Cardiac Safety Research Consortium: Can the thorough QT/QTc study be replaced by early QT assessment in routine clinical pharmacology studies? Scientific update and a research proposal for a path forward

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The International Conference on Harmonization E14 guidance for the clinical evaluation of QT/QTc interval prolongation requires almost all new drugs to undergo a dedicated clinical study, primarily in healthy volunteers, the so-called TQT study. Since 2005, when the E14 guidance was implemented in United States and Europe, close to 400 TQT studies have been conducted. In February 2012, the Cardiac Safety Research Consortium held a think tank meeting at Food and Drug Administration's White Oak campus to discuss whether "QT assessment" can be performed as part of routine phase 1 studies. Based on these discussions, a group of experts convened to discuss how to improve the confidence in QT data from early clinical studies, for example, the First-Time-in-Human trial, through collection of serial electrocardiograms and pharmacokinetic samples and the use of exposure response analysis. Recommendations are given on how to design such "early electrocardiogram assessment," and the limitation of not having a pharmacologic-positive control in these studies is discussed. A research path is identified toward collecting evidence to replace or provide an alternative to the dedicated TQT study. (Am Heart J 2014;168:262-72.)

Background

Delayed cardiac repolarization, measured in the surface electrocardiogram (ECG) as prolongation of the QT interval, can lead to proarrhythmic events. Drug-induced QTc prolongation has been associated with a rare, potentially fatal, ventricular arrhythmia known as Torsades de Pointes. As a result of an increased awareness that noncardiovascular drugs may cause QTc prolongation,[1,2] the International Conference on Harmonization (ICH) in May 2005 issued the guidance document E14: The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for NonAntiarrhythmic Drugs.[3,4] This guidance calls for a dedicated ECG study, commonly known as the TQT study, which should be conducted with most new systemically available drugs. The TQT study is often performed before phase 3 clinical development and includes a negative (placebo) control, a positive control, and ≥1 dose of the active compound, which is expected to produce plasma concentrations that cover the maximum systemic exposure anticipated in the target population ("worst case scenario," for example, increased exposure in patients with impaired clearance of the drug or due to drug-drug interactions). The TQT study is designed to exclude a "threshold" effect below which QTc changes are considered to have no significant clinical consequence. A drug is deemed to be of negligible proarrhythmic risk if QTc

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prolongation exceeding 10 milliseconds can be excluded, that is, if the upper limit of the one-sided 95% confidence interval (CI) around the mean placebo-corrected change in QTc (ΔΔQTc) is <10 milliseconds at all postdosing timepoints ("negative TQT study") after treatment with a supratherapeutic dose. As stated in the ICH E14 guideline, this definition is chosen to provide reasonable assurance that the mean effect of the study drug on the QT/QTc interval is not greater than around 5 milliseconds. In case this "threshold" effect cannot be excluded (a "positive TQT study"), the QTc effect and its consequences are required to be further studied in the targeted patient population, which usually includes intensive ECG monitoring in late stage trials.

More than 300 TQT studies have been performed and submitted to the Food and Drug Administration (FDA) (n = 332 in July 2013) after the implementation of the ICH E14 guideline. Extensive experience with TQT studies has confirmed their sensitivity. There is currently no known example of a drug with a negative TQT study that has been clearly associated with Torsades de Pointes. On the other hand, the TQT study is resource intensive and has been criticized for its low cost effectiveness,[5] implications for the timelines for drug development, lack of specificity (ie, not all drug-induced QT prolongation may be proarrhythmic), along with labeling consequences. Incorporation of robust ECG assessment into a routinely performed early clinical study, in which the highest plasma levels of the drug in preapproval studies are typically achieved, for example, First-Time-in-Human (FIH) studies, may represent a more effective approach in terms of resources, timing, and risk management in clinical development.

The Cardiac Safety Research Consortium (CSRC) is a public-private partnership developed to advance scientific knowledge on cardiac safety for new and existing medical products by building a collaborative environment based upon the principles of the FDA's Critical Path Initiative[6] as well as other public health priorities. In February 2012, the CSRC held a think tank meeting at FDA's White Oak campus to discuss how QT assessment could be performed without the use of a dedicated TQT study. As a spin-off from this meeting, a group of experts convened to discuss options to improve the confidence in QT assessment in early clinical development and to assess circumstances under which such "early QT assessment" could replace the TQT study. This article summarizes scientific discussions of members of the CSRC regarding possible approaches to consider for earlier evaluation of drug-induced QTc prolongation in drug development; it identifies areas where knowledge gaps exist and suggests a research path toward collecting evidence to support or refute the proposed approaches and options to replace the TQT study. The focus of this article is on clinical QT assessment. A section on how nonclinical assays could be improved and thereby play a greater role in the assessment of risk of not only QTc prolongation but also of its feared consequence, the proarrhythmias, has been included to give a more comprehensive picture of ongoing initiatives in this area.

