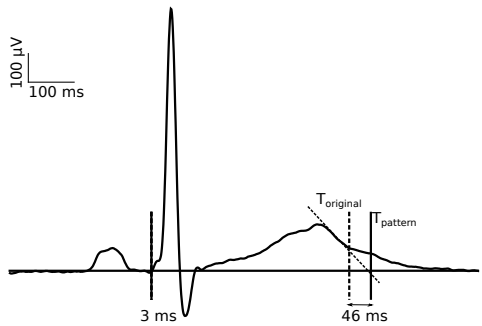

FIGURE 3.2 Example of the result of application of the pattern matching methodology by Hnatkova, et al.[111] Applying the pattern matching methodology for this electrocardiogram (ECG) changed the onset of the QRS complex by 3 ms and T-wave offset (original: $T_{original}$, adjusted: $T_{pattern}$) by 46 ms. Reprinted from Johannesen, et al.,[112] with permission from Springer Science+Business Media.

3.2 Pattern Matching (Studies I and II)

There is no gold standard for measurement of the QT interval on an ECG since the location of the fiducial points making up the QT interval, onset of the QRS complex and T-wave offset depends on the interpretation if the ECG. However, systematic measurement of the QT interval is important as changes between drug and placebo are compared. A methodology proposed by Hnatkova, et al.,[111] ensures consistent measurement of the QT intervals by re-adjustment of the measurements based on pattern matching. An example of application of the methodology is shown in Figure 3.2.

The basis of the pattern matching methodology is to extract the samples in a window around the onset of the QRS complex and of T-wave offset separately in a global lead (e.g. vector magnitude or root-mean-square lead) and align it with all other extracted windows from the same subject. Afterwards, all the extracted segments are compared to each other iteratively by taking one segment and aligning all other segments to that one segment based

on maximizing a cost function, e.g. cross-correlation. When the maximum cross-correlation is achieved the location of the onset of the QRS complex or T-wave offset in the segment the other segments were aligned to is updated based on a weighted average of the distance of each of the other segments to the first segment, where the weight is based on the cost function. The procedure is the then continued for all the other segments. This way the location of the QRS complex or T-wave offset will converge to some average location based on similarity to other waveforms. This methodology has been shown using drug-free data to reduce the variability of QTc.[111]

3.3 ECG Quality Metrics (Study II)

To evaluate quality of measurements of the QTc interval in TQT studies, e.g. to serve as a replacement for the positive control, a set of QTc quality metrics has been proposed.[91] The concept behind the quality metrics is to capture consistency in the QT/RR relationship between visits as well as intra- and inter-subject differences over time. This is done by computation of three types of tests.

The first test covers intra-subject variation in the heart rate corrected QT (QTc) interval, and is computed by the standard deviation of drug-free measurements of QTc per subject. The second test quantifies the day-to-day variability of QTc measurements by evaluating the difference per subject per time point using drug-free data. Finally, the third test computes the day-to-day variability per time point or average across time points of differences between all subject combinations.

3.4 ECG Simulations (Study III)

Simulations of the effects of various amounts of L-type calcium channel block combined with hERG potassium channel block was performed using a combination of a model of the human ventricular action potential, the O'Hara-Rudy model[113] and the ac-

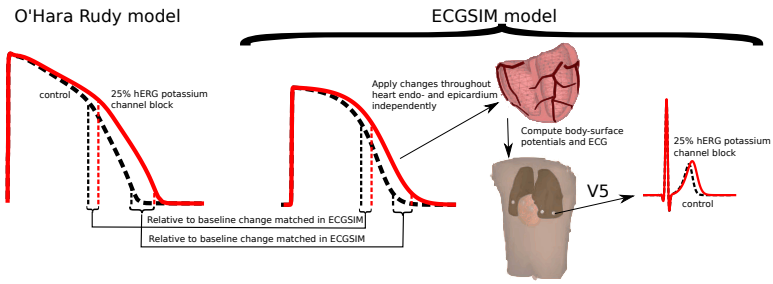

FIGURE 3.3 Example of simulated human ether-à-go-go-related gene (hERG) potassium channel block. The left panel shows a simulated action potential (control: black dashed, hERG potassium channel block: red) and the measurement of the relative (to control) changes in action potential from the O'Hara-Rudy model.[113] The relative changes from the O'Hara-Rudy action potential are then applied to the action potential in ECGsim[114] and a 12-lead electrocardiogram (ECG) is simulated. Reprinted from Johannesen, et al.,[118] with permission from John Wiley and Sons.

tion potential-to-body surface ECG model (ECGsim, `http://www.ecgsim.org`).[114]

In the first step the drug effect on the action potential was studied using the O'Hara-Rudy model using the *"conductance block model"*,[115] by reducing the maximum conductance of a current by x percent. The effect of 25% hERG potassium channel block or 25% reduction of the conductance relative to control is shown in Figure 3.3, where the red line is after block of the hERG potassium channel and the black dashed line is control.

Afterwards, changes in the action potential relative to control was measured and applied uniformly to the action potentials in the ECGsim model throughout the endo- and epicardium and the 12-lead ECG is computed using ECGsim.

Finally, the ECG intervals of interest (QT, J-T_{peak} and T_{peak}-T_{end}) are measured on the simulated ECGs using an automatic delineator.[116,117]

3.5 Assessment of ECGs (Studies IV-VI)

The primary analysis of ECGs from the two prospective clinical trials were performed on 10 s ECGs extracted during supine rest based on signal-to-noise ratio and heart rate stability.[30]

The extracted 10 s ECGs were then preprocessed by first upsampling to 1 kHz using cubic spline interpolation. Subsequently baseline wander was removed by estimating the baseline wander using cubic splines fitted through a point in the PQ segment.[119]

From each 10 s ECG a median beat was created by aligning each individual beat from the 10 s ECG on the R-peak and computing the median sample-by-sample to form the median beat for each lead.

Afterwards, the vectorcardiogram was constructed using the Guldenring transform.[120] Then, the magnitude of the resulting X, Y and Z leads were computed to form the vector magnitude lead (Figure 3.4). The vector magnitude lead represents a global lead and measurement of the QT interval using this lead has been shown to result in more consistent measurements.[121]

In Study IV the onset and offset of the QRS complex was measured using lead II. The QRS onset and offset was located in the Study V using a wavelet-based methodology using the vector magnitude lead.[104,116]

The T-wave offset is then measured in the resulting vector magnitude lead using the tangent method.[16] The tangent method defines the offset of the T-wave is the intersection between the steepest slope of the descending limb of the T-wave and the isoelectric or *"zero line"*. An example is shown in Figure 3.4.

The peak of the T-wave was defined as location of the maximum of the T-wave. In the case of notched or flat T-waves the peak was defined as the location of the first peak or in the middle of the T-wave respectively.

In both prospective clinical trials ECG readers blinded to treatment, time and subject information measured all ECG intervals. For ECGs where the measurement of any fiducial point (onset and offset of QRS, peak of T-wave or end of T-wave) was more than

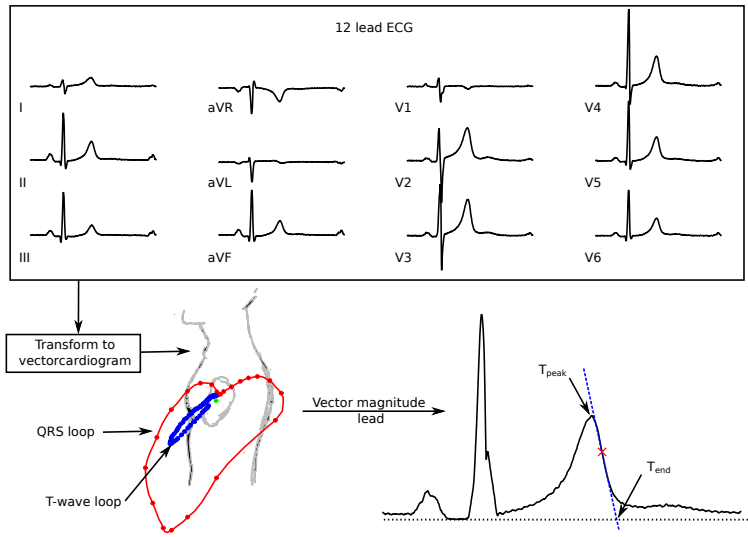

FIGURE 3.4 Example of assessment of the T-wave offset in the vector magnitude lead. First, the median beat from the 12-lead median beat electrocardiogram (ECG) is used to construct the X,Y and Z leads (vectorcardiogram) by applying the Guldenring transform.[120] Afterwards, the magnitude of the vectorcardiogram is computed sample-by-sample. Finally, the T-wave offset is located using the tangent method.[16] Reprinted from Johannesen, et al.,[122] with permission from John Wiley and Sons.

