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REVIEW

Update on the evaluation of a new drug for effects on cardiac repolarization in humans: issues in early drug development

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Following reports of death from cardiac arrhythmias with drugs like terfenadine and cisapride, the International Conference for Harmonization formulated a guidance (E14) document. This specifies that all new drugs must undergo a 'thorough QT/QTc' (TQT) study to detect drug-induced QT prolongation, a surrogate marker of ventricular tachycardia, especially torsades de pointes (TdPs). With better understanding of data from several completed TQT studies, regulatory requirements have undergone some changes since the E14 guidance was implemented in October 2005. This article reviews the implications of the E14 guidance and the changes in its interpretation including choice of baseline QT, demonstration of assay sensitivity, statistical analysis of the effect of new drug and positive control, and PK-PD modelling. Some issues like use of automated QT measurements remain unresolved. Pharmaceutical companies too are modifying Phase 1 studies to detect QTc liability early in order to save time and resources. After the E14 guidance, development of several drugs that prolong QTc by >5 ms is being abandoned by sponsors. However, all drugs that prolong the QT interval do not increase risk of TdP. Researchers in regulatory agencies, academia and industry are working to find better biomarkers of drug-induced TdP which could prevent many useful drugs from being prematurely abandoned. Drug-induced TdP is a rare occurrence. With fewer drugs that prolong QT interval reaching the licensing stage, knowing which of these drugs are torsadogenic is proving to be elusive. Thus, paradoxically, the effectiveness of the E14 guidance itself has made prospective validation of new biomarkers difficult.
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Introduction

The association of drug intake with prolongation of cardiac repolarization and potentially fatal cardiac tachyarrhythmias is known since 1964 when reports of quinidine-induced polymorphic ventricular tachycardia were published (Seltzer and Wray, 1964; Jenzer and Hagemeijer, 1976). Since then, several other cardiac and non-cardiac drugs have been implicated in causing prolongation of the QT interval (Table 1). The licenses to market some of these drugs have been withdrawn by regulatory authorities after they have been associated with sudden cardiac death and a rare form of ventricular tachycardia, torsades de pointes (TdPs) (Shah, 2002). To prevent such rare but serious adverse events, regulatory authorities now require innovator pharmaceutical companies to subject new drugs to stringent tests to detect potential cardiac toxicity.

The Committee for Proprietary Medicinal Products of the European Medicines Agency was the first to issue regulatory guidance on assessment of new drugs for drug-induced QT prolongation in 1997 (Committee for Proprietary Medicinal Products, 1997). This was followed by several years of debate between experts from regulatory agencies, cardiologists, pharmacologists and statisticians, ultimately resulting in the formulation of two important documents by International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): S7B guidance that addresses preclinical evaluation of cardiac

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Table 1 List of drugs with definite torsadogenic potential

Category	*Drug*	*Category*	*Drug*
Anti-arrhythmic agent	Amiodarone	GI stimulant/ Anti-emetic agent	Cisapride
	Disopyramide		Domperidone
	Dofetilide		Droperidol
	Ibutilide	Anti-infective agent	Clarithromycin
	Procainamide		Erythromycin
	Quinidine		Pentamidine
	Sotalol		Sparfloxacin
Anti-malarial agent	Chloroquine	Antihistamines	Astemizole
	Halofantrine		Terfenadine
	Quinine	Others	Levomethadyl
Anti-psychotic agent	Chlorpromazine		Methadone
	Haloperidol		Probucol
	Pimozide		Arsenic trioxide
	Thioridazine		Bepridil

Source: http://www.azcert.org/medical-pros/drug-lists/drug-lists.cfm

proarrhythmic risk (ICH Harmonized Tripartite Guideline S7B, 2005) and E14 guidance that deals with evaluation of proarrhythmic risk in humans (ICH Harmonized Tripartite Guideline E14, 2005). Following the experience with several 'thorough QT/QTc' (TQT) studies designed in accordance with the E14 guidance, regulatory requirements have undergone some changes, especially in the design, analysis and interpretation of study results (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). It is therefore imperative that pharmacologists involved in design and conduct of clinical studies on new drugs be aware of all the issues pertaining to this important step in new drug development. This article reviews the implications of the E14 regulatory guidance for new drugs under development for potential use in humans.

Physiology of cardiac repolarization and TdP

Most drugs that cause TdP prolong the action potential of cardiac myocytes by blocking potassium channels (Roden, 2004). The cardiac action potential has five phases namely Phase 0 to 4. Phase 4 refers to the membrane potential when the cell is not being stimulated (resting membrane potential). Electrical stimulation of the cell sets off a sequence of actions involving the influx and efflux of ions through specific channels on the cell membrane that together produce the action potential. The nomenclature of ion channels as per the Guide to Receptors and Channels are mentioned in parenthesis in this review (Alexander *et al.*, 2008). Phase 0 is the rapid depolarization phase which occurs because of the opening of fast Na^+ channels ($Na_v1.5$) with rapid influx of Na^+ into the cell. Phase 1 of the action potential represents the onset of cardiac repolarization, and occurs because of outward movement of K^+ and Cl^- ions along with inactivation of the fast Na^+ channels (Rudy, 2007). The resulting transient net outward current causes the small downward deflection of the membrane potential. Phase 2 or plateau phase of the cardiac action potential is sustained by a balance between inward movement of Ca^{++} through L-type calcium channels ($Ca_V1.2$) and outward movement of K^+ through the slow delayed rectifier potassium IKs channels ($K_V7.1$). This is followed by Phase 3 of the action potential, where the L-type calcium channels ($Ca_V1.2$) close, while the slow delayed rectifier IKs potassium channels ($K_V7.1$) are still open. This results in a net outward current which opens the rapid delayed rectifier potassium IKr ($K_v11.1$) channels and the inwardly rectifying potassium IK1 ($K_{ir}2.1$, K_{ir} 2.2, K_{ir} 2.3) channels. A rapid efflux of potassium occurs through these channels causing repolarization of the cell membrane. The IKr ($K_v11.1$) channels close when the resting membrane potential is restored, while IK1 ($K_{ir}2.1$, K_{ir} 2.2, K_{ir} 2.3) channels continue to conduct throughout Phase 4, contributing to resting membrane potential.

The sum of action potentials generated in the entire ventricle is represented on the surface electrocardiogram (ECG) tracing by the QRS complex, ST segment and the T wave (Figure 1). The QT interval, measured from the onset of the QRS complex to the end of the T wave, represents the duration of the ventricular action potentials. Prolongation of the QT interval because of abnormally prolonged ventricular repolarization, referred as long QT syndrome (LQTS), can occur as a consequence of altered function of some of these ion channels because of congenital defects (Antzelevitch, 2007). To date, 10 genotypes of congenital LQTS characterized by mutations in at least seven genes encoding for different ion channels or their structural anchoring proteins have been identified (Antzelevitch, 2007). Of these, genetic abnormality of the IKr ($K_v11.1$) channel results in the LQTS2, characterized by a prolongation of cardiac repolarization (QT interval >450 ms), decreased T wave amplitude and prolongation of terminal part of the QT interval with characteristic notching of the T wave (Vaglio *et al.*, 2008). These morphological changes are similar to that observed in acquired drug-induced LQTS (Ahmad and Dorian, 2007). As most drugs that prolong the QT interval do so by blocking the same IKr ($K_v11.1$) channel implicated in LQTS2, LQTS2 has been considered the genetic prototype of the drug-induced LQTS (Ahmad and Dorian, 2007).