Current state of nonclinical QT assessment and how confidence in nonclinical testing can be improved

The current nonclinical assessment to determine the potential of drugs to prolong cardiac repolarization is largely determined by the regulatory requirements laid out in ICH S7B.[7] These cardiovascular safety pharmacology studies are primarily designed to detect relatively large effects that may be a concern in the FIH studies. The core studies conducted include an assessment of drug effects on the human ether-à-go-go-related gene (hERG) potassium channel assay and an in-vivo assessment of the QT interval in a nonrodent (usually dog or monkey) cardiovascular study. Although there is considerable interlaboratory variability in the conduct of the nonclinical QT assays, recent retrospective analyses suggest that the nonclinical assays have fairly good predictivity for QT effects in humans. Analysis of 114 compounds compiled by a consortium of 7 pharmaceutical companies suggests good concordance between the single-dose telemetered dog studies and FIH studies with an estimated sensitivity and specificity of 80% and 70%, respectively.[8] Furthermore, a survey of 150 compounds suggests that the nonclinical assays have good predictivity of the results of the human TQT study, especially for drugs that produce maximal prolongation of the mean QTc interval that is >10 milliseconds in humans.[9] However, the ability of the nonclinical assays to predict the outcome of a clinical TQT study remains controversial with many regulatory authorities. The main reason for this controversy is that the magnitude of effect of concern in the TQT study is much smaller than for the nonclinical assays, which are generally not powered to detect such small changes.

The hERG patch clamp assay is conducted on all small molecule compounds before human testing. However, there are some significant challenges when using the data from this assay to predict the outcome of a TQT study. Firstly, there is no standardization of the hERG assay, and it is recognized that the hERG inhibitory potency of some drugs is dependent on the patch clamp protocol. An alternative approach would be to agree upon the outcomes (and plasma exposures) for a number of compounds tested in a TQT study, and in order for a sponsor to claim a negative result for new compounds, the sponsors would have to demonstrate that the selected patch clamp protocol detected these drug effects at appropriate concentrations in order to validate the assay. There are also other challenges to the interpretation of estimates of inhibitory potency from the hERG assay,

which include discrepancies between target and actual in vitro concentrations, related to loss of compound from the bathing solutions.

A number of compounds have multiple pharmacologic actions that can modify the translation of hERG blockade to QTc prolongation. A classic example is verapamil, which although a hERG blocker has little risk for QTc prolongation in humans because of its potent calcium channel-blocking properties. Thus, to predict the outcome of a TQT study finding, the hERG assay should be integrated with a "translation" assay, often a nonrodent in vivo assay. These latter assays have limited throughput, and there is an opportunity to validate other assays to define earlier in the discovery paradigm whether compounds have other pharmacologic properties that may "block" the translation of the hERG inhibitory activity and therefore improve the predictivity for a clinically significant proarrhythmic effect in humans of the hERG patch clamp assay. The profile of compounds such as verapamil has also resulted in a proposal that future nonclinical testing paradigms should include a broader panel of cardiac ion channels and computer simulations to better predict proarrhythmic liability.

A QTc prolongation of approximately 10 milliseconds in the dog or the monkey is unlikely to stop compound progression to human trials but could be considered a risk for a positive TQT study. As with the patch clamp assay, there is no industry standard protocol for the conduct of these studies, and of greater concern, there are no accepted criteria for assay sensitivity. Sample size is typically small (n = 4-8), and concurrent positive control treatment arms are often not included. Recently, the Safety Pharmacology Society organized a "best practices" meeting to discuss the nonrodent cardiovascular study, and the recommendations of this meeting are critical to improving the concordance between the nonclinical and clinical QT studies.[10] If sponsors claim that a compound is negative in the nonclinical cardiovascular assay and therefore has a low risk of a positive result in the TQT study, they must provide evidence that the relevant study had the statistical power to detect a QTc effect of concern (ie, 5 milliseconds as evidenced by an upper bound of the 90% CI of 10 milliseconds QTc prolongation). In the absence of statistical data to support the appropriate assay sensitivity, it is not possible to know whether a compound is a genuine "negative" or the drug effect was less than the assay sensitivity (often approximately 20 milliseconds because many sponsors define a positive effect as 10% prolongation of the QTc interval).

Pharmacokinetic/pharmacodynamic (PK/PD) modeling is widely used in clinical studies to better define the exposure-response relationship for QTc prolongation both in phase 1 studies and in the TQT study. However, these techniques are only just starting to be used in the nonclinical discipline.[11] One of the advantages of the nonclinical studies is that much higher doses can be studied, increasing the chances of detecting an effect on the QT interval. If QT effects are detected, PK/PD modeling can be applied to predict expected effects at clinically relevant exposures.