5 ms different or there were disagreement about the presence of notch or whether or not the T-wave was measurable, the ECGs were re-assessed blinded to the reason for re-assessment and previous measurements. If the two readers disagreed after the second read, a blinded expert ECG reader was consulted.

3.6 Holter Analysis (Study VI)

To facilitate analysis of beat-by-beat changes in the QT interval and to quantify changes in the rate dependency of QT or markers of QT interval instability (see section 3.7) a series of processing steps was implemented (Figure 3.5).

The steps for this process consists of:

I. Holter QRS detection:
   a) Automatic detection of QRS complexes using a previously developed QRS detector.[104]
   b) Adjudication of automatic delineation near windows of interest starting 5 min before supine rest and ending at the end of the postural maneuvers using Holterlab.[105]

II. Extraction of non-overlapping 10 s ECG from periods of supine rest and postural maneuvers.

III. Preprocessing of the extracted 10 s ECGs and generation of median beats similar to the standard assessment of ECGs (see section 3.5).

IV. Semi-automatic detection of the onset of the QRS complex and T-wave offset:
   a) Automatic determination of the onset of the QRS complex and T-wave offset similar to the primary ECG assessment (see section 3.5).
   b) Application of pattern matching to the onset of the QRS complex to ensure systematic measurements. This process was necessary as the onset detector of the QRS was not as reliable as the T-wave offset and application of pattern matching reduced the overread burden significantly.[111]

V. Project locations of fiducial points from median beats onto the beats used to create the median, in the Holter recording, using cross-correlation.[123]

3.7 Dynamic Repolarization Markers and Reverse Use Dependence (Study VI)

Changes in the relationship between heart rate and the QT interval were quantified by evaluating the linear relationship between

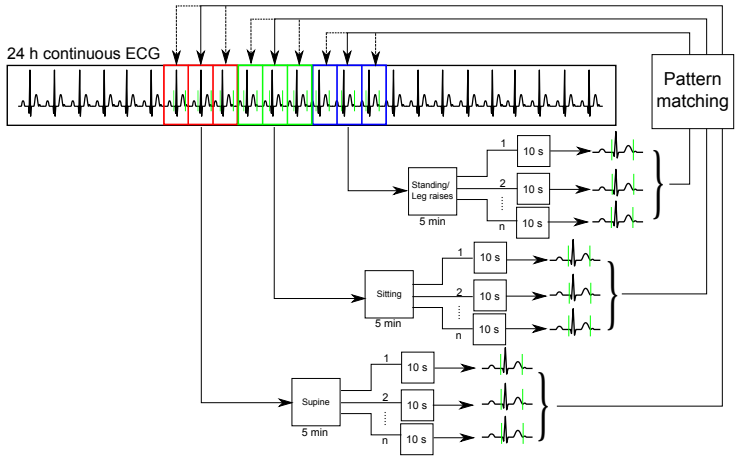

FIGURE 3.5 Overview of the methodology used to measure beat-by-beat QT/RR measurements, following detection and adjudication of QRS detections. First step is to extract non-overlapping 10 s electrocardiograms (ECGs), and create representative median beats. Afterwards, each median beat is annotated and pattern matching is applied to ensure consistent identification of the onset of the QRS complex and the measurements are projected back onto the continuous ECG.

the square root of the preceding RR interval in seconds and the QT interval in seconds as proposed by Okada and colleagues.[124] An increase in the slope of this relationship indicates the presence of reverse use dependence.

Several methodologies exist for quantifying changes in QT interval variability as described in a recent systematic review by Niemeijer and colleagues.[125] In addition, analysis of QT variability indices typically only include a few measures of QT interval variability making it difficult to compare and contrast methods.[125] As a result, three methods were included in this work:

- QT variability index (QTVI) as proposed by Berger et al.[126]
  - $\text{QTVI} = log10\left(\frac{QTv/QTm^2}{HRv/HRm^2}\right)$
- Median absolute deviation of QT (MADqt)

  - MADqt $= median(|QT_i - median(QT)|)$

- Short term variability of QT (STVqt)

  - STVqt $= \sum_{i=0}^{n-1} \frac{|QT_{i+1} - QT_i|}{n\sqrt{2}}$

These three methods were selected either because they had been used to study drug-induced effects[127] or quantify the beat-to-beat variation in QT similar to methods used in preclinical studies. It is worth noting that MADqt and STVqt are not corrected for changes in heart rate but QTVI is.

In addition to considering three indices of QT interval variability, the indices are computed for each of the three different maneuvers independently because a recent study by Hnatkova, et al., suggests that measures of QT interval variability might depend on postural maneuver.[128]

3.8 Statistical Analysis

As several ECG intervals exhibit heart rate effects, they have to be corrected for heart rate before statistical analysis. However, because only drugs without substantial heart rate changes were studied, the QT interval was corrected using Fridericia's correction,[18] which is appropriate in the absence of substantial heart rate changes.[129]

In addition to the QT interval, heart rate dependent changes have also been observed for the J-T_{peak} interval[118] and minimal heart rate effects near resting heart rates for PR, QRS and T_{peak}-T_{end}.[22,23,118]

The effect of heart rate on the J-T_{peak} interval was modeled using measurements from a subset of subjects (n = 431) with the widest span in heart rate (55 to 75 bpm) using an exponential model, similar to Bazett[17] or Fridericia.[18] This resulted in the following correction equation for J-T_{peak}:[118]

$$\text{J-T}_{\text{peak}}\text{c} = \frac{\text{J-T}_{\text{peak}}}{(\text{RR}/1000)^{0.58}}$$

The other ECG intervals (PR, QRS, T_{peak}-T_{end}) were not corrected for heart rate.

Studies I and II

As there is no gold standard definition of the QT interval, the impact of applying the pattern matching methodology to reduce QT interval variability was evaluated by a linear mixed effects model:

$$\Delta\Delta QTc_{i,j} = (\alpha + \alpha i) + (\beta + \beta_i) \times conc + \epsilon$$

Where $\Delta\Delta QTc_{i,j}$ represents the jth baseline, and placebo-adjusted ($\Delta\Delta$) QTc measurement from subject i, α and β is the intercept and slope respectively. Subscripts i for α and β indicates that it is the random effect for that subject.

In the described model above the residual represents the consistency of the QTc measurements as moxifloxacin concentrations are linearly correlated with changes in the $\Delta\Delta$QTc.[130]

This model was fitted for each study using PROC MIXED in SAS 9.2 (SAS Institute, Cary, NC, USA). The residuals between different methods for computing the QTc was then compared using a paired t-test in R 2.15.3 (R Foundation for Statistical Computing, Vienna, Austria).

In both Studies I and Study II a Mann-Whitney test was used to compare the intra-replicate heart rate (Study I) or QTc quality metrics (Study II) by different groups using R.

Study III

Study III included the retrospective assessment of 34 TQT studies on several ECG biomarkers: PR, QRS, T_{peak}-T_{end} and QTc (see section 3.1).

For the QTc interval the ICH E14 guidelines defines a threshold of regulatory concern of 10 ms,[60] but for the other ECG intervals no thresholds exist. To define a set of thresholds for all the studied ECG intervals in the same way, the day-to-day variability (QTc quality test 2, see section 3.3) was quantified using data from a

set of 20 cross-over studies with multiple drug-free baseline days. The day-to-day variability per study was summarized by taking the average lower to upper bound of variability and taking the maximum rounded absolute bound evaluated in R.[131]

Afterwards, if the maximum absolute effect of a drug by time within the first 12 hours excluded the day-to-day variability for each ECG biomarker it was considered to be significant. Of note, the time point for different biomarkers were not forced to be the same, as it is possible that different mechanisms are driving the different biomarkers.

Lastly, Fisher's exact test was used to evaluate the relationship between block of a given cardiac ion channel (peak sodium, hERG potassium or L-type calcium) and effects on PR, QRS, T_{peak}-T_{end} or QTc using R.

Studies IV-VI

Studies VI and V were based on two prospective clinical trials (see section 3.1), which consisted of ECG measurements and drug concentrations for each drug. The main analysis for the data from these two studies were either a by-time or concentration dependent analysis.

