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Thorough QT Studies
Questions and Quandaries

Marek Malik,[1] *Christine E. Garnett*[2] and *Joanne Zhang*[3]

1 Division of Cardiac and Vascular Sciences, St George's, University of London, London, UK
2 Pharmacometrics Staff, Office of Clinical Pharmacology, Center of Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA
3 Division of Biometrics VI, Office of Biostatistics/Office of Translational Sciences, Center of Drug Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland, USA

Abstract

The International Conference on Harmonisation E14 Guidance was successful in largely standardizing the conduct of the so-called thorough QT/QTc studies (TQTS). Nevertheless, there is still a spectrum of frequently encountered problems with details of design, conduct and interpretation of TQTS. Several of these challenges are reviewed here, starting with explaining that the TQTS goal is only to identify drugs for which the proarrhythmic risk might be considered excluded for the purposes of regulatory benefit-risk assessment. Suggestions are made on how to categorize and quantify or exclude proarrhythmic risk if the TQTS is positive. There is a conceptual need for TQTS, and this is discussed, together with reasons why restricted clinical registries cannot prove the absence of proarrhythmic liability of any drug.

Appropriate drug doses investigated in TQTS should be derived from the maximum clinically tolerable dose rather than from the known or expected therapeutic dose. With the help of concentration-QTc modelling, the standard therapeutic dose can be omitted from TQTS, especially if the study is expected to be negative. Conditions for single-dose TQTS acceptability are reviewed. The role of the so-called positive control is assessed, contrasting the role of a same-class comparator for the investigated drug. A single 400 mg dose of moxifloxacin is advocated as the present 'gold standard' assay sensitivity test. The necessity of careful placebo control is explained and the frequency of ECG assessments is considered. The central tendency and outlier analyses are discussed, together with the correct approaches to baseline adjustment.

The review concludes that the design and interpretation of TQTS must not be approached with mechanistic stereotypes, and highlights the importance of relating the QTc changes to drug plasma levels.

The necessity of conducting the so-called thorough QT/QTc study (TQTS) for most new pharmaceutical compounds (as well as for new indications and new administration routes of existing compounds) is now well recognized. In the current regulatory practice, TQTS are not required for monoclonal antibodies, large therapeutic proteins, non-bioavailable compounds or highly localized products (e.g. for ophthalmology applications), but other pharmaceuticals need to

undergo this testing. The International Conference on Harmonisation (ICH) E14 Guidance[1] is well known, including the related question and answer ICH initiative.[2] The topic of TQTS has also been the subject of a number of reviews and methodological suggestions.[3-5] Because of this, the conduct of TQTS is presently fairly standardized. However, there are frequently asked questions and problems repeatedly encountered when designing TQTS. Having these in mind, the following review addresses some of the recurrent dilemmas.

1. Convention of Thorough QT Studies (TQTS)

The goal of TQTS is frequently misunderstood. These studies are not aimed at quantifying the risk of drug-induced torsade de pointes (TdP) tachycardia. Rather, the purpose of TQTS is to exclude proarrhythmic risk due to the study drug. In other words, these studies are conducted to establish whether the investigated drug has any involvement in myocardial repolarization that might potentially be the basis of drug-induced TdP.

As is well known, TQTS is considered positive if the upper bound of the one-sided 95% confidence interval (CI) of the baseline and placebo-corrected QTc changes exceeds 10 ms for at least one timepoint of the study. A negative TQTS (i.e. one that is not positive) is interpreted as proof that extensive ECG safety evaluation during the later stages of drug development for the investigated drug is not necessary, and that the collection of baseline and periodic on-therapy ECGs is sufficient. This is because, based on regulatory experience, a negative TQTS is taken as a reasonable confirmation that the investigated drug will not cause TdP tachycardia with a frequency exceeding very rare isolated instances.

This notion might require a detailed discussion. There are approved drugs that do not prolong QTc, while having been reported to cause TdP in extremely rare, exceptional cases.[6-8] Nevertheless, these isolated cases occur so infrequently (e.g. only one reported case related to a drug with many tens of millions of exposures) that they can be neither predicted nor considered during regulatory review. To distinguish between the extremely rare TdP instances and the frequency of TdP induction that should be considered during regulatory drug review, the concept of 'regulatory awareness threshold' is occasionally introduced.[9] This 'awareness threshold' refers to the minimum drug-induced TdP frequency that is larger than the background TdP incidence and that should be considered by the regulators during benefit-risk evaluation of the drug. The awareness threshold is not (and cannot) be exactly defined. Judging from the TdP incidence associated with drugs that have previously been withdrawn because of TdP risk or labelled as potential TdP inducers, the threshold is somewhere between one case of TdP among 10^5–10^6 drug exposures.

Hence, using this concept, a negative TQTS is taken as an indication that the drug would not cause TdP more frequently than the awareness threshold. Consequently, regulatory considerations of TdP liability of the drug are judged unnecessary if TQTS is negative.