Although a majority of compounds that prolong the QTc interval do so through inhibition of the hERG channel, there are other mechanisms that may be a cause for concern. Mild vasodilators, such as the phosphodiesterase type 5 inhibitors, have been shown to prolong the QTc interval in humans,[12] although some authors believe that this is a consequence of an inappropriate correction for the heart rate increases observed with these compounds[13] or other autonomic effects. Other pharmacologic classes that cause or have been associated with QTc prolongation through mechanisms other than direct hERG channel block also include β2 and β3 adrenoceptor agonists, gonadotropin-releasing hormone receptor antagonists and superagonists, somatostatin analogues, and sphingosine-1-phosphate receptor modulators. These compounds have not been observed to affect the QTc interval in the conscious dog or monkey, raising questions about interspecies differences, sex hormone effects, or the above mentioned compounding effects on heart rate. QT/QTc prolongation resulting from such mechanisms can, however, be detected in adequately designed clinical studies, and therefore, any strategy designed to replace the TQT cannot rely entirely on nonclinical studies. There are also certain other mechanisms that cause QTc prolongation that are also not readily detected in the standard nonclinical studies, for example, hERG trafficking inhibitors, for example, pentamidine and arsenic trioxide.[14-17] Although these effects can be detected in specific in vitro assays, compounds are not routinely tested against this specific target because the incidence of hERG traffic inhibition at clinically relevant concentrations is quite low. However, there is an opportunity to make better use of existing nonclinical studies, namely, the repeat dose nonrodent toxicology studies. Given that multiple doses may be required to demonstrate the effect of hERG trafficking inhibitors on the QTc interval, these compounds should be detected in the repeat dose toxicology studies if ECG effects are appropriately monitored.

In conclusion, because the introduction of ICH S7B mandating the evaluation of small molecules on the cardiovascular system, systematic nonclinical evaluation of the effect of drugs on the QT interval is now conducted. It is recognized that this approach has greatly reduced the numbers of compounds entering clinical trials with an associated risk of a large effect on the QT interval. The emerging data also suggest that the nonclinical studies have good but not absolute predictive value for the outcomes of a TQT study. Concerns have been raised that using such preclinical tests, which are focused on QT prolongation as "gatekeepers" might impede the development of pharmacologic compounds

with a favorable benefit to risk relationship. This might be ameliorated in the future by developing assays focused on proarrhythmia instead of QTc prolongation. This has been the subject of recent detailed discussions to include the use of computer simulations and a broader panel of cardiac ion channels in the nonclinical safety evaluation.

The following recommendations may be considered to improve our confidence in the ability of nonclinical assays to predict the outcome of the TQT study:

1 To improve confidence in the hERG assay, agree on a panel of compounds with known outcomes in the TQT study (including exposures), which sponsors would test to demonstrate that their selected protocol has appropriate sensitivity and specificity.
2 Validation of new action potential assays that allow differentiation between mixed ion channel blockers that prolong the QT interval and those that do not.
3 If sponsors wish to use the nonclinical data to make a claim that the test compound is negative on the QT interval, then the recommendations from the cardiovascular best practice meeting should be applied.[10]
4 Consider the wider use of PK/PD modeling in the nonclinical studies to better define the exposure-response relationship for any effects on the QT interval, thereby improving the translation to humans.

Use of early clinical studies to assess risk of QTc prolongation

Early clinical trials present an opportunity to confidently evaluate QTc prolongation risk, and several authors have demonstrated the potential for these studies to replace the TQT study.[18-20] The choice of doses for the TQT study is of paramount importance, and critical PK data must therefore be generated before the TQT study. To determine the "worst-case" exposure scenario in the target patient population (due to eg, drug interactions, hepatic, or renal impairment, etc), it is recommended that the full PK profile of the novel drug and the therapeutic and supratherapeutic doses are identified before the TQT study is conducted. This information is usually available only by the end of phase 2 or later and often necessitates the frontloading of complex, resource-intensive phase 1 studies such as the human mass balance, drug interactions, and hepatic or renal impairment. The highest dose level in the phase 1 SAD study will depend on the nonclinical toxicology and safety pharmacology data for an individual drug as well as the treatment-emergent human safety and tolerability profile in the ongoing study. If permitted by these data, the dose escalation in the SAD study may proceed to the maximum tolerated dose (MTD) and exposure in humans. This often represents the highest human dose and exposure that will ever be evaluated in clinical studies[20]; indeed, the plasma exposure at the MTD in the SAD study will often exceed the estimated "worst-case" plasma exposure in the target patient populations, except for drugs that are poorly tolerated by healthy subjects or that exhibit prominent accumulation in plasma upon repeat dosing to steady state. Albeit in a smaller sample size, the PK, QTc, and cardiac safety data (including other ECG effects) at the MTD in the SAD and/or MAD studies would thus have the potential of providing analogous information to the supratherapeutic dose in the TQT study. Moreover, the range of doses and exposures in the SAD and other phase 1 studies are often wider than what can be explored in the TQT study, which would facilitate concentration—QTc concentration effect modeling (CEM) analysis.

Many of the early phase studies are conducted according to the same robust experimental conditions and in the same clinical sites that perform TQT trials. All of the same ECG acquisition and processing procedures as well as QT measurement algorithms can be applied to both TQT and early phase data. Data-based simulations suggest that the application of CEM could provide sufficient power to exclude a QTc increase of 10 milliseconds in a phase 1 study with a typically small sample size.[21] Appropriate design of the study, QT interval analysis, and statistical approach to the concentration effect modeling can provide valid quantification of the QTc effect of a novel drug candidate based upon phase 1 study data. This early clinical assessment can be combined with nonclinical data in a totality of evidence approach that provides negative predictive value that could match or exceed that of a TQT trial.

Most SAD studies follow a sequential parallel group design, where each subject on active treatment receives only one dose of the novel drug, whereas the complete crossover design is very rarely used because the large number of treatment periods (5-8 dose levels) increases the duration of the study and the risk of subjects dropping out. However, the alternating panel crossover design is also commonly used in SAD studies and allows for similar information as in the parallel SAD studies to be obtained from fewer subjects. Alternating panel crossover SAD studies could result in the distinction between the interindividual and intraindividual QTc variability and a more precise estimation of PK and QTc central tendency results.