The by-time analysis modeled the ΔQTc as a function of time, treatment, time and treatment interaction, period and sequence and a random effect on the intercept by subject using PROC MIXED in SAS. Afterwards, the $\Delta\Delta$QTc by drug and treatment was computed as the difference in the least squares means of treatment minus placebo by time.

The concentration dependent analysis was performed using a linear model as in the analysis of Study I (see section 3.8), allowing each subject to have an individual intercept and slope. The concentration dependent analysis was also performed using PROC MIXED in SAS. The assumption of the linearity was evaluated using a visual predictive check by comparing the data binned into 10 bins (deciles) and overlaying the population prediction from the model.

The difference in the relationship between the J-T_{peak}c and T_{peak}-T_{end} concentration dependent relationship (slope) was compared by using a paired t-test on the individual slopes between the two intervals using R.

The effect of mexiletine or lidocaine to reduce dofetilide induced effects on repolarization was evaluated by computing the difference of the least-squares estimates of mexiletine or lidocaine + dofetilide and the dofetilide arm. Similarly, the effect of diltiazem on moxifloxacin was compared to evaluate the effect of diltiazem to reduce moxifloxacin-induced QTc prolongation.

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CHAPTER 4

Results and Comments

4.1 Evaluation of Factors Influencing the Ability to Detect Effect of Moxifloxacin (Study I)

Comparisons of different methodologies for computing the heart rate corrected QT (QTc) interval and how it effects the residual variability of a linear concentration QTc model (see section 3.8) are shown in Figure 4.1. The analysis includes evaluation of four different QTc calculations: data 1 (original QT/RR), data 2 (original QT, average RR), data 3 (pattern adjusted QT, original RR) and data 4 (pattern adjusted QT, average RR).

The use of average RR lowered the residual of the model compared to the QT/RR measurements (data 1: 8.43 ± 2.00 ms versus data 2: 8.23 ± 1.88 ms; $P < 0.001$). An even greater reduction of the residual was achieved when the QT measurements were corrected (data 3) compared to just averaging the RR measurements (data 2: 7.55 ± 1.86 ms, data 3 versus data 2; $P < 0.001$). Lastly, combining average RR with pattern adjusted measurements (data 4) led to a further decrease in the residual (data 4: 7.12 ± 1.62 ms, data 4 versus data 3; $P < 0.001$).

This suggests that the residual is driven by reader variability, as shown by a reduction in the residual after application of pat-

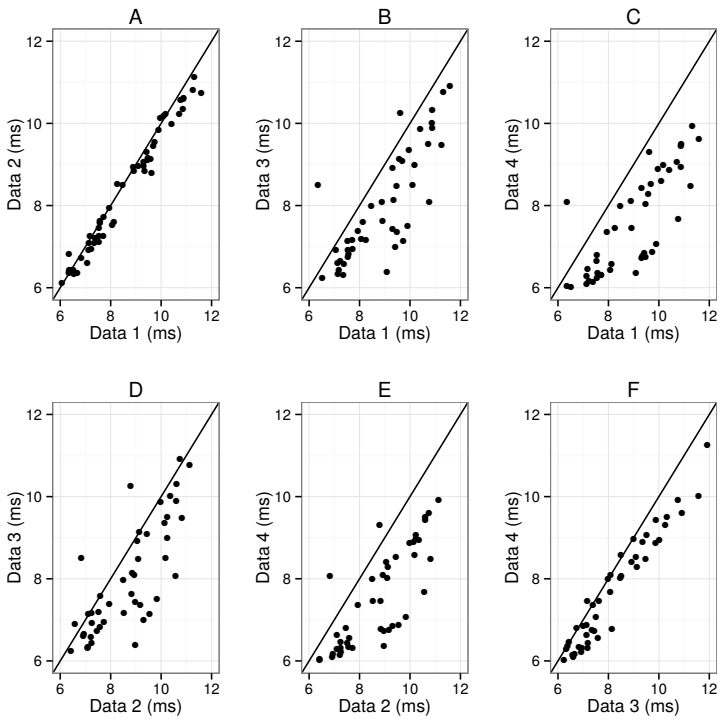

FIGURE 4.1 Comparison of residual variability of linear-concentration QTc models for four different QTc calculations: data 1 (original QT/RR), data 2 (original QT, average RR), data 3 (pattern adjusted QT, original RR) and data 4 (pattern adjusted QT and average RR). Reprinted from Johannesen, et al.,[112] with permission from Springer Science+Business Media.

tern matching. In addition, a reduction in the residual was also observed when using the average RR for the 10 s. It is, however, worth noting that the original RR could either be the average for 10 s or the average of three beats. Which one it is depends on whether or not the QT interval was measured on the median beat.

These findings are consistent with previous observations of reduction in intra-subject variability after applying pattern match-

ing[111,132] or averaging of the RR measurements.[77] This supports the notion that consistency of QT interval measurements and stable heart rates impacts the ability to detect the QTc prolongation associated with moxifloxacin.

While these results support that pattern matching algorithms are helpful to reduce reader variability, it is unknown how these observations translate to cases where significant morphological changes are present in the T-wave.

It is also worth noting that the intercept of the concentration QTc relationship tended to decrease after applying pattern matching and averaging of the RR. This sugests that the intercept could be an artifact of data variability (intercept: original QT/RR 2.25 ± 2.58 ms and pattern adjusted QT and average RR: 1.68 ± 1.84 ms). Similarly, there was a slight increase in the slope after applying pattern matching and averaging of the RR.

In addition, the intra-replicate heart rate range was evaluated as a measure of study conduct and compared between studies that were able to demonstrate assay sensitivity to studies that failed to demonstrate assay sensitivity (Figure 4.2). This analysis shows that studies that failed to detect the QTc prolongation associated with moxifloxacin had a higher variability in heart rate as seen by the increased range of intra-replicate heart rates (original RR:5.98 ± 2.19 bpm versus 3.86 ± 1.41 bpm and averaged RR: 5.60 ± 2.42 bpm versus 3.47 ± 1.13 bpm; $P < 0.001$ for both). This is consistent with the previous findings that averaging RR measurements improved the precision by lowering the residual of a concentration QTc analysis. These results suggest that using the median intra-replicate range of heart rates can serve as a study quality metrics and likely reflects study conduct.

Moreover, studies that failed to demonstrate assay sensitivity also tend to have lower maximum concentrations compared to studies with assay sensitivity (2.3 ± 0.6 µg/mL (n = 61) versus 1.8 ± 0.2 µg/mL (n = 4); $P = 0.33$). While pharmacokinetic information for moxifloxacin was not available in all studies that failed to demonstrate assay sensitivity, it seems plausible that reduced concentrations of moxifloxacin would impact the ability to detect

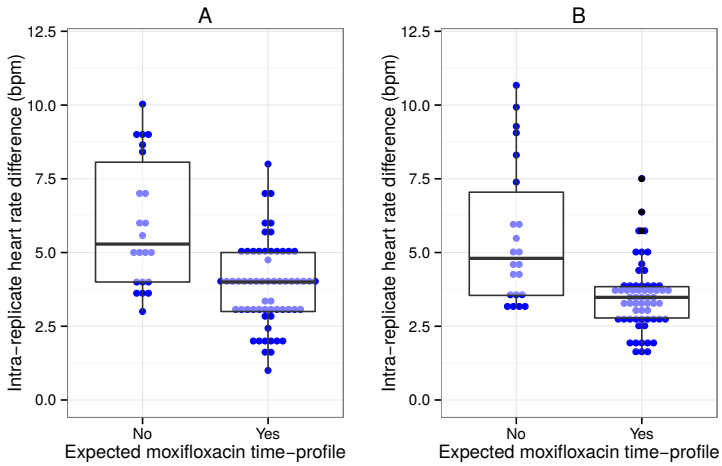

FIGURE 4.2 Comparison of the median intra-replicate heart rate between studies that detected the expected time-profile of moxifloxacin (assay sensitivity) compared to studies that did not using original RR (**A**) or 10 s average RR (**B**). Reprinted from Johannesen, et al.,[112] with permission from Springer Science+Business Media.

assay sensitivity as it reduces the magnitude of the QTc prolongation. With a reduced magnitude of QTc prolongation, the impact of inconsistent QT measurements with unstable heart rates likely has a greater impact.

4.2 Factors of ECG Data Quality in Thorough QT Studies (Study II)

Evaluation of the impact of different QTc calculations and previously proposed QTc quality tests[91] is shown in Figure 4.3. Similar to the results from Study I shown in Figure 4.1, there is a reduction for all QTc quality metrics when applying pattern matching.