The three dimensional structure of the IKr ($K_v11.1$) channel reveals a relatively wide mouth situated on the inner aspect of the cell membrane which permits many drugs to gain access to the channel and interfere with its function (Roden, 2008a). This wide portion of the channel is made up of α subunits which have an abundance of aromatic residues, and are encoded by the human ether-a-go-go-related gene (hERG/KCNH2 gene) located on long arm of chromosome 7 (Roden, 2008a). Most drugs that prolong the QT interval block the IKr ($K_v11.1$) channels by binding to the α subunits. However, some drugs like arsenic trioxide and pentamidine prolong the QT interval by reduced number of IKr ($K_v11.1$) channels on the myocardial cell membrane by causing abnormal trafficking of proteins which form the IKr ($K_v11.1$) channel (Eckhardt *et al.*, 2005; Kuryshev *et al.*, 2005). Although the delayed rectifier IKr ($K_v11.1$) channel is the major contributor to Phase 3 of the action potential (Antzelevitch, 2007), it is believed that IKs ($K_V7.1$) channels may compensate for reduced function of the IKr ($K_v11.1$) channels because of mutation or drugs and may form the hypothetical 'repolarization reserve' which prevents development of cardiac arrhythmias despite QT prolongation (Roden, 2008a).

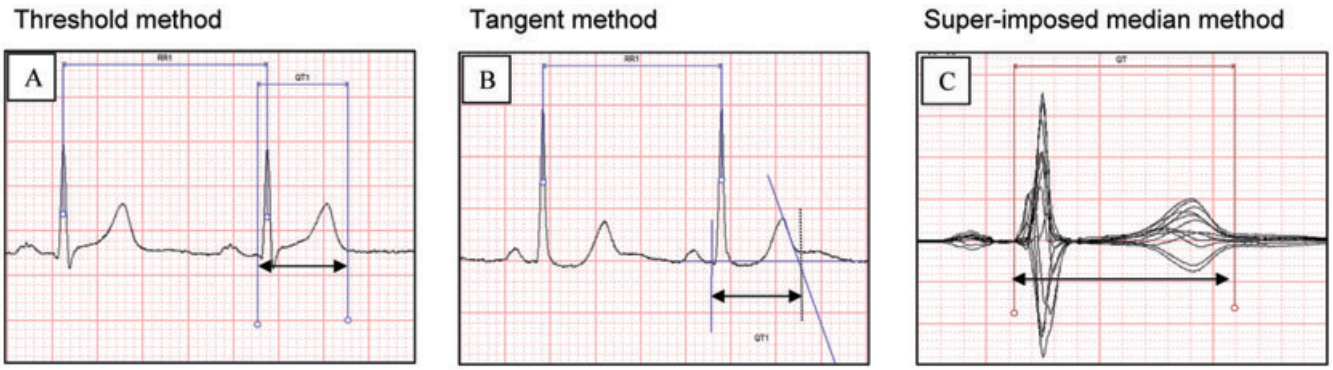

Figure 1 Methods of QT interval measurements. (A) In the threshold method, the end of the T wave is determined as a point where the descending limb of the T wave meets the isoelectric baseline. (B) In the tangent method, a line drawn through the peak of the T wave and the maximum slope point of the descending limb of the T wave is termed as tangent. The end of the T wave is determined by the point where this tangent intersects the isoelectric baseline (dotted vertical line in panel B). (C) In super-imposed median beat method, a computer-averaged representative beat is created for each of the 12 leads. The 12 representative beats (median beats) are then superimposed and the earliest onset of the Q wave and the latest T offset are used to mark the QT interval.

TdPs

In 1966, Francois Dessertenne described a form of polymorphic ventricular tachycardia, which he termed 'torsades de pointes' (Dessertenne, 1966). This irregular polymorphic ventricular tachycardia has a rate of 180–250 bpm and the morphology and vector of consecutive QRS complexes seems to revolve around the electrical axis (Ben-David and Zipes, 1993). Common manifestations of TdP are palpitation, symptoms of impaired cerebral circulation such as dizziness, syncope or seizures. In 20% of cases, TdP can subsequently degenerate into ventricular fibrillation with mortality of around 10% (Fung *et al.*, 2000). TdP is usually preceded by a prolongation of QTc interval and the association of QTc prolongation with TdP is so strong that ventricular tachyarrhythmias, even when meeting the morphologic criteria of TdP are not labelled as TdP unless preceded by QTc interval prolongation (Stratmann and Kennedy, 1987).

Prolonged cardiac repolarization is often followed by activation of an inward depolarization current, known as an early after-depolarization (EAD). This EAD may promote triggered activity resulting in the occurrence of a ventricular premature beat followed by a compensatory pause. The sinus beat that follows the pause has a greatly prolonged QT interval and an exaggerated U wave. TdP may then occur if another ventricular extrasystole coincidentally falls on the exaggerated U wave (Yap and Camm, 2003).

QT prolongation alone does not cause TdP (Antzelevitch, 2007). The ventricular myocardium is composed of at least three electrophysiologically and functionally distinct cell types: epicardial, mid-myocardial (M) and endocardial cells (Anyukhovsky *et al.*, 1996). If the action potential is prolonged by exactly the same extent in all layers of myocardial cells, the risk of developing TdP is relatively low, a phenomenon seen with the anti-arrhythmic drug amiodarone. Prolongation of repolarization by variable extent in these three layers is an important precondition for developing TdP (Anyukhovsky *et al.*, 1996). This transmural dispersion of repolarization is common with drugs that block the IKr (K_v11.1) channel like cisapride, because the number of IKr (K_v11.1) channels may vary from cell to cell. Another important factor predisposing to TdP in the presence of QT prolongation is a relatively slow heart rate (negative rate dependency) (Lokhandwala and Toal, 2003). Only a few individuals receiving drugs that block the IKr (K_v11.1) channel develop significant QT prolongation and potentially fatal TdP (Roden, 2004). Predisposing factors include interactions with concomitantly used drugs resulting in supra-therapeutic drug levels, female gender, advanced age, bradycardia, hypokalemia, hypomagnesaemia, ventricular hypertrophy, renal failure, central nervous system lesions, low-salt diet, congestive heart failure and nutritional disorders like prolonged starvation and anorexia nervosa (Antzelevitch, 2007). It is believed that some patients experiencing TdP may have genetic polymorphisms of genes coding for cardiac ion channels (Roden *et al.*, 2006).

The 'TQT study'

During new drug development, prior to the initiation of Phase 3, typically the drug will have been administered to about 200 to 300 subjects in about 10 Phase I and Phase II studies. These studies are designed to assess safety and tolerability of the drug in humans. Although ECGs are recorded in all these studies, they are not powered to detect a clinically significant QT prolongation. Not surprisingly, the risk of TdP with many previously marketed drugs was not detected by these studies. The ICH-E14 guidance therefore lays down the framework under which all new drugs with systemic bioavailability have to undergo a TQT study designed primarily to detect drug-induced QT prolongation (ICH Harmonized Tripartite Guideline E14, 2005). A TQT study may also be needed if a marketed drug is developed as a new formulation, in a different dosage, for new indications, or for a new target population.

Objectives

While drug-induced QTc prolongation of less than 5 ms is considered as not pro-arrhythmic, a prolongation of more

than 20 ms is considered a definite risk factor for TdP (Shah, 2002). The objective of the TQT study is to detect a mean placebo-adjusted QTc prolongation of ≥5 ms or a one-sided 95% upper confidence interval of ≥10 ms with an investigational drug (ICH Harmonized Tripartite Guideline E14, 2005).