The appropriate number of ECG timepoints for the statistical analysis of the central tendency of QTc prolongation in the TQT study has been recently addressed by Zhang and Stockbridge,[22] who pointed out the regulatory expectation for the 24-hour postdose ECG timepoint to assess the impact, if any, on hERG channel trafficking (transport of hERG proteins or components thereof, from the endoplasmic reticulum to the cell membrane), which results in delayed-onset QTc prolongation.[14,15,23] As expected from the temporal concentration-QTc effect relationship, Shah and Morganroth[24] have found that the largest mean placebo-corrected QTc change from baseline (90% CI) in a sample of 30 published TQT studies was observed during the time of occurrence of the peak plasma concentration and 3 additional timepoints thereafter. In addition, a few subsequent timepoints for PK and ECG assessments would strengthen the CEM analysis by

describing the decreasing plasma concentration during the elimination phase. This approach would normally result in 6 to 8 timepoints to evaluate the ECG effect of the drug during the first 24 hours after dosing in the early clinical study. Only rarely would the plasma PK profile in the elimination phase warrant the extension of ECG and PK sampling beyond 24 hours to support the adequate CEM analysis.

The baseline QTc value in the crossover single-dose TQT study is typically a single predose QTc data point on the dosing day in each treatment period (usually the mean QTc from 3 repeat ECG timepoints with replicate ECGs at each timepoint, obtained within 90 minutes before dosing) because each subject has his/her own time-matched placebo data to account for diurnal effects.[25-28] In contrast, parallel designs typically use serial predose time-matched ECGs during the full day before dosing as baseline to account for the diurnal effects. For phase 1 studies that have a placebo cohort, regardless of design (ie, crossover or parallel), a predose baseline value could be used when the data are analyzed by CEM approaches that incorporate a time component in the model to account for the diurnal effects.

To attain the greatest consistency and reduce variability in the QTc data from phase 1 studies intended to optimize signal detection and support the claim for the TQT waiver, the experimental conditions of clinical conduct and ECG acquisition must be as robust and carefully standardized and monitored as they would be in the TQT study. In particular, changes in heart rate, autonomic tone, and stress should be avoided. Subjects should be resting quietly in a supine position for $\geq$10 minutes before timepoints scheduled for ECG extraction from the continuous ECG recording. It is important to eliminate any ambient noise in the clinic (TV, radio, and conversation) both during the pre-ECG rest and during the recording of each ECG segment targeted for extraction. The vital sign assessments and any type of blood draws should always occur after the nominal timepoint for ECG extraction whenever these study procedures coincide. If possible, ECGs should be collected $\geq$2 hours after a meal, and the time of meals should be standardized between study days and treatment periods. Identical ECG lead placement in every treatment period as well as on multiple study days within one treatment period must be ensured.

Pharmaceutical companies today routinely use continuous digital 12-lead ECG (Holter) acquisition and extraction of replicate ECGs in the vast majority of TQT studies. The same approach would offer advantages over the acquisition of replicate standard resting 12-lead ECGs in phase 1 studies intended to support the claim for the TQT waiver. Continuous ECG recordings allow for the extraction of high-quality, replicate ECGs at times when QT interval duration has adapted for the preceding increases or decreases in heart rate, thus reducing the confounding effect of the QT/R-R hysteresis. When necessary, many more replicate ECGs (eg, 5-10) can be extracted from the recording at each nominal timepoint than would be cost effective with the standard resting ECGs, and continuous digital recordings are available for retrospective analysis at critical timepoints after dosing that are difficult or impossible to capture by advance scheduling (eg, deviation in the time of occurrence of maximum plasma concentration of the parent drug and cardioactive metabolites, clinical adverse events possibly related to QTc prolongation), as long as the stability of the R-R interval at such unscheduled timepoints is acceptable. Continuous ECG recordings eliminate the need to disconnect and reconnect lead electrode cables before each of the nominal ECG timepoints, which significantly decreases the effort on part of the clinic staff.

Replicate ECG strips extracted from continuous ECG (Holter) at each nominal timepoint are recommended to allow the phase 1 QTc data to support the claim for a TQT waiver; the need to decrease the biological variability of the QTc interval and the measurement error is as important here as in the TQT study. Optimized extraction of replicate ECGs from tracings with verified stable QT/R-R relationship in the appropriately short time window (eg, 5 minutes) around the nominal timepoint will minimize variability, while improving quality.

The available methods for QT interval duration measurement have been described by the ICH E14 Implementation Working Group in 2008,[3] and the properties and pros versus cons of individual methods are beyond the scope of this article.