In addition, a slight improvement when combining pattern adjusted QT measurements with average RR measurements was observed (data 1 versus data 4, $P < 0.001$ for all). These findings support that the proposed QTc quality metrics, which quanti-

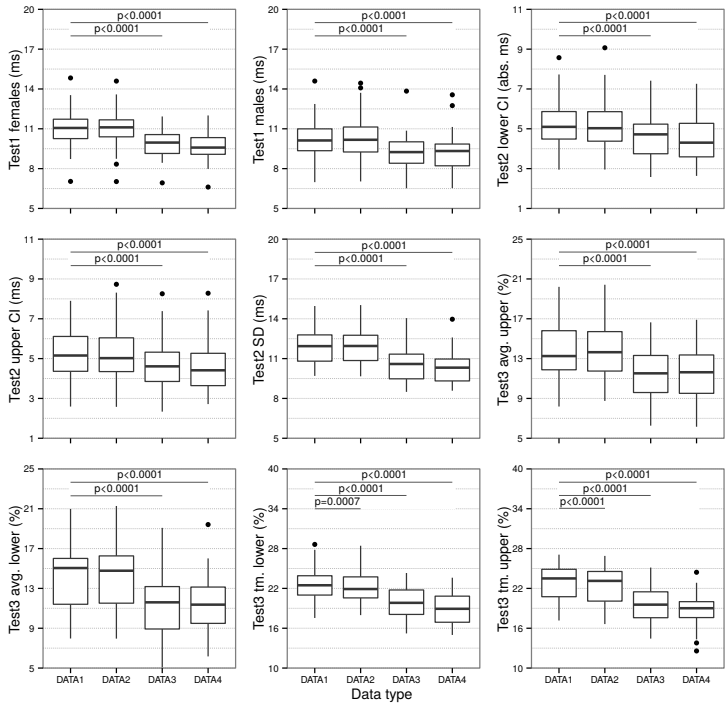

FIGURE 4.3 Comparison of effect of different QTc calculations on previously proposed QTc quality metrics: data 1 (original QT/RR), data 2 (original QT, average RR), data 3 (pattern adjusted QT, original RR) and data 4 (pattern adjusted QT and average RR). Reprinted from Johannesen, et al.,[133] with permission from Springer Science+Business Media.

fies not just intra-subject variability but also consistency of inter-subject differences across time and periods, is influenced by reader variability and heart rate variability.

A comparison of the QTc quality metrics from cross-over studies grouped by QT measurement methodology or recorder type is shown in Table 4.1. The table shows that the QTc quality metrics tend to be lower (more consistent QTc measurements) for studies with semi-automatic QT measurements in comparison to studies using manual QT measurements.

TABLE 4.1 QTc quality test[91] values grouped by protocol factors. Reprinted from Johannesen, et al.,[133] with permission from Springer Science+Business Media.

	Recorder type		QT measurement methodology		
	Continuous (N=22)	Standard (N=12)	Manual (N=12)	Semi-automatic (N=11)	Unknown (N=11)
Test 1					
Female (ms)	11.14 (1.54)	10.91 (1.67)	11.55 (1.67)	10.21 (1.34), $P = 0.03$	11.41 (1.38)
Male (ms)	10.50 (1.57)	9.84 (0.99)	10.53 (1.01)	9.50 (1.46)	10.82 (1.45)
Test 2					
Lower CI (ms)	-5.17 (1.14)	-5.44 (1.57)	-5.50 (1.16)	-5.16 (1.53)	-5.11 (1.26)
Upper CI (ms)	5.08 (1.06)	5.75 (1.64)	5.23 (1.45)	5.47 (1.32)	5.25 (1.26)
SD (ms)	11.92 (1.43)	11.86 (1.20)	12.21 (1.43)	11.16 (1.17)	11.16 (1.17)
Test 3 - average					
Proportion < -10 ms (%)	13.86 (2.84)	14.51 (3.46)	15.33 (2.86)	11.30 (1.45), $P < 0.01$	15.52 (2.59)
Proportion > 10 ms (%)	13.58 (3.38)	15.43 (3.50)	15.28 (3.83)	11.66 (2.58), $P = 0.02$	15.66 (2.55)
Test 3 - time-matched					
Proportion < -10 ms (%)	22.75 (2.62)	22.12 (2.08)	23.46 (2.37)	20.52 (1.55), $P < 0.01$	23.52 (2.08)
Proportion > 10 ms (%)	22.50 (3.10)	22.90 (2.33)	23.19 (3.13)	20.98 (2.58)	23.69 (2.07)

This would suggest that semi-automatic QT measurements provide more consistent measurements. How many QT measurements that were corrected during the semi-automatic overread or which criteria were used to determine if adjustment of a QT measurement was necessary is not known so no definitive conclusion can be made.

No differences were observed between studies using standard electrocardiogram (ECG) recording methodology when compared to studies that used continuous Holter recordings. This is in contrast to prior work by Badilini and colleagues,[30] which suggested that extracting ECGs from a Holter recording allows for obtaining 10 s ECGs of higher quality and more stable heart rates, leading to less variable QTc measurements. One possible explanation for this conflicting observation is that some of the studies using continuous recording methodologies extracted ECGs at fixed time points from the Holter, e.g. 1, 2 and 3 min after supine rest, thus ignoring the stability of the heart rate and the ECG signal quality.

Overall, these findings suggest that the proposed QTc quality metrics capture consistency of QT measurements. In addition, the evaluation of QTc quality metrics in a set of 20 cross-over studies suggests that using semi-automatic QT measurement techniques improves QT measurement consistency. It is, however, worth noting that the sample size of this study was relatively small, particularly when the set of studies was split into different subsets based on measurement methodology or recording methodology. Moreover, for 11 of the studies there was no mentioning of the specific measurement methodology in the study report and these were labeled as unknown which could have influenced the results. That being said, the concept of using intra- and intersubject stability of QTc measurements seems to have merit and how it can be expanded to be applicable to studies without multiple drug-free baseline days deserves further study.

4.3 Retrospective Analysis of Drug-Induced ECG Patterns (Study III)

This study evaluated the relationship between changes in different ECG intervals and electrophysiological mechanisms: PR interval (L-type calcium), QRS (peak sodium) and T_{peak}-T_{end} (human ether-à-go-go-related gene (hERG)). The relationship between the previously proposed ECG intervals and cardiac ion channel effects is shown in Figure 4.4.

All the drugs that had been shown preclinically to block the L-type calcium (n = 4) prolonged the PR interval (Figure 4.4) more than the day-to-day variability (±5 ms). Similarly all drugs that blocked the peak sodium current prolonged the QRS duration (Figure 4.4) more than the day-to-day variability (±3 ms), except for one drug. The one exception is a peak sodium current blocker. It was is not expected to prolong the QRS duration clinically as it preferentially blocks the sodium channel in its inactivated state and has similar disassociation kinetics to Vaughan Williams class Ib antiarrythmics (e.g., lidocaine).

There was also good agreement (18/26) between hERG potassium channel block and prolongation of the T_{peak}-T_{end} interval. However, there were a better agreement between prolongation of the QTc interval and hERG potassium channel block (20/26). The improved consistency with QTc interval prolongation and hERG potassium channel block compared to T_{peak}-T_{end} and hERG potassium channel block is likely because prolongation of the QTc interval is divided into T_{peak}-T_{end} prolongation and QRS onset to T-wave peak prolongation, thus reducing the signal.

Interestingly, evaluation of the concentration dependent relationship from drugs that are selective hERG potassium channel blockers and multichannel blockers (Figure 4.5) showed that selective hERG potassium channel block prolongs both the J-T_{peak}c interval as well as the T_{peak}-T_{end} interval. In contrast, drugs that block the hERG potassium channel as well as inward currents (L-type calcium) showed no prolongation (drug 6) or shortening (drug 11) of the J-T_{peak}c interval.