Timing of a TQT study
Typically TQT studies are not performed until the safety of the new drug is demonstrated in a Phase 1 study. The pharmacokinetic profile of the drug along with that of its active metabolites, if any, needs to be thoroughly studied, along with an estimate of the proposed therapeutic dose. By this time Phase 2 studies are already in progress. The TQT study should be performed sufficiently early so as to make the 'go or no-go' decision for further development of the drug before starting expensive Phase 3 studies. Usually TQT studies are performed in parallel to Phase 2b studies, after proof-of-concept and the therapeutic dose have been established.

Subjects
The TQT study is performed in healthy normal volunteers in the age group of 18–45 years to avoid confounding because of underlying disease, co-morbidities or concomitant medications. Drugs like neuroleptics may not be tolerated by healthy volunteers. The TQT study with these drugs may then be performed in patients with the disease against which the drug is targeted. Cardiac safety of rotigotine was evaluated in patients with advanced Parkinson's disease rather than in healthy subjects (Malik *et al.*, 2008a) and combretastatin A4 phosphate was studied in patients with advanced carcinoma (Cooney *et al.*, 2004). However, designing a TQT study in patients, especially for oncology drugs, poses definite medical and ethical challenges (Rock *et al.*, 2009).

Physiological influences on QT interval
Physiological conditions that affect QT interval are important confounding factors that must be accounted for in the study design. The most important of these is the effect of heart rate on the QT interval, which is discussed separately later.

Gender. QT interval corrected for heart rate (QTc) is longer in females probably because androgens shorten the QT interval in males (Stramba-Badiale *et al.*, 1997). The density of potassium ion channels differs in males and females and QTc-prolonging drugs produce greater QT prolongation in women (Stramba-Badiale and Priori, 2005). As a result, women account for 70% of reported cases of TdP (Makkar *et al.*, 1993). The ICH-E14 states that all major clinical studies should have an adequate representation of female subjects and that in a positive TQT study, data from vulnerable subgroups like females are of particular interest. Of the 21 studies listed in Table 1, 40 to 50% of subjects were females in 17 studies; three study enrolled only males as these drugs were for use only in males and one study had only female subjects. A recent recommendation states that it is appropriate to perform the TQT study in male or female healthy volunteers (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008).

Diurnal variation. There is a small but significant circadian variation in the QTc interval ranging from 75 to 115 ms which increases during sleeping hours and is longest in the early morning (Molnar *et al.*, 1996).

Diet. QT interval increases after consumption of a meal (Nagy *et al.*, 1997). Consumption of protein supplements, caffeine, xanthine-containing products, chocolate, cocoa-containing drinks, alcoholic beverages or grapefruit juice also affect the QT interval and should be avoided by subjects in a TQT study (Zitron *et al.*, 2005).

Autonomic activity. Sympathetic and vagal activities affect both heart rate and the QT interval (Magnano *et al.*, 2002). Therefore, subjects are required to rest for 5 min in supine position before all ECG recording time-points in TQT studies. Moreover, blood is drawn only after the ECG has been recorded to avoid any confounding autonomic changes.

Study design
Thorough QT/QTc studies should have a double-blind, randomized design, and include placebo (negative control) and a positive control.

Negative control (placebo). Placebo control is included in the TQT study, to account for the spontaneous diurnal and day-to-day variation in QT interval. It is common to observe 'significant' QT/QTc interval prolongation because of spontaneous variability even with placebo treatment (Pratt *et al.*, 1996). Therefore, QT/QTc change from baseline (ΔQTc) observed with a new drug is adjusted for the time-matched change seen with placebo by appropriate statistical modelling, to obtain a 'placebo-adjusted change from baseline' in the QTc interval (ΔΔQTc).

Active control. A positive or active control is included to demonstrate assay sensitivity (Zhang, 2008). A study that does not detect the QT prolonging effect of the active control is unlikely to detect that of a new drug. The active control should produce a mean QTc prolongation of about 5 ms and the TQT study should be able to demonstrate that the lower bound of the one-sided 95% confidence interval is above 0 ms (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). Positive controls used in previous studies include drugs like moxifloxacin or ibutilide. Non-pharmacologic positive controls such as autonomic maneuvers like change in posture, isometric exercise or food ingestion have been evaluated but have not been found to be reliable (Sahashi *et al.*, 2006).

Moxifloxacin is the most commonly used positive control. It produces a peak QTc prolongation of approximately 10–14 ms after a single oral or intravenous dose of 400 mg (Démolis *et al.*, 2000). As the mean QTc prolongation with moxifloxacin is much greater than the 5 ms threshold specified by the ICH, regulatory authorities, especially the US Food and Drug Administration (FDA), now expect that the TQT study should be able to demonstrate that the lower 95% one sided confidence limit of the moxifloxacin effect should be >5 ms at one or more time-points to establish assay sensitivity (Zhang, 2008). The response to moxifloxacin has been so well

characterized that regulatory authorities also expect the study to demonstrate the characteristic time course of drug effect – with onset of action at 30 min, peak effect between 1 to 4 h and a gradual decline to baseline QTc value over the next 24 h (Stass *et al*., 1998). An alternative approach has been to show the effect of the positive control at a time-point other than the peak effect, at which the mean placebo-adjusted QTc prolongation is closer to 5 ms (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008).

Use of a double-blind positive control is not considered to be necessary if ECG analysis is performed in a blinded manner and all other study procedures are performed exactly as with the study drug or placebo (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). However, some regulatory agencies have been encouraging double-blinding of moxifloxacin by use of double dummy or by blindfolding subjects when the medication is administered (Strnadova, 2008). Over-encapsulation is another alternative, but alteration of pharmacokinetics may be an issue (Strnadova, 2008; Mason, 2009).

Concern has been voiced over the use of moxifloxacin (a fluoroquinolone antibiotic) as an active control in TQT studies for oncology drugs that are performed in cancer patients, as this may preclude their use to treat infections later in the disease. The 5-HT3 receptor antagonist, ondansetron, could be used instead. It has the additional advantage of causing only a transient QT prolongation, permitting its use on the pretreatment day without a significant carry-over effect (Rock *et al*., 2009).

Ibutilide, in a dose of 0.002 $mg \cdot kg^{-1}$ infused intravenously over 10 min was used as a positive control for assessing effect of tadalafil on QT interval (Beasley *et al*., 2005). It has the advantages of having a very short half-life, dose-dependent controlled QT prolongation, and the option of rapidly reversing its effect on QT interval by calcium infusion in case a volunteer develops cardiac arrhythmias. However, inconvenience of intravenous infusion and problems with blinding are concerns.

Dose of new drug. Two doses of the new drug are often studied – the therapeutic dose, and a supra-therapeutic dose which would simulate levels seen in hepatic or renal failure or with metabolic inhibitors – usually 2–6 times the therapeutic dose. The exact dose used depends on the maximum tolerated dose of the drug determined during early studies. In a recently published TQT studies, the supra-therapeutic dose of tolterodine (4 mg) was just two times the therapeutic dose because of dose-limiting anti-cholinergic side effects (Malhotra *et al*., 2007) while a fivefold higher dose was used for tadalafil (Beasley *et al*., 2005) and levetiracetam (Hulhoven *et al*., 2008) and a sixfold dose was used for levoceterizine and maraviroc (Hulhoven *et al*., 2007; Davis *et al*., 2008). If for some reason a supra-therapeutic dose cannot be used, use of metabolic inhibitors to achieve the supra-therapeutic concentrations of study drug is an alternative option. Ketoconazole, which inhibits cytochromes oxidase enzymes that metabolize many drugs, should be avoided as ketoconazole itself can prolong the QT interval (Mok *et al*., 2005).