The phase 1 MAD study could evaluate the QTc prolongation of the parent and/or the cardioactive metabolite(s) after dosing to steady state in plasma; the dose levels could often be escalated to the MTD in repeat dosing. The MAD studies would be particularly useful for the early clinical QTc assessment of drugs that exhibit prominent accumulation of the parent or metabolite(s) in plasma on repeat dosing to steady state, if parent drugs at steady state form metabolite(s) at a different rate than after the single dose, or if the nonclinical ECG assessment or the 24-hour ECG measurement in the SAD study suggests a potential QTc prolongation through a delayed mechanism, such as inhibition of synthesis of hERG channels or their trafficking to the plasma membrane.[24] For other drugs, robust QTc assessment in SAD studies could produce sufficient high-quality data on the concentration-QTc relationship to support the claim for the TQT waiver.

Instead of using either SAD or MAD study data alone to support the claim of the TQT waiver, sponsors could potentially conduct both the SAD and MAD using the same robust clinical conduct and intensive ECG and PK monitoring so that the integrated QTc assessment in early clinical development could be made based on the CEM analysis of the pooled SAD plus MAD study data, thus

Table I. Concentration effect modeling plays a key role in regulatory decisions for drugs that prolong QTc interval

Drug	Role of CEM
Anzemet (dolasetron)[29]	Project the QTc prolongation in elderly and renally impaired patients in the product label.
Zofran (ondansetron)[30]	The lower dose intravenous regimen of 0.15 mg/kg every 4 hours for 3 doses is recommended in adults for chemotherapy-induced nausea and vomiting, with no single intravenous dose of ondansetron to exceed 16 mg due to the risk of QTc prolongation. Single dose of 32 mg intravenous removed from the label due to risk of QTc prolongation.
Celexa (citalopram)[31,32]	Project the QTc prolongation at the 40 mg dose, which was not directly evaluated in the TQT study. In the general patient population, this dose is labeled as the highest dose to be used clinically based on benefit-risk assessment.
Caprelsa (vandetanib)[33]	Characterize QTc prolongation in the patients from ECGs obtained in phase 3 trials for the product label.
Ranexa (ranolazine)[34]	Characterize QTc prolongation in patients with highly variable pharmacokinetics. Project QTc prolongation in patients with hepatic impairment.
Saphris (asenapine)[35]	Magnitude of QTc prolongation in label (2-5 milliseconds) is that predicted by CEM and not the mean values reported from the IUT analysis of TQT data (5-10.5 milliseconds).
Sertindole[36]	Project QTc prolongation in patients who are CYP2D6 poor metabolizers for benefit-risk assessment.

Abbreviation: *CYP2D6*, Cytochrome P450 2D6.

overcoming the possible limitations from the smaller sample size in either individual phase 1 study. There is only one published report from such pooled QTc assessment based on the uniformly robust ECG monitoring across phase 1/2A studies and the use of CEM in the pooled PK and QTc data,[19] but recent anecdotal evidence from pharmaceutical companies indicates that the approach is quite commonly used when warranted by the perceived QT liability based on the preclinical and clinical data. This approach could be limited in rare cases when the ratios of QT active metabolites are substantially different between the SAD and MAD studies.

To summarize, carefully conducted early phase trials that include ECG and QT measurement and CEM analysis as the primary assessment of QTc prolongation are feasible and have the power and validity to exclude a QTc effect at the level of regulatory concern.

The role of CEM in QT assessment

The role of CEM of QTc data has been the topic of multiple scientific meetings, presentations, and manuscripts and is described as "an important component of a totality of evidence assessment of the risk of QT prolongation" in the recently released (March 2014), latest version of ICH E14 questions and answers document (question 5.1 in[3]). The benefit of using concentration to quantify drug effects on the QTc interval is that it accounts for the presence of intersubject variability in the pharmacokinetics of a compound. Incorporating all concentration and QTc data across treatments in the analysis makes more efficient use of the data and permits prediction of effects on the QTc interval under alternate treatment conditions, for example, doses not directly studied, increased exposures due the effect of intrinsic, and extrinsic factors, etc, which are often encountered during drug development.

Since the implementation of the ICH E14 guidelines, CEM has played a key role in the regulatory review of drugs and has been conducted in almost all of the reviews of data from TQT studies. It has been invaluable for interpreting the results of positive TQT studies by translating the mean QTc effects at therapeutic and supratherapeutic doses into something that can be used in clinical and regulatory decision making. Table I provides some examples, where QTc effects derived from CEM have been used in drug labels or in benefit-risk evaluation.

Another role of CEM is to clarify ambiguous results in the TQT study. One shortcoming of the primary statistical end point derived using the intersection union test (IUT) is the existence of a high false-positive rate in the absence of a drug effect, depending upon study design.[37] This false-positive rate may in part be due to the positive bias inherent in the IUT,[38] which has been shown to be as high as 4 to 5 milliseconds.[38,39] The asenapine TQT trial was a randomized, placebo-controlled, double-blind, multicenter, parallel-group trial, in which subjects with schizophrenia or schizoaffective disorder received asenapine 5/10 mg twice daily (BID), asenapine 15/20 mg BID, placebo, or quetiapine 375 mg BID for 16 days.[35] The QT-IRT review stated that a dose-response relationship was not observed for asenapine, as shown in Table II, but with the small sample size (<35 subjects per arm), the study was not powered to detect a dose-response relationship using the primary end point. Concentration effect modeling analyses conducted by both the sponsor and FDA reviewers showed that asenapine prolonged the Fridericia-corrected QTc (QTcF) interval in a concentration-dependent manner. The sponsor's model-predicted mean placebo-corrected change-from-baseline QTcF at a mean peak plasma level of 10.7 ng/mL, which corresponds to an asenapine dose of 20 mg BID, was 4.9 milliseconds (2.5, 7.7 milliseconds; 90% bootstrap CI). The current asenapine US label states: "SAPHRIS was associated with increases in QTc interval ranging from 2 to 5 milliseconds compared with placebo," which is the magnitude predicted from the CEM analysis.[40]