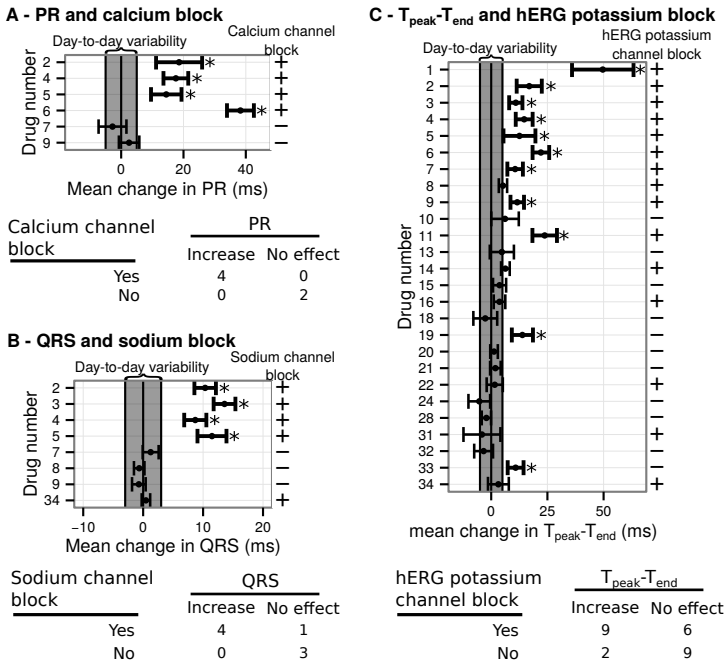

FIGURE 4.4 Relationship between maximum effect by-time (see section 3.8) for drugs tested for L-type calcium channel block (**A**), peak sodium current block (**B**) and human ether-à-go-go-related gene (hERG) potassium channel block (**C**) and PR, QRS and T_{peak}-T_{end} intervals respectively. Reprinted from Johannesen, et al.,[118] with permission from John Wiley and Sons.

An example ECG of this observation is shown in the bottom panel of Figure 4.5 where drug 6 (multichannel blocker) prolongs the QTc interval overall but by shortening of J-T_{peak}c and prolonging of T_{peak}-T_{end}. In contrast drug 7, a selective hERG potassium channel blocker, prolongs both the J-T_{peak}c and T_{peak}-T_{end} intervals. This suggest that hERG potassium channel block prolongs both J-T_{peak}c and T_{peak}-T_{end}, and that T_{peak}-T_{end} is a more specific marker of hERG potassium channel block.

The impact of L-type calcium channel block on ECG sub-

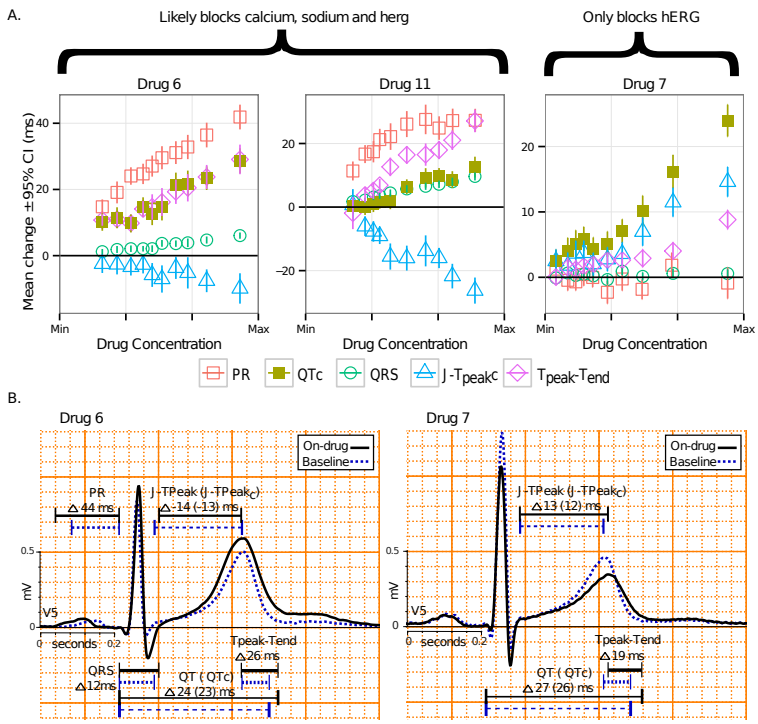

FIGURE 4.5 Top panel (**A**) shows the results of a concentration dependent analysis for two drugs from the same class that block multiple cardiac ion channels (drug 6, 11: human ether-à-go-go-related gene (hERG), L-type calcium and sodium) on the left and a selective hERG potassium channel blocker drug 7 on the right. The bottom panel (**B**) shows an example electrocardiogram (ECG) from drug 6 (multichannel blocker) and drug 7 (selective hERG potassium channel blocker). Reprinted from Johannesen, et al.,[118] with permission from John Wiley and Sons.

intervals was studied in a simulation study (Figure 4.6). The simulation study shows that increasing amount of L-type calcium channel block reduced QT prolongation from hERG potassium channel block by shortening of the J-T_{peak} interval. This is consistent with the observations in the clinical trials and supports the use of the J-T_{peak} interval as a marker of inward current block.

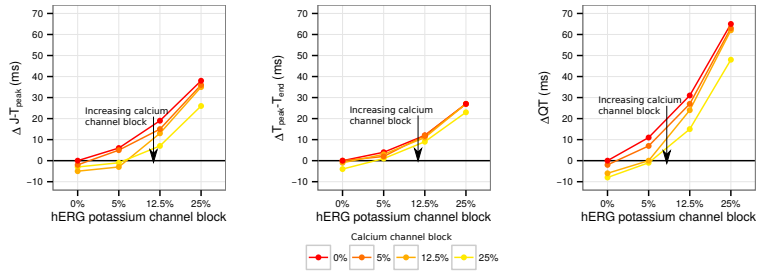

FIGURE 4.6 Results from a simulation study of varying amounts of human ether-à-go-go-related gene (hERG) potassium channel block (0, 5, 12.5 and 25%) combined with either 0, 5, 12.5 and 25% block of the L-type calcium channel on different intervals measured on the electrocardiogram (ECG): J-T_{peak}, T_{peak}-T_{end} and QT. Reprinted from Johannesen, et al.,[118] with permission from John Wiley and Sons.

The results of this combined retrospective analysis and simulation study supports the use of the ECG subinterval J-T_{peak}c to detect the presence of inward current block. However, the risk for torsade de pointes (TdP) for the studied drugs is not known and only a small subset of the included drugs had been evaluated for other cardiac ion channels than the hERG potassium channel. In addition, the assessment of effects on cardiac ion channels varied between the included drugs. Lastly, in the simulation study we applied relative changes observed in simulations using the O'Hara-Rudy model[113] to action potentials in the heart-to-body-surface ECG simulator ECGsim,[114] which is a simplification and has its limitations and assumptions.

4.4 Differentiating Selective hERG Potassium Channel Block from Multichannel Block (Study IV)

A prospective clinical trial of 22 healthy volunteers (11 female) was conducted to prospectively study the ECG signatures of four drugs: dofetilide, quinidine, ranolazine and verapamil. The concentration dependent relationships are shown in Figure 4.7.

The concentration dependent analysis of dofetilide showed an

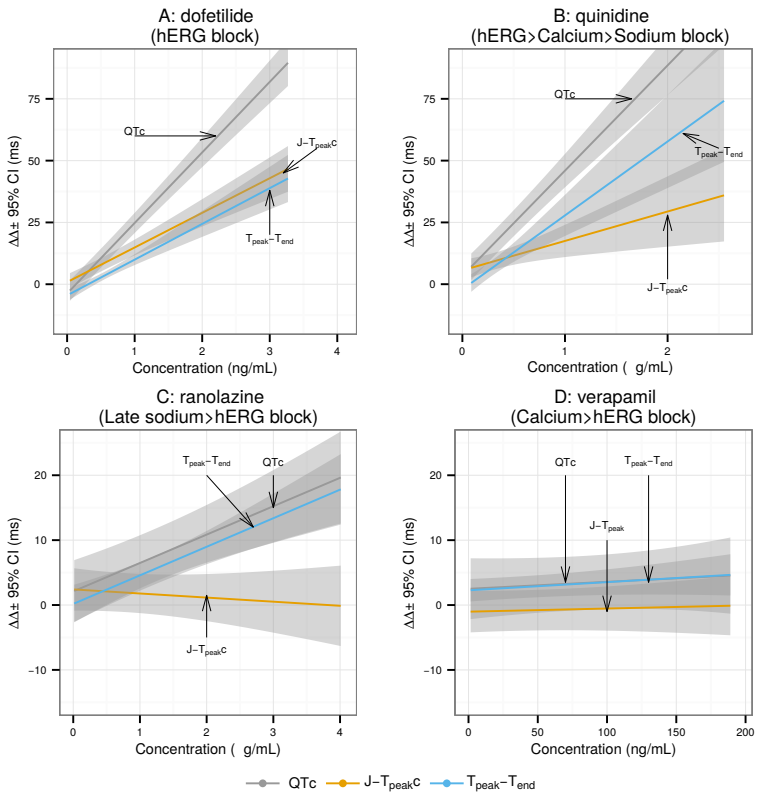

FIGURE 4.7 Concentration dependent analysis of QTc (gray), J-T_{peak}c (orange) and T_{peak}-T_{end} (blue) for a selective human ether-à-go-go-related gene (hERG) potassium channel blocker (**A**, dofetilide) and hERG potassium channel blockers that block either the L-type calcium channel (**B**, quinidine; **C**, verapamil) or the late sodium current (**D**, ranolazine). Reprinted from Johannesen, et al.,[122] with permission from John Wiley and Sons.

equal prolongation of both J-T_{peak}c and T_{peak}-T_{end} (J-T_{peak}c versus T_{peak}-T_{end} $P = 0.89$). These observations are consistent with those in Study III, that selective hERG potassium channel blockers prolong both J-T_{peak}c and T_{peak}-T_{end}.