The decision to use a single dose or multiple doses of the drug in the TQT study depends on the pharmacokinetic characteristics of the parent drug and its metabolites. Single dosing has the advantages of being cost-effective by reducing the duration of the study as well as allowing use of a convenient crossover design. Drugs with short half-lives and no active metabolites could be administered as a single dose. Drugs with a longer half-life, like brivaracetam which has an elimination half-life of 7–8 h, require multiple dosing (Rosillon *et al*., 2008). Multiple dosing should also be used for drugs with accumulation of active metabolites. In multiple dosing studies, it is not necessary to administer multiple doses of the positive control; a single dose of moxifloxacin preceded by multiple doses of placebo will suffice (Strnadova, 2008).

Crossover or parallel design. A crossover design has the advantage of a smaller sample size, but should be used only for drugs with a short half-life. A four-way crossover design is the most common design used in TQT studies (Hulhoven *et al*., 2007; Hulhoven *et al*., 2008). However, if a large washout period is required, the number of dropouts will increase; also a substantial spontaneous variation in the QTc interval may occur in the same individual over the study period of weeks. Studies using multiple doses of a drug with long half-life, like lamotrigine, usually have a parallel design (Dixon *et al*., 2008). This design requires a larger sample size, but shortens the study duration per subject.

Choice of baseline. As there is considerable physiological variation in QTc in the same individual, there are two possible ways of defining a baseline (Bonate and Russell, 1999). In the time-matched baseline approach, ECGs are recorded at the same time-points on the day prior to drug administration as those at which post-dose ECGs are planned. Subtracting each post-dose QTc value from the corresponding time-matched baseline QTc value permits adjustment for diurnal variation. Alternatively, QTc interval from ECGs recorded 0, 30 and 60 min prior to drug administration can be averaged to give a single baseline value with which all post-dose values are compared.

A recent study showed that both methods of baseline assessments were equally good in detecting moxifloxacin-induced QTc prolongation in crossover studies (Tyl *et al*., 2008). A single predose baseline has the advantage of reducing number of ECGs recorded, reduces stay in the Phase 1 unit and study cost. It is, however, acceptable only in crossover studies, where the same subject receives placebo as well as the study drug (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). In parallel studies, however, where different subjects receive placebo and the study drug, a single pre-dose baseline will not fully account for diurnal variation in QTc interval and may under estimate the true magnitude of the QTc prolongation (Bonate and Russell, 1999). Time-matched baseline ECGs recorded on pre-dose day are therefore preferred in parallel studies (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008).

ECG acquisition technology

The E14 guidance has specified the limit of regulatory concern to be 5 ms (ICH Harmonized Tripartite Guideline

E14, 2005), which is equal to one-eighth of a millimeter of a standard ECG tracing. Therefore, digital ECG recorders are used for acquiring the ECG in TQT studies. The digital ECG tracing is like a series of dots, rather than a line. The precision of measurement corresponds to the sampling frequency of the ECGs, or the time interval between consecutive dots in milliseconds. ECGs with a sampling frequency of 500 Hz (dots or samples are 2 ms apart) to 1000 Hz (dots or samples are 1 ms apart) are used. These ECGs can be displayed on computer screens with a substantial magnification (about 10 times) allowing precise identification of the Q-onset and T-offset. Sometimes, prints of ECGs are scanned and converted to digital ECGs (Moss *et al.*, 2001); although superior to measuring QT interval directly on the print, digitized ECGs may differ from the original digital ECGs (Hingorani *et al.*, 2008). Another benefit of digital ECGs is the ability of computer algorithms to perform automated QT interval measurements by mathematical analysis of the ECG waveforms.

Standard 10-s recordings of the 12-lead ECG are often used. However, this involves repeated connection and disconnection of the patient cable. A digital 12-lead Holter ECG device can be used instead, which records the digital ECG continuously over a 24-h period. Ten second snapshots of the ECG are then extracted from the recording at specified time-points. This has the advantage of permitting the selection of tracings which are free of artifacts, at a stable heart rate, and at time-points of interest that are identified retrospectively. Holter ECGs have been found to be as accurate as 12-lead digital ECGs in the assessment of drug-induced change in QT and RR intervals (Sarapa *et al.*, 2004). Until recently, sampling rate of Holter ECGs was considerably lower than that of 12-lead ECGs because of technological limitations. Newer Holter recorders with sampling rates similar to those of 12-lead ECG devices are now available.

Timing of ECGs

Electrocardiographs are recorded at several time-points decided by the pharmacokinetics of the study drug and its metabolites. It is important to record several ECGs close to the Tmax in order to study the peak effect on the QT/QTc interval. ECG recording should continue even after the Tmax to study any delayed effects of the drug or its metabolites on cardiac repolarization. ECGs are also recorded close to the Tmax of the positive control so as to demonstrate assay sensitivity. In order to retain double-blinding of the positive control, ECGs should be recorded at the same time-points in all treatment groups.

ECG measurement

Central laboratory. There is considerable variability in identifying the end of the T wave, as it gradually merges with the baseline. To maintain consistency in QT measurement, and to prevent reader bias, the E14 guidance states that electrocardiographs should be read in a central laboratory by a small group of trained readers blinded to both treatment and patient identity (ICH Harmonized Tripartite Guideline E14, 2005; ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). All ECGs from a single subject should be read by the same cardiologist, thereby avoiding the problem of inter-reader variability. Some ECGs should be reread to quantify inter- and intra-reader variability and these results are submitted to the regulatory authorities (ICH Harmonized Tripartite Guideline E14, 2005). High reader variability also contributes to large within-subject and between-subject variability in QT interval, which in turn increases the calculated sample size of a TQT study. Therefore, central ECG laboratories have stringent quality control processes to maintain high standards of ECG interpretation. Automated QT interval measurements by computer algorithms have been explored as an alternative to manual measurement, since machine-read ECGs would show greater consistency. However, manual QT intervals measurements or manual over-read of automated annotations are still preferred over automated algorithms (Patterson *et al.*, 2005; ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008).

QT measurement. Traditionally, lead II has been used for QT interval measurement because in this lead, the vectors of repolarization usually result in a long single wave rather than discrete T and U waves (Garson, 1993). The highest accuracy for arrhythmic events is predicted by the longest QT interval in the 12-lead ECG (Lund *et al.*, 2002) which is usually found in precordial leads V3 and V4 (Malik and Camm, 2001; Sadanaga *et al.*, 2006). Nonetheless, lead II is still commonly used for QT interval measurement in TQT studies. Difficulties in delineating the end of T wave are encountered when the T wave is flat, bifid, biphasic or overlapping on a U wave (Deshmukh *et al.*, 2008). Whether repolarization time includes the entire Q–TU complex is a subject of controversy (Ritsema van Eck *et al.*, 2005). When a U wave interrupts the T wave before it returns to baseline, the QT interval is measured as the nadir between T and U waves (Moss *et al.*, 2001). Where the end of the T wave is obscured by a U wave, QT interval measurement by the tangent method may be more reliable (Figure 1). An alternative to the single lead approach is to generate a computer-averaged representative beat for each of the 12 leads. These beats can then be superimposed on-screen and the earliest onset of the Q wave and the latest T offset identified (Figure 1) (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008).