Table II. FDA statistical analysis: placebo-corrected change from baseline QTcF (ΔΔQTcF, milliseconds) across asenapine dose groups

Treatment	Time, h	Mean ΔΔQTcF*, milliseconds	90% CI†, milliseconds
Asenapine 5 mg BID, n = 30	3	5.0	–1.5, 11.4
Asenapine 10 mg BID, n = 27	2	10.5	4.5, 16.5
Asenapine 15 mg BID, n = 33	3	8.7	3.0, 14.4
Asenapine 20 mg BID, n = 29	4	4.9	–1.9, 11.6

* Confidence interval derived from the IUT.
† Placebo-corrected change-from-baseline QTcF.

Application of CEM to QTc interval data has several favorable attributes not present in the IUT. The IUT provides estimates of QTc interval effects by treatment group (dose) and time. However, both dose and time are regarded simply as categorical variables without magnitude. In terms of the IUT analysis, groups of subjects receiving different doses are equally likely to have a QTc interval prolongation regardless of the magnitude of difference between the doses, and those prolongations are equally likely to occur at any measured timepoint. This is implausible from a pharmacologic perspective because drug concentration at the site of action drives the potential to prolong the QTc interval either directly or indirectly. Drug concentration in serum or plasma is a closer surrogate to the biologically relevant concentration than dose and by the nature of serial PK sampling, implicitly incorporates the time course of drug concentration. Therefore, a CEM approach represents a more biologically plausible analysis to quantify drug effects on the QTc interval, as opposed to IUT, which ignores time course and influence of drug concentration. This is exemplified in the TQT study for Januvia (sitagliptin), which evaluated a therapeutic dose of 100 mg and supratherapeutic dose of 800 mg. At one timepoint after the peak plasma level, the upper confidence limit for the 800 mg dose failed to exclude 10 milliseconds resulting in a positive TQT study. However, a linear concentration-QTc relationship had a shallow slope, which predicted mean QTc effects <5 milliseconds for the 800 mg dose. Because the exposure margin with the supratherapeutic dose was 11-fold and had a shallow concentration-QTc relationship, the TQT study was considered negative in the product label.[41] This case may represent the improvement of the power to find a true effect using CEM or provide an example, where multiplicity issues inherent in the IUT approach led to a false-positive.

Although there are numerous benefits to the CEM approach, there are also notable concerns, which have precluded its use by regulators as the primary end point in the TQT study. The main concern is the potential for underprediction of the QTc estimate if the model assumptions of a linear model are invalid.[42] This concern can be addressed by objectively testing the model assumptions using prespecified criteria and goodness-of-fit assessments. An important assumption to be tested is that a direct relationship between plasma drug concentrations and increases in QTc interval duration exists. There is potential for a delay or hysteresis between plasma drug concentrations and changes in the QTc interval due to infrequent events such as the presence of an active metabolite or inhibition of hERG channel trafficking. Such delays can be detected using standard diagnostic plots and accounted for by PK/PD models, for example, effect compartment models.[20,43] In addition, the complexity and lack of consistent applications of CEM methodology have also been raised as concerns.[20,42] Critics of the CEM approach have noted that PK/PD models are complex, and their application requires specialized computer software and skilled pharmacometricians.[44] However, complex PK/PD models are not necessary in most cases.

A consistent application of the CEM approach across the pharmaceutical industry can be implemented by developing a standardized methodology, which uses prespecification of the modeling approach along with objective decision criteria, including criteria for the presence of hysteresis of the QTc effect. A standardized, prespecified analysis plan will address the concern that model development is operator dependent[42] and will enable CEM to be more accepted by regulators as a primary end point in QTc evaluation.

Most work done in this area has been targeted toward application of CEM in TQT studies. However, as stated previously, early phase clinical studies such as the SAD and/or MAD studies provide a unique opportunity to explore concentrations that are often much higher than those achieved after anticipated therapeutic doses, and these studies are therefore ideally suited for evaluating the relationship between drug concentration and QTc interval. Based on considerations outlined in the "use of early clinical studies to assess the risk of QTc prolongation" section, it can be expected that ECG data will be of equivalent quality to those collected in TQT studies. Using all available data pooled across similar studies (eg, SAD + MAD) enables efficient use of data and may provide a single, unified understanding of the QTc signal from phase 1 studies.

Assay sensitivity

To provide confidence of the estimated impact of the investigated drug on cardiac repolarization, the ECG component of a clinical study needs to show not only that it is capable of detecting differences in QTc interval

measurement but also that the detection of QTc interval differences is sufficiently sensitive to identify repolarization changes that are considered of regulatory concern. Therefore, a proof of such capability needs to be an integral part of any such study. This proof is usually termed *assay sensitivity*, and there are several ways and levels at which it might be provided.