In contrast, quinidine prolonged the QTc similarly to dofetilide (dofetilide: 79.3 ms (95% confidence interval (CI): 72.2 to

86.3 ms); quinidine: 78.1 ms (95% CI: 70.9 to 85.2 ms)), but quinidine preferentially prolonged the T_{peak}-T_{end} interval (J-T_{peak}c versus T_{peak}-T_{end}, $P = 0.025$). This observation is consistent with the electrophysiological mechanism of quinidine at the studied drug concentrations, where it is expected to block both the hERG potassium channel and the L-type calcium channel.

The QTc prolongation observed with ranolazine was entirely due to prolongation of the T_{peak}-T_{end} interval (J-T_{peak}c versus T_{peak}-T_{end} $P < 0.001$). Thus, supporting that the J-T_{peak}c interval is influenced not only by hERG potassium channel block but also inward current block. Specifically, that ranolazine's late sodium current block shortened J-T_{peak}c while ranolazine's hERG potassium channel block prolonged J-T_{peak}c resulting in no net change.

No changes in QTc, J-T_{peak}c and T_{peak}-T_{end} were observed with verapamil, likely due to a balance of hERG potassium channel and L-type calcium channel block. A significant increase in the PR interval (32.1 ms (95% CI: 26.7 to 37.4 ms; $P < 0.001$)) was observed after verapamil administration, suggesting the administered dose was high enough to elicit an electrophysiological response.

The findings of this study confirm the hypothesis that hERG potassium channel block prolongs both J-T_{peak}c and T_{peak}-T_{end}, and inward current block (L-type calcium or late sodium) preferentially shortens the J-T_{peak}c interval. These observations are similar to those made for electrolyte abnormalities[134] and genetic abnormalities.[135] Specifically, abnormalities in the hERG potassium channel (long QT type 2)[135] or hypokalemia result in a prolongation of both J-T_{peak}c and T_{peak}-T_{end} as well changes in the T-wave[136,137] similar to the observations with hERG potassium channel block in this study.

In contrast, hypocalcemia has been shown to prolong the QT interval without T-wave changes (via prolongation of the ST-segment),[134] and hypercalcemia shortens the action potential akin to L-type calcium channel block.[138] Similarly, abnormalities in the sodium channel associated with long QT syndrome type 3 prolong the QT interval without changes in the T-wave.[135]

Changes in the PR interval were only observed after administration of verapamil, as previously noted, but no changes were observed with quinidine which is expected based on the L-type calcium channel block associated with quinidine. This is likely due to the autonomic effects of quinidine.[139] A slight change in QRS was observed for ranolazine (2.7 ms (95% CI: 0.5 to 4.9 ms; $P = 0.018$) and verapamil (2.6 ms (95% CI: 0.4 to 4.8 ms; $P = 0.020$)). Finally, an increase in heart rate was observed for both quinidine (9.4 bpm (95% CI: 6.4 to 12.4 bpm; $P < 0.001$)) and verapamil (9.8 bpm (95% CI: 6.8 to 12.9 bpm; $P < 0.001$)). In addition, a slight increase in heart rate was observed for ranolazine (4.2 bpm (95% CI: 1.2 to 7.2 bpm; $P = 0.007$)).

Dofetilide and quinidine were both associated with substantial QTc interval prolongation and changes in the morphology of the T-wave.[140] This makes determination of the peak of the T-wave and end of the T-wave difficult. Two independent reviewers agreed, however, in 98.6 % of the ECGs (less than 5 ms, or presence of notch). This suggests, that despite the significant changes in morphology that the measurements of the ECG intervals were consistent. In addition, quinidine and verapamil increased the heart rate which confounded the use of a population-based correction, such as Fridericia.[18,129] However, it is unlikely that the differences between J-T_{peak}c and T_{peak}-T_{end} are due to the heart rate effects of quinidine. Moreover, a slight increase in QTc for verapamil may have been missed due to heart rate effects.

4.5 Late Sodium Current Block for Drug-Induced Long QT (Study V)

Following the first prospective clinical trial described in the previous section, a second prospective clinical trial was conducted to evaluate the ability of late sodium current block (mexiletine, lidocaine) or calcium channel block (diltiazem) to shorten QTc prolongation associated with hERG potassium channel block (dofetilide, moxifloxacin).

Similar to the results of the first prospective clinical trial, do-

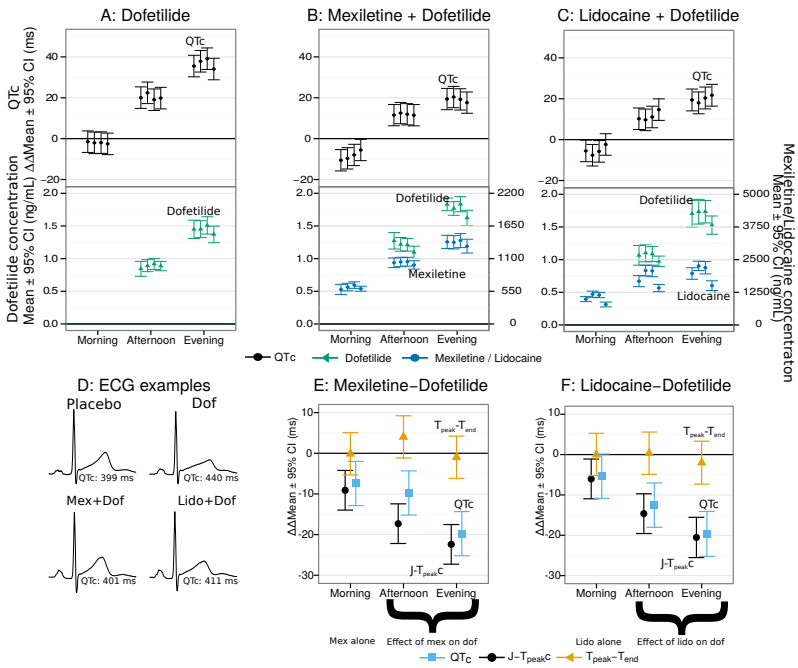

FIGURE 4.8 The top row of panels show the time-dependent effects on baseline, and placebo-adjusted changes ($\Delta\Delta$) in QTc (black) or concentration (green: dofetilide, blue: mexiletine/lidocaine) for the dofetilide alone arm (**A**), mexiletine + dofetilide arm (**B**) or lidocaine + dofetilide arm (**C**). Representative electrocardiograms (ECGs) examples from the same subjects are shown in panel (**D**). Panels **E** and **F** show the difference in the changes from baseline between the dofetilide alone arm and the mexiletine + dofetilide and lidocaine + dofetilide arms for (blue: QTc, black: J-T_{peak}c, orange: T_{peak}-T_{end}). Reprinted from Johannesen, et al.,[110] with permission from John Wiley and Sons.

fetilide dosing resulted in prolongation of the QTc interval in the evening (39.1 ms (95% CI: 33.9 to 44.4 ms; $P < 0.001$) Figure 4.8) that was split between prolongation of the J-T_{peak}c interval (24.0 ms (95% CI: 19.2 to 28.9 ms; $P < 0.001$)) and the T_{peak}-T_{end} interval (16.5 ms (95% CI: 11.7 to 21.3 ms; $P < 0.001$)).

The slightly increased J-T_{peak}c over T_{peak}-T_{end} is consistent with the relative effects between the two intervals for lower con-

centrations of dofetilide observed in the first prospective clinical trial (Figure 4.7). This suggests that there is not necessarily a perfect balance in the increases in the two intervals for weak selective hERG potassium channel blockers.