QT Correction for heart rate

The QT interval shortens with increasing heart rate (Figure 2) (Viitasalo and Karjalainen, 1992). Therefore, heart rate corrected QT interval or QTc is used to study cardiac repolarization. QTc is the QT interval that would observed in the same ECG if the heart rate was 60 beats per min (i.e. RR interval is 1 s) (Lokhandwala and Toal, 2003). Many methods have been developed to calculate QTc. The Bazett's formula ($QTcB = QR/RR^{1/2}$) (Bazett, 1920) and Fridericia's formula ($QTcF = QT/RR^{1/3}$) (Fridericia, 1920) assume that the relationship between QT and RR is exponential, while the Framingham formula [$QTcL = QT + 0.154 \times (1 - RR)$] (Sagie *et al.*, 1992) assumes a linear relationship. As these formulae do not adequately correct for the effect of heart rate (Malik, 2001), other formulae too have been described (Figure 2). One such formula is a study-population-specific correction formula

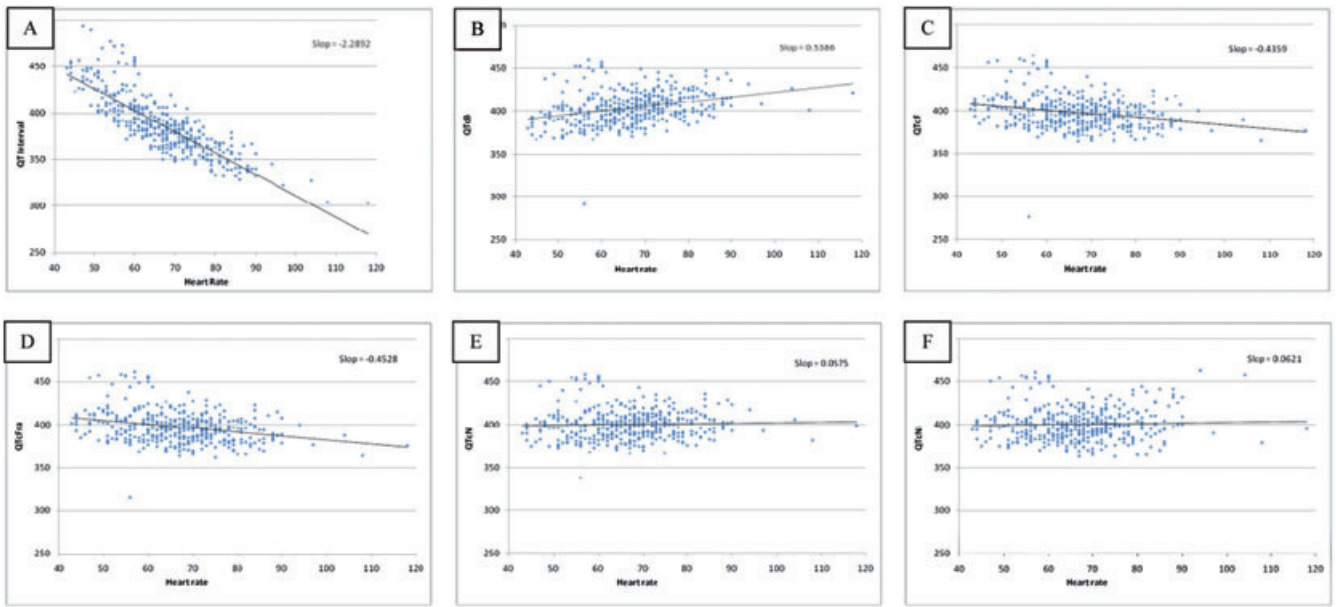

Figure 2 An example of the effect of heart rate on QT and QTc intervals from a study where multiple ECGs were recorded over 24 h in 23 normal healthy subjects. Note that as heart rate increases, there is progressive shortening of the uncorrected QT interval (A). An ideal correction formula would be one where the regression line of QTc versus heart rate is horizontal (has a slope of zero). The Bazett's formula (QTcB; B) overcorrects (positive slope) while the Fridericia's formula (QTcF; C) and Framingham Formula (QTcFra; D) undercorrect (negative slope). The study-population-specific formula (QTcN; E) and individual subject formula (QTcNI; F) have a slope closest to horizontal.

(Hnatkova and Malik, 1999), where several baseline ECGs are recorded in each subject in the study. ECGs from all subjects are pooled and the QT–RR relationship is determined. The formula thus obtained is used to correct for the heart rate effect in all study ECGs. Malik *et al*. have gone a step further and recommended that individual subject-specific correction formulae should be used in TQT studies (Malik *et al*., 2002). Twenty to fifty drug-free ECGs recorded in each subject over a wide range of heart rates are needed to obtain the individual correction formula (Batchvarov *et al*., 2002; Hollister and Montague, 2005). The ICH-E14 guidance emphasizes that although these correction methods are 'most suitable for the "thorough QT/QTc study" and early clinical studies . . .', '. . . QT interval data corrected using Bazett's and Fridericia's corrections should also be submitted in all applications' (ICH Harmonized Tripartite Guideline E14, 2005).

Hysteresis. The QT interval gradually adapts to a change in heart rate over a period of 2–3 min (Lau *et al*., 1988). Mathematical modelling of this hysteresis is complex and is still a subject of research (Malik *et al*., 2008b). To avoid issues in correcting for hysteresis, subjects should rest in supine position for 5 min so that the heart rate remains stable at each ECG time-point.

Statistical issues

Test

The primary objective of a TQT study is to test whether or not the new drug, when compared with placebo, causes a QTc prolongation of ≥10 ms. A negative study would be one where the 95% one-sided upper confidence bound of the placebo-adjusted QTc prolongation is <10 ms at all time-points at which ECGs are recorded (ICH Harmonized Tripartite Guideline E14, 2005). This definition is chosen to provide reasonable assurance that the mean effect of the study drug is not greater than around 5 ms. While statistical testing for this effect requires multiple comparisons, adjustment for multiplicity is not performed in this analysis as the test relies on the intersection-union test.

When analysing the effect of the positive control, the objective is to prove its 'superiority' when compared with placebo. To establish assay sensitivity and reject the null hypothesis, at least one lower bound of 95% one sided CI with moxifloxacin should be above 5 ms. If each time-point-specific comparison between moxifloxacin and placebo is tested separately, it is called a local test of significance (Zhang, 2008). As local tests will be performed at many time-points, loss of alpha because of multiplicity of tests needs to be adjusted for. Using a more conservative value of alpha would increase the likelihood of a type II error. Hence, one can anticipate *a priori* a few time-points at which maximum QTc prolongation occurs with the positive control (Zhang, 2008). After a single dose of 400 mg of moxifloxacin, the maximum QTc effect of around 10 ms occurs 1 and 4 h after oral administration (Démolis *et al*., 2000). Selection of three time-points falling within this time period would minimize the extent of correction of alpha. Another approach involves the use of a so-called global test instead of performing multiple local tests with alpha adjustment (Tsong *et al*., 2008; Zhang, 2008).

Sample size

The sample size of a study depends on the estimated difference between the means and the variability between observa-

tions. The difference between the means has been defined by the E14 guidance to be 5 ms. Therefore, efforts are made to decrease all possible sources of variability in QT interval measurement in order to reduce sample size; ECGs are recorded only after adequate rest, at precise time-points, using high-quality digital ECGs and are read in a central laboratory by a small group of trained individuals (Dmitrienko *et al.*, 2008). Precise on-screen techniques also increase accuracy of QT interval measurement (Dmitrienko *et al.*, 2008). QT values may vary by as much as 25 ms over 10 consecutive complexes (Malik and Camm, 2001). Moreover, there is considerable minute-to-minute variation in the QT interval. Hence, recording three to five replicate ECGs at each time-point and measuring QT intervals in three or more complexes in each ECG helps decrease variability further (Morganroth, 2007).