In presently conducted TQT studies, pharmacologic assay sensitivity assessment is used, usually by measuring the QTc responses to a single oral 400 mg dose of moxifloxacin. This level of assay sensitivity addresses not only the QT interval measurements but also, partly, drug dosing, experimental conditions, and adherence to the randomization schedule. The same approach to assay sensitivity could be incorporated into early clinical studies. However, using an active pharmacologic control would substantially increase the complexity and cost of the phase I studies. Thus, although the use of pharmacologic controls would permit demonstration of assay sensitivity of early phase I studies, it cannot be universally recommended for such a purpose. Although QTc changes due to nonpharmacologic provocations (eg, food effect or postural provocations[45,46]) are possible alternative approaches, it is difficult to recommend these strategies at this time because their operator characteristics are not sufficiently described; they are associated with confounding heart rate perturbations (which lead to methodological interpretation issues),[47] and sufficient uniformity in small sample sizes has not been demonstrated. Furthermore, such provocations might interfere with the conduct of early clinical studies.

A different level of ensuring assay sensitivity might potentially be based on certifying laboratories providing the ECG measurement, similar to what is done for biochemical analyses. However, such certification is challenging and unlikely to be successful in that the analysis includes human interpretations, and the individual readers as well as their interpretations over time are not constant, being subject not only to random variability but also systematic bias. In biochemical analyses, the procedures and equipment used are frequently calibrated to demonstrate stability of results. These repeated calibrations are meaningful only if it can be shown that the calibrated processes are faithfully reproduced in each measurement procedure and over time. This is difficult if not impossible with manual interventions and individual judgment of ECG waveforms.

The need of repeated proofs of assay sensitivity could be avoided only if a highly reliable and fully automated ECG analysis system was created, validated, and shown to be independent of different computer implementations, similar to the current computer routines for complex mathematical calculations. Such a system would always deliver identical readings and would need to function at an acceptable level when there are drug-induced changes in ECG morphology. Likewise, it would need to be able to handle artifacts and signal noise. Although substantial advances in ECG computer processing have been made, there is presently no ECG measurement algorithm that has been shown to be sufficiently reliable without human intervention and regardless of the signal circumstance.

Instead of replicating predictable effects of known drugs (such as single moxifloxacin doses), the proof of assay sensitivity might rely on the handling and measurements of the study ECGs. Such a proof of assay sensitivity is less direct than the pharmacologic proof but might still provide evidence that the study subjects and the collected ECGs have been handled and measured with sufficient precision necessary for the identification of repolarization impacts that are of regulatory concern. This approach to assay sensitivity proof might be seen as more approximate compared with the standard pharmacologic assay sensitivity assessment. However, even the standard moxifloxacin-based proof of assay sensitivity does not validate the accuracy of the study entirely, for example, in situations when the investigated drugs affect heart rate and/or cardiac autonomic status.[47] An alternative approach seems to be the most practical option for early (and usually small) clinical studies and may parallel the proposal made previously for crossover TQT studies.[48] This was based on the premise that if QT/QTc measurements are accurately and systematically made in the study, small drug-induced changes in QTc duration will be detected. In terms of the stability and accuracy of QT measurements, 2 scenarios might be distinguished: if the early clinical study contains both full-day drug-free baseline and on-treatment recordings for each subject, as is sometimes done in MAD studies, the analysis can investigate the stability between drug-free baseline and on-placebo recordings in the same subjects. This involves demonstrating the stability of QT measurements by showing that different subjects have different QT profiles and that the differences between subjects found during baseline are reproduced with sufficient accuracy in the on-placebo recordings. Although the approach would need to consider the conditioning effects by study conduct,[49] it has been repeatedly shown that under standardized conditions, the QT profiles show both substantial intersubject differences and high intrasubject reproducibility.[50] The limits at which the intrasubject reproducibility and intersubject differences need to be demonstrated may be related to the study size because it is related to the power with which the study can prove negative conclusion. However, considering the fact that the early clinical studies are usually much smaller than the typical crossover TQT studies, the necessary limits of agreement might need to be tighter compared with the previous proposal.[48]

If the early clinical study contains only on-treatment ECG recordings, extrapolation of the same approach might be derived from theoretical deductions. Although there is little experience with this possibility at the

current time, conceptual considerations suggest that it might be equally practical. The on-placebo recordings might be divided into 2 halves (eg, morning and afternoon sections) and processed in the same way as repeated drug-free recordings, again showing systematic differences between different on-placebo subjects, that is, showing stability of QTc profiles in both parts of the on-placebo recordings in the same subject together with similar differences between any pair of subjects in both parts of these recordings. To appropriately demonstrate the expected result, the separation of the placebo recordings into 2 different halves would need to be made independent of those involved in the ECG analysis. This would entail additional data management requirement, but these should not be prohibitively complex or expensive.