Interestingly, in the morning when a selective late sodium current blocker was administered by itself, the QTc interval was changed by (-10.6 ms (95% CI: -15.8 to -5.4 ms; $P < 0.001$)) with mexiletine and a change in the QTc interval of (-6.6 ms (95% CI: -15.8 to -2.4 ms; $P = 0.005$)) with lidocaine. The shortening of the QTc interval was due to shortening of the J-T_{peak}c interval (mexiletine: -9.3 ms (95% CI: -14.1 to -4.6 ms; $P < 0.001$); lidocaine: -6.2 ms (95% CI: -11.0 to -1.4 ms; $P = 0.012$)), and no changes in the T_{peak}-T_{end} interval (mexiletine: $P = 0.85$, lidocaine: $P = 0.97$).

This is consistent with the effects observed with ranolazine and other drugs thought to block the late sodium current as well as the effects of lidocaine and tocainide in long QT type 3 patients.[109]

In the afternoon and in the evening when the selective late sodium current blocker was coadministered with dofetilide the QTc was shortened in a dose-dependent fashion (Figure 4.8). The shortening of the QTc interval was due to by shortening of the J-T_{peak}c interval (mexiletine: -23.2 ms (95% CI: -28.0 to -18.3 ms; $P < 0.001$); lidocaine -20.5 ms (95% CI: -25.5 to -15.5 ms; $P < 0.001$)). Thus, resulting in a similar ECG signature as observed with ranolazine in the first prospective clinical trial.

An example of an ECG is shown in Figure 4.8 (panel D), where the QTc is prolonged by $\approx$40 ms after dofetilide, and the T-wave is flatter and asymmetric. After co-administration with mexiletine or lidocaine, the dofetilide-induced QTc prolongation of $\approx$40 ms is shortened by $\approx$40 ms for mexiletine or $\approx$30 ms for lidocaine. In addition, the mexiletine ECG shows signs of normalized T-wave morphology.

When evaluating the plasma concentrations from the study there was a slight increase in the plasma concentrations of dofetilide when co-administered with mexiletine (1.8 ± 0.3 vs 1.5 ± 0.3 ng/mL, $P < 0.001$) or lidocaine (1.8 ± 0.4 vs 1.5 ± 0.3, $P = 0.068$).

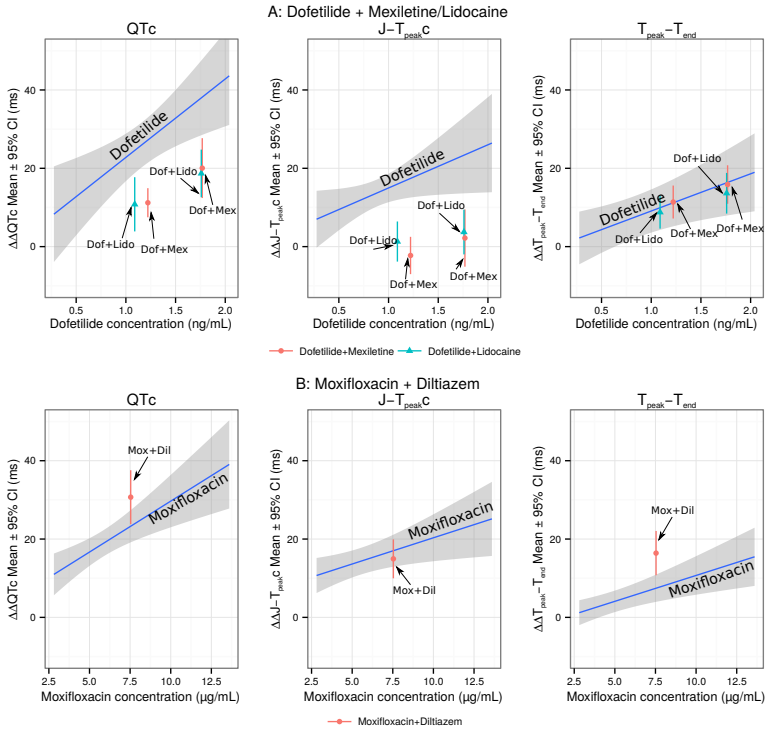

FIGURE 4.9 Differences between the changes in QTc (left panel), J-T_{peak}c (middle panel) or T_{peak}-T_{end} (right panel) for dofetilide alone (**A**) or moxifloxacin alone (**B**) represented by the line and shaded area compared to dofetilide + mexiletine (**A**, red), dofetilide + lidocaine (**A**, blue) or moxifloxacin + diltiazem (**B**, red). Reprinted from Johannesen, et al.,[110] with permission from John Wiley and Sons.

To account for these differences in the analysis of the effect of mexiletine or lidocaine, a concentration dependent analysis was also performed (Figure 4.9), which confirmed what was observed in the time-dependent analysis (Figure 4.8).

Similarly, the plasma concentrations were higher in the evening compared to the afternoon for moxifloxacin which is accounted for in the concentration dependent analysis (Figure 4.9). Unexpectedly, no shortening of the QTc interval or the J-T_{peak}c interval was

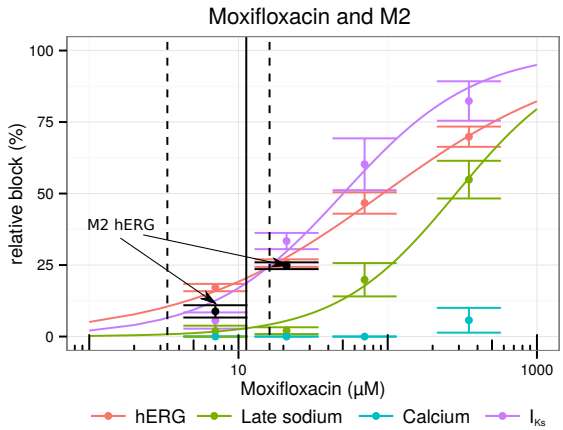

FIGURE 4.10 Effects of moxifloxacin on four different cardiac ion channel or currents (red: I_{Kr}, green: $I_{Na,L}$, blue: $I_{Ca,L}$ or purple: I_{Ks}). The black error bars represent human ether-à-go-go-related gene (hERG) effect of the moxifloxacin M2 metabolite. The error bars represent mean ± standard error and the lines are the result of fitting a sigmoidal line through the raw data points. Reprinted from Johannesen, et al.,[110] with permission from John Wiley and Sons.

observed when moxifloxacin was co-administered with diltiazem, a L-type calcium channel blocker. This could be due to a potential accumulation of the M2 metabolite of moxifloxacin which also blocks the hERG potassium channel (Figure 4.10). Additionally, moxifloxacin also blocks the slow potassium current (I_{Ks}), the effect of which could be enhanced by diltiazem-triggered autonomic changes and confound the analysis (Figure 4.10).

4.6 Drug-Induced Reverse Use Dependence and QT Variability (Study VI)

Using the data from the postural maneuvers in the first prospective clinical trial (Study IV), changes in the QT/RR relationship or QT interval dynamicity were evaluated.

This relationship is summarized for all subjects in Figure 4.11,

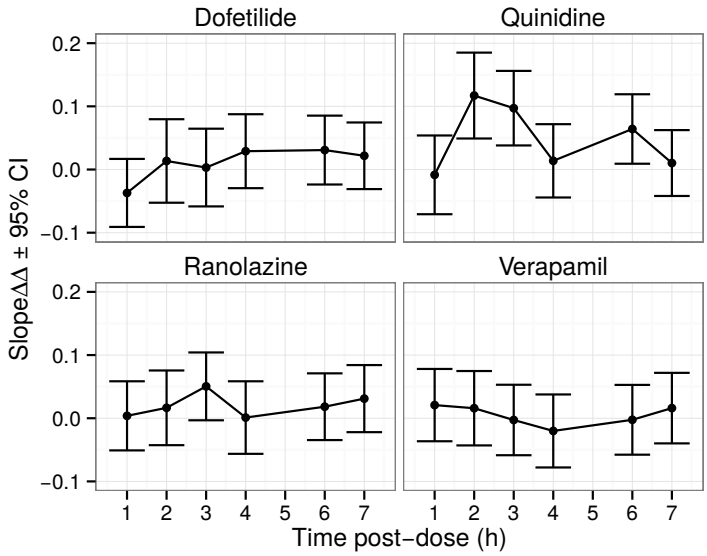

FIGURE 4.11 Time-course of the baseline, and placebo-adjusted changes ($\Delta\Delta$) for the slope of the QT/RR relationship (see section 3.7) for dofetilide, quinidine, ranolazine and verapamil.

which shows that quinidine was the only drug that increased the relationship and caused reverse use dependence ($P < 0.001$).