For a parallel study, the between-subject variability (usually 9–14 ms) is important, while within-subject variability (usually 5–10 ms) is important in crossover studies (Julious, 2004). As a result, sample size in crossover studies can be as low as 40% of that in parallel studies (Julious, 2004). Therefore, wherever pharmacokinetics of the new drug permits, a crossover design is preferred for TQT studies; only eight of the 21 TQT studies published to date had a parallel design (Table 2). The sample size selected should also be adequate to demonstrate assay sensitivity with the positive control as the value of alpha to be used for analysis of positive control data may require multiplicity adjustment (Zhang, 2008).

Outliers

Besides looking for mean QTc prolongation, QT/QTc prolongation in individual subjects beyond specified cut-off limits is also important. Previous studies have shown that the 40-year risk of cardiac arrest or sudden death is <20% in individuals with a QTc <450 ms, and 80% in persons with QTc ≥500 ms (Morganroth *et al.*, 1991; Garson, 1993; Priori *et al.*, 2003). There is also general agreement that QTc prolongation by >30 ms should raise concerns, with greater concern when the QTc exceeds >60 ms (Fenichel *et al.*, 2004). The ICH E-14 guidance specifies that all subjects with QTc interval between 450 to 479 ms, 480 to 499 ms and those with QTc interval ≥500 ms must be reported. Similarly, individuals with QTc prolongation of 30–59 ms and ≥60 ms must be reported as outliers (ICH Harmonized Tripartite Guideline E14, 2005).

Concentration–QTc analysis

Regulatory review of a TQT study is not complete without an assessment of concentration–QTc relationship. An evaluation of the concentration–QT relationship takes into account individual responses instead of averaging the QT response at each time-point (Bloomfield and Krishna, 2008). In addition to this, concentration–QTc analysis helps in predicting QT prolongation at doses of study drug other than those used in TQT study. It is also useful in situations where a new drug may show marginal QTc prolongation at a single time-point as a type I error; concentration–QTc modelling can confirm that the drug is devoid of a positive QTc effect. Concentration–QTc analysis can also quantify incremental risk in special populations; one such analysis showed that the QTc prolonging effect of ranolazine in patients with hepatic insufficiency is about thrice that seen in healthy normal individuals (Garnett *et al.*, 2008). The drug was therefore considered to be contraindicated in liver disease. Concentration–QTc modelling can also help to differentiate whether excessive QT prolongation observed in a few subjects is due to high drug concentration or due to differential susceptibility in these subjects (Rock *et al.*, 2009).

Usually a linear mixed effect model is used to assess concentration–QTc relationship. Most studies have fitted a simple linear model to the concentration–QT data, giving a line of best fit, with 90% two sided upper and lower confidence ranges. The predicted value of ΔΔQTc prolongation at mean Cmax is obtained from the regression; if the ΔΔQTc exceeds 5 ms or the upper 90% confidence exceeds 10 ms, then the drug is considered to cause significant QTc prolongation (Garnett *et al.*, 2008). Assuming a linear relationship between plasma concentration of moxifloxacin and ΔΔQTc interval from baseline, moxifloxacin produces a 3.9-ms increase in QTc for every 1000 ng·mL^{-1} increase in plasma concentration (Stass *et al.*, 1998). Similarly, ranolazine produces 2.6-ms prolongation of QTc per mg·mL^{-1} increase in plasma concentration (Garnett *et al.*, 2008). Concentration–QT analysis has also been used to identify the QT prolonging effect of an chemotherapeutic drug in cancer patients using QT and concentration data from Phase 1 studies because a formal TQT study was not feasible in these subjects (Garnett *et al.*, 2008).

Data submission to FDA warehouse

In addition to submission of the clinical study report of the TQT study and individual subject data, the FDA also required submission of the analysed digital ECGs with fiduciary markers indicating the PR, RR, QT intervals and QRS duration. The annotated ECG data file is submitted in an FDA-specified HL7-compatible XML file format (Zareba, 2002). The FDA then uses computer software to review selected ECGs for accuracy. This warehouse of digital ECGs stored in a uniform format also permits scientific research on pooled data in an effort to find better biomarkers of cardiac proarrhythmia.

Impact of positive TQT studies

QTc prolongation demonstrated in a thorough QT study does not necessarily mean that the drug will not get regulatory approval. QTc prolongation and the risk of TdP will be judged in the context of potential benefits to determine licensing and appropriate labelling (Shah, 2002). However, these drugs will require more stringent ECG monitoring during Phase II and Phase III studies. A recent regulatory recommendation also mentions that manual ECG analysis or manual over-read of automated annotations will be required in late phase clinical trials for drugs with a positive TQT study, while less expensive automated analysis of ECGs may be adequate for drugs that do not prolong the QT interval (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2008). Pharmaceutical companies often make a conscious decision to stop further

Table 2 Summary of thorough QT/QTc studies published until April 2009

Author	*Drug groups*	*Study Design*	*T1/2 (h)*	*Total subjects*	*ΔΔQTc at supra-therapeutic dose*	
					Mean	*UCL*
Barriere *et al.*, 2004	Placebo, Telavancin 7.5 mg·kg^{-1} or 15 mg·kg^{-1}, Moxifloxacin 400 mg	Parallel	9	160	4.5	NA
Morganroth *et al.*, 2004	Vardenafil (10 and 80 mg), Sildenafil (50 and 400 mg), Moxifloxacin 400 mg, Placebo	Crossover	V = 4–5 S = 3–4	58	V = 10 S = 9	V = 11 S = 11
Beasley *et al.*, 2005	Tadalafil 100 mg, ibutilide 0.002 mg·kg^{-1} IV, Placebo	Crossover	17.5	99	3.5	5.5
Harris *et al.*, 2005	Buprenorphine transdermal system 10 mg b.i.d., 20 mg b.i.d., placebo, oral moxifloxacin 400 mg	Parallel	20–70	132	5.9	8.4
Serra *et al.*, 2005	Darifenacin 15 mg or 75 mg, Placebo or Moxifloxacin 400 mg OD × 7 days	Parallel	13–19	188	–1.2	2.2
Hulhoven *et al.*, 2007	Placebo, Moxifloxacin 400 mg, Levocetirizine 5 mg, 30 mg	Crossover	6–10	52	1.1	3.9
Malhotra *et al.*, 2007	Placebo, Moxifloxacin 400 mg, Tolterodine 2 mg, 4 mg	Crossover	1.9–3.7	48	5.6	9.8
Zhang *et al.*, 2007	Placebo/Duloxetine 60–200 mg BD, Moxifloxacon 400 mg single dose	Crossover with ascending doses of Duloxetine	12.5	117	–2.7	-0.6
Ayalasomayajula *et al.*, 2008	Aliskiren 300 mg or 1200 mg, Moxifloxacin 400 mg, Placebo OD × 7 days	Parallel	24	298	5.2	8.1
Davis *et al.*, 2008	Placebo, Moxifloxacin 400 mg, Maraviroc 100, 300, 900 mg	Crossover	14–18	61	3.6	5.8
Dixon *et al.*, 2008	Lamotrigine 25 mg to 200 mg b.i.d. per day, Placebo/Moxifloxacin 400	Parallel with ascending doses of Lamotrigine	24–34	152	–2.81	0.2
Hulhoven *et al.*, 2008	Placebo, Moxifloxacin 400 mg, Levetiracetam 1000 mg, 5000 mg	Crossover	6–8	52	4.1	8.1
Iwamoto *et al.*, 2008	Placebo/Moxifloxacin 400 mg, Raltegravir 1600 mg single oral dose	Crossover	9	31	–0.4	3.1
Kubitza *et al.*, 2008	Rivaroxaban 15 mg, 45 mg, Moxifloxacin 400 mg, Placebo	Crossover	7–11	54	–0.91	1.52
Malik *et al.*, 2008a	Placebo/Moxifloxacin 400 mg, Rotigotine 4–24 mg·day^{-1}	Parallel with ascending doses of Rotigotine	5–7	130	2.84	4.76
Peeters *et al.*, 2008	Etravirine 200 mg BD, Etravirine 400 mg OD, Moxifloxacin 400 mg, Placebo for 8 days	Crossover	41	41	–0.2	2.1
Rosillon *et al.*, 2008	Brivaracetam 75 mg b.i.d., brivaracetam 400 mg b.i.d. or placebo b.i.d. for 6.5 days, or single-dose moxifloxacin 400 mg	Parallel	7–8	184	–1.1	3
Sarapa *et al.*, 2008	Placebo/Moxifloxacin 400 mg, Ritonavir 100 mg	Crossover	3–5	65	0.16	1.69
Damle *et al.*, 2009	Nelfinavir 1250 mg b.i.d. on days 1–4, Nelfinavir 1250 mg b.i.d. on days 1–3 plus 3125 mg on day 4, Placebo, and Moxifloxacin 400 mg qd on days 1–4	Crossover	3.5–5	68	3	6
Modi *et al.*, 2009	Dapoxetine 60 mg or 120 mg or 240 mg, Moxifloxacin 400 mg, Placebo	Crossover	16–20	96	–1.9	3.2
Sechaud *et al.*, 2009	Deferasirox 20 mg·kg^{-1}, Deferasirox 40 mg·kg^{-1}, Placebo, Moxifloxacin 400 mg	Parallel	8–16	182	–0.5	1.2