A path forward

It can be argued that the E14 guidance has served the main purpose to improve the characterization of the QTc effect of drugs deemed sufficiently medically valuable to justify their further development and approval. This is likely to be the result of actions undertaken by both sponsors and regulators, such as a carefully monitored, risk-averse drug development approach with discontinuation of drugs that have a potential for QTc prolongation complemented by a set of regulatory actions (eg, label warnings, letters to health care providers, boxed warnings, and withdrawals of drugs from the market or refusal of authorizations). It is, however, also important to acknowledge the negative impact that comes from overly cautious discontinuation of development programs for drugs that may provide substantial therapeutic benefit, as not all drugs that prolong the QTc interval are known to be proarrhythmic. The requirement for and the design of the TQT study have been debated extensively since the adoption of the ICH E14 guidance in 2005; notwithstanding this debate, an alternative path might encompass a set of minimum standards for evaluation of a drug's effect on the QT/QTc interval, including (*a*) clinical study data that allow the exclusion of a QTc prolongation effect exceeding the threshold of regulatory concern, currently approximately 5 milliseconds, as shown by the upper bound of the 2-sided 90% CI being <10 milliseconds; and (*b*) the demonstration of the study's ability to detect a small QTc effect.

The analysis of the relationship between drug concentrations and QTc effect of a drug through CEM has proven to be an added asset in characterizing the QT/QTc effect, whether in laboratory animals, healthy volunteers, or in patients. Concentration effect modeling evaluates the QTc effect as a function of drug concentrations, and all data from all subjects/patients at all timepoints are used in the model; the statistical power of CEM to exclude small QTc effects is therefore greater than the time-matched analysis. This feature enables its application to routinely performed clinical pharmacology studies (eg, standard SAD studies with 6 to 8 subjects per dose level), which may represent a more efficient way of performing clinical QT assessment than a dedicated TQT study. A comparative evaluation of the ability of TQT studies and "early QT assessment" to detect small QTc changes would be essential to understand and define the pros and cons of different approaches. In this context, a project undertaken in collaboration between the CSRC and the Clinical Pharmacology Leadership Group of the Consortium for Innovation and Quality in Pharmaceutical Development is worth describing. Five marketed drugs, which all cause QTc prolongation, have been identified in discussions with the FDA. These drugs will, in addition to a "QT-negative" drug, be prospectively studied in healthy volunteers in a setting similar to a standard SAD study.[51] Each drug will be given to 9 subjects in 2 doses; for the "QT-positive" drugs, the lower dose is expected to result in approximately 8- to 12-milliseconds QTc prolongation and the higher dose in approximately 15- to 20-milliseconds effect. Serial replicate ECGs will be extracted from continuous recordings and paired with measurements of drug plasma concentrations to allow analysis using CEM. The concordance of the results from this "SAD-like" study and previous QT assessment for these drugs will then be evaluated against prospectively defined success criteria; if these TQT study–positive drugs also come out positive in the prospective study, it would provide supportive evidence for replacing the TQT study with early QT assessment (aka the TQT waiver).

Irrespective of the approach used to exclude a clinically concerning QTc effect of a new drug, whether a TQT study or "early QT assessment," a negative result has major implications for subsequent patient studies in terms of determining the intensity of ECG monitoring. It is therefore critical to understand to what extent the QT evaluation was able to exclude or demonstrate a small effect, should there be one. On a practical scale, it is unlikely that a pharmacologic positive control will be used in early phase studies to provide reassurance of "assay sensitivity." Alternatively, sufficiently robust methods of establishing assay sensitivity may therefore be required. As many of the components of clinical QT assessment are still highly dependent on human interventions and will vary over time. it is unlikely, in our view, that demonstration of assay sensitivity will be replaced by "accreditation" of sites/ECG laboratories. It therefore seems reasonable to assume that data to confirm or refute a study's sensitivity will have to come from each study separately, that is, generated from the study itself (see assay sensitivity section).

Replacing the thorough QT study with "early QT assessment"

There is growing interest in finding alternatives to the conventional TQT studies for a variety of reasons such as

cost, large sample size needed to detect the small change using conventional E14 analysis, and the routine use of moxifloxacin outside its indication. If replacement of the TQT study were to be achieved, it is implicit that the components discussed above are fulfilled and harmonized across the industry and regulators. The path forward about replacing the TQT study with clinical QT assessment applied to routine clinical pharmacology studies will rely on the demonstration that this new approach maintains the ability to exclude small ECG changes, whereas making more efficient use of human resources. If planned efforts from sponsors, academicians, and regulators convincingly demonstrate that early QT assessment can provide data with the same level of confidence as the TQT study, this approach could then serve as an alternative. In some programs, it seems likely that a TQT study might remain the most appropriate approach, whereas for others, "early QT assessment" might be used in lieu of performing a TQT. In the future, it is possible that we will see a combination of some programs opting for the newer methods using CEM and others using the conventional TQT study.

The potential for standardized, enhanced, and novel nonclinical assays focused on directly assessing the proarrhythmic potential of a new chemical entity to replace the TQT study are currently also under debate and remains to be established.[52] There is an opportunity to use an integrated approach of combining CEM bridging nonclinical and clinical data, which could provide additional valuable information, reducing the need for a specific TQT study. Enhancing the predictivity of nonclinical studies is likely to involve establishing novel assays (combination of hERG and other ion channel evaluations and/or newer methodologies including human cell cultures and tissues as well as in silico modeling).[53] Although there are data in support of this approach,[10,54,55] it should be recognized that realization of this potential will require concerted effort, time, and evidentiary base.

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