Reverse use dependence has previously been shown for dofetilide[124,141], which was not observed in this study ($P = 0.18$). Previous studies,[124,141] however, used a higher dose of dofetilide (250 and 750 µg twice daily compared to single dose of 500 µg), which may explain the discrepancy.

It is, however, unlikely that the lower dose is the only explanation as a study by Lande and colleagues showed that a single dose of 500 µg dofetilide[142] increased the slope of the QT/RR relationship. However, methodological differences exist between the study by Lande, et al., and the current study. Most notably, that the study by Lande et al. only included all QT measurements with stable heart rates around a 4 h window whereas in the current

study beat-by-beat measurements were performed.

Cappato and colleagues[143] only observed a change in the relationship for quinidine after complete autonomic blockade. By comparison, this study found a change without autonomic blockade (0.12 (95% CI: 0.05 to 0.19; $P < 0.001$)), likely a result of the longer RR intervals compared to those studied by Cappato et al. (RR: 400 to 600 ms versus RR: 600 to 1000 ms).

Interestingly, quinidine caused an increase in the slope of the relationship which was not observed with dofetilide (Figure 4.11), despite causing equal amounts of QTc prolongation (≈80 ms) at the studied doses. These differences could potentially be explained by a difference in the hERG potassium channel block mechanism,[144] difference in hERG potassium channel block potency,[140] or autonomic effects of quinidine.

The lack of changes in the QT/RR relationship for ranolazine ($P = 0.065$) is consistent with *in vitro* observations that block of the late sodium current could normalize the QT/RR relationship.[145] Similar observations were also made for mexiletine in a patient with Timothy syndrome (L-type calcium channel abnormalities).[146]

No changes in the QT/RR relationship were observed for verapamil ($P = 0.47$) in this study. However, a study of verapamil in patients with paroxysmal atrioventricular nodal reentrant tachycardia had a slight reduction of the slope of the QT/RR relationship.[147] This discordance could be due to methodological differences (ambulatory recordings vs Holter extraction for periods of postural maneuvers), dosing (effects following single dose compared to multiple dosing) or QT measurement methodology.

Interestingly, the study by Fauchier and colleagues with verapamil in patients observed a more profound change in the relationship between RR and Q-T_{peak}, which in the absence of QRS changes is similar to the J-T_{peak}/RR relationship. This is consistent with Study III and IV, suggesting greater influence of L-type calcium on the J-T_{peak} interval.

The drug-induced effects on different QT interval indices either accounting for heart rate (QT variability index (QTVI)) or not

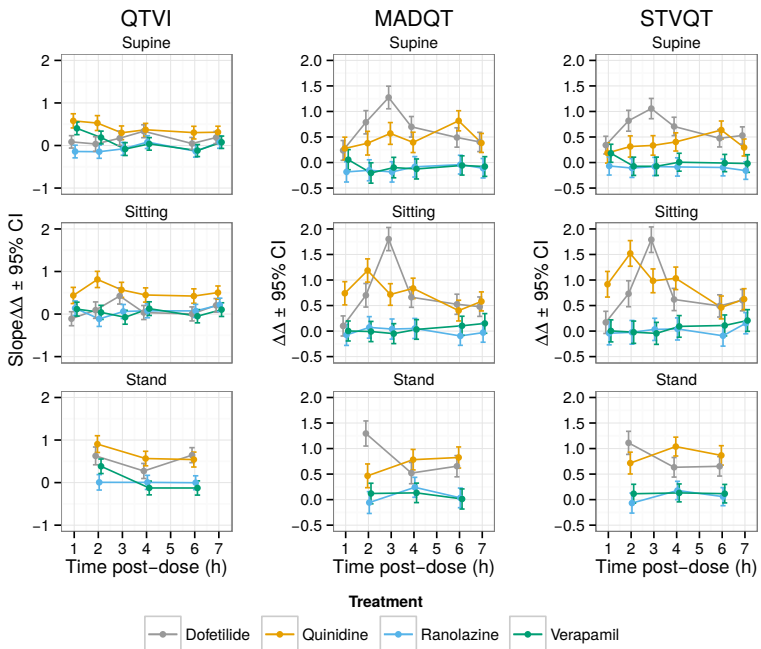

FIGURE 4.12 Time-course of the baseline, and placebo-adjusted changes ($\Delta\Delta$) for different methods of quantifying the variability in QT and RR (see section 3.7) for dofetilide (gray), quinidine (orange), ranolazine (blue) and verapamil (green) during supine rest (first row), unsupported sitting (middle row) or standing (bottom row).

accounting for rate (median absolute deviation of QT (MADqt), short term variability of QT (STVqt)) was also evaluated for each of the three postural maneuvers and the results are shown in Figure 4.12.

Figure 4.12 shows an increase in the different variability indices for dofetilide and quinidine ($P < 0.001$ for all) that tend to be maximized near the maximum drug concentrations (2 to 4 h). In addition, an increase was also observed for verapamil near the time of maximum verapamil concentration (1 h), which was largest for QTVI in the supine and standing positions ($P < 0.001$).

These observations are similar to those for intravenous cocaine[127] (I_{Kr} blocker with autonomic effects) and sertindole[148] (I_{Kr} blocker).

The changes are larger for standing and unsupported sitting compared to supine, supporting that evaluation of variability of the QT and RR intervals for different postural maneuvers should not be combined.[128]

Interestingly, the results of this study suggest that QT variability assessed using beat-by-beat measurements is more sensitive to drug-induced effects than the slope of the QT/RR relationship. This could be due to using beat-by-beat measurements of QT and RR. Because, while the beat-by-beat measurements contain information relevant to detection of changes in QT interval dynamicity, it likely adds noise to the assessment of rate dependent changes.[142]

However, measurement of the QT interval on a beat-by-beat basis, as needed for assessment of QT interval dynamicity, is not always practical and susceptible to measurement error. Alternatively, assessment of the QT/RR relationship could be performed using limited data from stable beats as proposed by Lande and colleagues.[142] However, the inclusion of only stable beats necessitates the use of a wider time window, which reduces the ability to link the effects with drug concentrations.

Finally, while both methodologies explored in Study VI detect changes in QT interval dynamicity associated with strong hERG potassium channel block, they do not appear to be as sensitive and specific to detect mild hERG potassium channel block and detect the presence of multichannel block.

CHAPTER 5

Conclusions

The studies in this thesis suggest that by evaluation of the J-T_{peak}c and T_{peak}-T_{end} intervals it is possible to differentiate between selective human ether-à-go-go-related gene (hERG) potassium channel blockers with or without additional inward current block (L-type calcium or late sodium). Detection of additional inward current block is relevant to differentiate between drugs with high risk for torsade de pointes (TdP) such as dofetilide (selective hERG potassium channel blocker) in contrast to low TdP risk such as ranolazine (multichannel blocker). In addition, work in this thesis suggests that the most important markers of electrocardiogram (ECG) data quality are reader variability and heart rate stability, a surrogate of study conduct.

The major conclusions for each study were:

I. The ability to detect QTc interval prolongation associated with moxifloxacin is mostly influenced by QT reader variability and stability of the heart rate.

II. ECG quality metrics focusing on intra- and intersubject differences of QTc are influenced by reader variability and QT

measurement methodology. No differences were observed between standard 12-lead ECGs and ECGs from continuous recordings, likely due to extraction methodology.

III. Drugs that only block the hERG potassium channel prolong the QTc interval as well as the J-T_{peak}c and T_{peak}-T_{end} interval. In contrast, drugs that block the L-type calcium channel and/or late sodium current in addition to the hERG potassium channel show preferential prolongation of T_{peak}-T_{end}, and potential shortening of the J-T_{peak}c interval.

IV. The observed ECG patterns of Study III were prospectively confirmed in a clinical trial of four drugs that are either selective hERG potassium channel blockers (dofetilide) or multichannel blockers: L-type calcium (quinidine and verapamil) or late sodium (ranolazine).

V. Co-administration of a selective late sodium current blocker shortens drug-induced QTc prolongation of dofetilide resulting from hERG potassium channel block. In addition, the QTc shortening is entirely driven by shortening of the J-T_{peak}c interval consistent with Studies III and IV.

VI. hERG potassium channel block causes reverse use dependence and instability of the QT interval, which can be detected by using postural maneuvers. In addition, markers of QT interval variability might be more sensitive than changes in the QT/RR relationship.

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USA, 2015

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Studies I–VI