All studies were performed in normal healthy subjects, except the study with rotigotine, which was performed in patients with Parkinson's disease.
NA, not available; UCL, upper confidence limit.

drug development when a candidate drug is found to produce QT/QTc prolongation beyond the acceptable limit. Some drugs like ranolazine, with features that make them unique despite a significant QT effect, are developed further and receive regulatory approval with the addition of a warning and precautionary statement in the package insert (FDA cardiovascular and renal drugs advisory committee review of ranolazine, 2003). Some drugs like moxifloxacin and alfuzosin prolong the QTc interval beyond the limit of regulatory concern, but these drugs have already been in clinical use for several years and after millions of doses have been prescribed, the incidence of TdP with these drugs has been rare, and hence these drugs are still marketed, albeit with a warning in the drug label [FDA review for Uroxatral (alfuzosin HCL tablets), 2003].

QT assessment in Phase I studies

After the implementation of the ICH-E14 guidance in 2005, development of several promising drugs has been abandoned because of significant QT prolongation. Earlier detection of a QT liability of drugs could potentially save time and resources spent on development of potentially unsafe compound. Recording and analysis of ECGs with the stringent quality control as in the TQT study could also be performed during 'First time in humans' single ascending dose and multiple ascending dose studies. Although cardiac safety alone is not the primary objective of these early human studies, recording ECGs at multiple time-points that include the predicted Tmax will permit detailed QT assessment. Designed to find the highest tolerable dose of the drug, these early studies provide an opportunity to study the effect on QT interval at doses higher than those that may eventually be studied in a formal TQT study. While these studies are underpowered to detect a mean QTc prolongation of 5 ms, they provide adequate data for concentration–QTc modelling. If a QT prolonging effect is found, it may be prudent to conduct a TQT study earlier in the drug development process, if not stop further development altogether. Conversely, the cost of collecting high-quality ECG data at an early stage may not be worthwhile for a class of drugs with high failure rates because of noncardiac toxicity (Rock *et al*., 2009).

Concordance with preclinical studies

Regulatory requirements for the preclinical assessment of a drug for its effect on ventricular repolarization include an *in vitro* electrophysiology-based IKr (K_v11.1) assay (also known as the hERG assay) to study the effect of the drug on ionic current through the channel, and an *in vivo* QT assay to study the effects of single ascending doses on the QT interval in intact laboratory animals (ICH Harmonized Tripartite Guideline S7B, 2005). The ratio of the 50% inhibitory concentration (IC50) for the hERG assay or serum concentration that produces a 10% prolongation of the QT interval in *in vivo* studies to free plasma concentration of drug required for clinical efficacy is an index of pro-arrhythmic potential. The ICH-S7B guideline does not specify thresholds to stratify pro-arrhythmic risk. In the European Union, a ratio of <30 is considered to be indicative of a positive QT effect (Pollard *et al*., 2008; Shah, 2008), values between 31 to 100 are borderline, while a value >100 is considered safe; other authors define a safe limit as a ratio >300 (Shah, 2008; Vik *et al*., 2008). In general, the larger the safety margin, lesser the likelihood that the drug will fail in later stages (Pollard *et al*., 2008). There is reasonable correlation between preclinical assay data and the results of the TQT study in humans (Shah, 2008). Pollard *et al*. state that out of 17 new compounds subjected to thorough QT studies in man (10 positive and seven negative), results of preclinical assays correctly predicted outcomes in all except one case, where the preclinical data were negative, but human data were positive (Pollard *et al*., 2008). The US FDA has reported that eight out of 10 clinically positive drugs were identified as positives in preclinical assays (Pollard *et al*., 2008).

Regulatory approach to preclinical assay data is mixed: European and Japanese regulatory authorities have more flexible approach on the need for a thorough QT evaluation when preclinical assays are negative. On the other hand, the US FDA and Health Canada require TQT studies for all drugs, regardless of the preclinical assessment (Health Canada, 2006; Shah, 2008; Rock *et al*., 2009). Whenever the results of preclinical assays differ from those of a TQT studies in humans, ICH-S7B recommends additional follow-up studies to evaluate the reason for the discrepancy. Discrepancies may result from poor reproducibility of assay techniques (Yao *et al*., 2008), inadequate power of preclinical studies to detect a modest drug effect, inadequate blinding (Rock *et al*., 2009), interspecies differences, and effects on heart rate and autonomic nervous system in humans (Table 3). Lastly, *in vivo* preclinical studies may miss a QT effect as 10% prolongation of the QTc interval is commonly used as a cut-off in animals while the ICH-E14 guidance defines 2.5% prolongation (10 ms QTc prolongation assuming baseline QTc to be 400 ms) as the threshold for pro-arrhythmic risk (Pollard *et al*., 2008). Nevertheless, a positive TQT study would require extended ECG monitoring in subsequent studies although preclinical data show that the drug is safe. Conversely, even though the TQT study is negative, if preclinical data are strongly positive, regulators may still insist on extensive ECG monitoring in later phase studies (Shah, 2008).

QT assessment in oncology and protein therapeutics

Assessment of QT effects of anti-cancer drugs is challenging as it may not always be possible to design studies in accordance with the E14 guidance. Consequently, many deviations have been proposed, although there is no consensus on the acceptability of these and it is advisable to discuss these with regulatory authorities before starting a study. Drug toxicity makes it unethical to do these studies in healthy volunteers. Dose limiting toxicity may preclude use of a supra-therapeutic dose. Up to 15% of patients with advanced cancer may have QTc values >450 ms at baseline; hence, baseline QTc values >470 ms are more appropriate for exclusion of subjects from

dedicated QT studies rather than >450 ms as recommended for healthy volunteers (Sarapa and Britto, 2008; Rock *et al*., 2009). Extended drug-free washout periods may be unethical for patients with advanced cancer. The design of a dedicated QT study with sunitinib in patients with advanced solid tumours addressed many of these concerns (Sarapa and Britto, 2008; Sutent, 2008, Product Monograph). Baseline ECGs were recorded on day 1, a single dose of moxifloxacin was administered on day 2, placebo on day 3, followed by sunitinib from days 4 to 10 (Sutent, 2008, Product Monograph). Where it is not feasible to design definitive QT studies, data from Phase Ia, Ib and Phase II studies may be used to estimate risk by concentration–QTc modelling. Analysis of outliers and arrhythmic events in Phase III is also important (Rock *et al*., 2009). Finally, an upper confidence limit of mean baseline-adjusted QTc prolongation which exceeds 20 ms may be considered clinically significant for anticancer drugs (rather than 10 ms for other drugs), while a value between 10 and 20 ms may indicate moderate risk (Sarapa and Britto, 2008). Where clinical benefit is substantial, considerable QT prolongation may be acceptable. Therefore, the decision about further drug development must take into account the fact that the risk of death because of advanced cancer may exceed the risk of drug-induced TdP even if the drug causes some QT prolongation (Rock *et al*., 2009).

Whether large molecules like 'biologics' should be studied for their effect on cardiac repolarization is being hotly debated. Most small molecule drugs that prolong the QT interval do so by entering the myocardial cell and binding to the IKr channel (K_v11.1) on its intracellular aspect (Vargas *et al*., 2008). Large proteins like monoclonal antibodies cannot enter cells and bind only to specific cells against which they are targeted. Thus their ability to alter cardiac repolarization is being questioned. There is substantial preclinical data that show that monoclonal antibodies have no effect on hERG channel activity in the *in vitro* hERG assay (Vargas *et al*., 2008). Based on these data some researchers argue that a thorough QT study may not be required for these agents. However, large protein fractions from venoms of some snakes, scorpions and anemone can alter IKr function by binding to a 'toxin-binding site' on the outer aspect of this ion channel (Stockbridge, 2008). Anti-Ro/SSA autoantibodies too may prolong QT interval by possibly altering trafficking of the ion channel (Lazzerini *et al*., 2004). More recently, a depsipeptide (FK-228) with molecular weight of 540 Da, has been reported to cause significant QT prolongation probably by blocking the IKr channel after entering myocardial cells (Piekarz *et al*., 2006; Vargas *et al*., 2008). Therefore, regulatory authorities want to see more clinical data before they make any exception to the approval process for these molecules. ECGs may have to be recorded for a longer period than in studies with small molecule drugs, to look for effects on ion channel lifecycle, if any.

Post marketing surveillance

Apart from these specific measures to detect the propensity of new molecules to cause QT prolongation prior to approval, extensive post-marketing surveillance data and spontaneous reporting of adverse events by patients and physicians are expected by regulators. However, this system of spontaneous reporting has been found to under-report the true incidence of drug-induced TdP by a factor of as much as 10 (Wysowski and Bacsanyi, 1996; Darpö, 2001).

All drugs that prolong QT may not cause TdP

Despite millions of prescriptions, alfuzosin, which prolongs the QT interval, has rarely been associated with TdP (Borer and Armstrong, 2003). Amiodarone too prolongs QT duration but does not increase risk of TdP (Antzelevitch, 2005). Similarly, the anti-anginal drug ranolazine increases QT duration but simultaneously decreases heterogeneity of repolarization. Both amiodarone and ranolazine are mild calcium and sodium channel blockers; this property might compensate for the propensity to proarrhythmias because of QT prolongation (Antzelevitch *et al*., 2004). Verapamil and sodium pentobarbital are other useful agents that prolongs the QT interval but do not increase the risk of TdP (Shimizu *et al*., 1999; Vik *et al*., 2008).

Table 3 Reasons for lack of correlation between data from preclinical studies and results of thorough QT/QTc studies in humans. Preclinical studies include an *in vitro* hERG assay that quantitates the effect of the drug on ionic current through the IKr channel, and an *in vivo* QT assay where the effects of ascending doses of the drug on the QT interval in intact laboratory animals are studied

In vitro *hERG assay*	In vivo *assay*	*Thorough QT/QTc study in humans*	*Reasons for the discrepancy*
Negative	Positive	Positive	Active metabolites (but not drug) cause QT prolongation Abnormal trafficking of ion channel proteins Limited water solubility of drug in *in vitro* assay Drug causes QT prolongation due to effect on autonomic nervous system or sex hormones QT prolongation caused by blockade or modulation of other ion channels., e.g., IKs channel (Kv7.1)
Positive	Negative	Negative	Drug acts on multiple cardiac channels which compensate for the effect on IKr channel (K_v11.1)
Positive	Negative	Positive	Low power of *in vivo* assay to detect QT prolongation Threshold of *in vivo* QT prolongation (10% prolongation) is more than that used in TQT studies (approx. 2.5% prolongation)
Negative	Negative	Positive	Active metabolites in human different from those in animals Genetic predisposition in human subjects Autonomic nervous system effects of drug differ in humans
Positive	Positive	Negative	High exposures used in preclinical assays not achieved in TQT study

Thus, not all drugs that prolong the QTc interval to a similar degree carry the same risk of inducing TdP (Roden, 2004), although the risk tends to be higher with extreme QT prolongation (Antzelevitch, 2007). This has prompted extensive research to find a better biomarker for drug-induced TdP. Most biomarkers are identified by studying ECGs from patients with congenital LQTS2, and looking for these in ECGs with drug-induced QT prolongation. In congenital LQTS2 and in some individuals with drug-induced QT prolongation, prolongation of M cell action potential results in broad, low-amplitude T waves which are often deeply notched or bifurcated (Yan and Antzelevitch, 1998). In one study, appearance of abnormal morphology of T waves on the ECG was a more sensitive marker of drug-induced IKr (K_v11.1) channel inhibition than QTc prolongation (Graff *et al.*, 2008). Recent studies support Tpeak–Tend (Tp-e) interval as another index of transmural dispersion and vulnerability. It is believed that epicardial repolarization is coincident with the peak of the T wave and repolarization of M cells coincides with the end of the T wave (Yan and Antzelevitch, 1998). The normal Tp-e/QT ratio in precordial ECG leads ranges from 0.15 to 0.25; a value greater than 0.28 was strongly associated with risk of developing TdP in patients with acquired LQTS (Shimizu *et al.*, 2002; Gupta *et al.*, 2008). T wave alternans is the periodic beat-to-beat variation in T wave amplitude which is known to predisposes to TdP. Computer algorithms can now detect beat to beat variations T wave amplitude as small as one-millionth of a volt (microvolt T-wave alternans); increased microvolt T wave alternans has been shown to increase the risk of cardiac arrhythmias and death (Kroll and Gettes, 2002). However, validation of these biomarkers in drug-induced QT prolongation has been difficult because drug-induced TdP is becoming rare because all new drugs that prolong the QT interval have a detailed warning in the package insert and product monograph, because of which these drugs are prescribed infrequently and administered with extra precautions.

Conclusion

The ICH-E14 guidance was formulated in response to a need to detect drugs that predispose to TdP at an early stage of drug development, rather than after they were licensed for marketing. Since it came into effect, many new drugs have been found to cause 'significant' QT prolongation and their further development has been abandoned. To that extent, the E14 guidance seems to have served its purpose. However, not all drugs that cause QT prolongation >5 ms would actually increase risk of TdP. A search for better biomarkers of drug-induced TdP is on, and development of a more specific biomarker would prevent many useful drugs from being prematurely abandoned. Paradoxically, the effectiveness of the E14 guidance itself has placed a road-block in the validation of new biomarkers. In the absence of better markers, the QT interval remains at the best available surrogate marker of drug-induced TdP (Roden, 2008b) and all new drugs with systemic bioavailability will have to be subjected to a formal TQT study.

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