Consistent with this, a positive TQTS does not mean that the drug is automatically judged proarrhythmic. Rather, it means that further data are needed for possible regulatory considerations of TdP liability. In practice, this means that additional ECG monitoring might be needed in further clinical studies to characterize the safety profile of the drug in patients and susceptible populations. The intensity of ECG monitoring in subsequent studies is guided by effect size, dose- and exposure-response relationships, and intended patient population. Each case of positive TQTS needs to be evaluated individually and detailed discussion between the pharmaceutical sponsor and the regulators is warranted. In special cases, detailed evaluation of the benefit-risk ratio might also lead to the approval of a clearly torsadogenic drug, naturally with an appropriate label.

Thus, in terms of torsadogenic risk quantification, TQTS is the first step. If a properly conducted TQTS is negative, the possibility of torsadogenic risk does not need to be included in regulatory benefit-risk evaluations. On the

contrary, if the TQTS is positive, further ECG studies are needed in populations susceptible to proarrhythmia, such as patients with hepatic or renal impairment, or patients with heart failure. ECG monitoring of these patients allows not only the detection of arrhythmia episodes (which might not be frequent) but also quantifying and categorizing cases of substantially altered ventricular repolarization indicative of proarrhythmic risk.

2. The Need for TQTS

Some pharmaceutical specialists still consider TQTS to be merely an unnecessary burden of a box-ticking exercise. Even safety concerns about drugs that have been firmly established as proarrhythmic are occasionally dismissed or their implications disputed.[10] From the regulatory and public safety point of view, this is a somewhat neglectful opinion. In terms of drug safety, public perception, and thus the charge given to the regulators, is much more tolerant to false positives (i.e. restricting approval of safe drugs) than to false negatives (i.e. approval of harmful compounds).

In the past, when focused investigations of the TQTS type were not performed regularly, cardiac repolarization liability of some drugs was found far too late – only after fatal complications were observed.[11,12] Typical examples are that of terfenadine and cisapride, for which even large population registries of many tens and hundreds of thousands of patients failed to identify the torsadogenic proarrhythmic risk[13-15] that was subsequently well recognized.[16,17] Unfortunately, these lessons appear to be slowly learned. Registries with fewer than 20 000 patients are still quoted as definite proofs of the absence of proarrhythmic liability.[18]

Occasionally, criticism is also made pointing out that while possible TdP liability needs to be thoroughly investigated, other rare adverse reactions are not considered during benefit-risk evaluations for drug approval. It is true that there are other rare adverse drug reactions with a similar or possibly even greater overall risk than that of the drug-induced torsadogenic proarrhythmia, which are not regularly investigated thoroughly. Indeed, for drug complications such as aplastic anaemia[19,20] or allergies,[21] no counterpart to TQTS exists. This is because, at present, there is no investigative surrogate for these rare reactions that might be tested for during the early stages of drug development. Should such a surrogate be found, it is likely that corresponding thorough investigations would be required.

QTc interval prolongation is also frequently criticized as the clinical surrogate of torsadogenic proarrhythmia. As a predictor of drug-induced TdP, this surrogate is indeed not perfect.[22] While every drug that has so far been observed to cause TdP tachycardia in a meaningful number of cases (i.e. above the regulatory awareness threshold) has also been found to prolong QTc interval substantially if administered at high doses or metabolically multiplied,[22] the opposite is not true. There are drugs that cause moderate QTc interval prolongation while not readily triggering TdP.

In TQTS, however, QTc interval prolongation is not used as a surrogate of TdP risk. It is used as a surrogate of drug-induced changes of cardiac repolarization that might require further investigations in susceptible patients. This is the reason for the interpretation of positive and negative TQTS, as already discussed.

There is also an ongoing discussion on the question of whether TQTS are needed for drugs that have a negative preclinical profile in terms of TdP liability. In isolated cases, the opinions of different regulatory agencies on this question might possibly differ. Ranges of preclinical tests indeed offer probes into the mechanisms by which the drugs might alter myocardial repolarization. Nevertheless, since the concordance between TQTS results and preclinical investigations is not entirely persuasive, and since the discussions are ongoing,[23] TQTS investigations are needed irrespective of the result of preclinical studies.

From time to time, suggestions are also made to replace QTc interval prolongation in TQTS with the study of other ECG characteristics, mostly concentrating on various T-wave morphological descriptors. Different studies are usually quoted showing that mortality or arrhythmia risk

prediction in cardiac patients was found to be more accurate based on different T-wave morphology quantifiers compared with the prolonged QTc interval.[24-27] To a large extent, these suggestions are missing the point. Detection of drug-induced repolarization changes in TQTS is needed rather than quantification of general risk in cardiac patients. Also, it is not obvious whether a change in T-wave morphology observed with one drug would be equally applicable to other drugs.[28] Thus, while future regulatory use of T-wave morphological investigations cannot be excluded, these studies presently play a mostly research role.

3. Drug Dose

Study designers frequently query the appropriate dose to be investigated in TQTS. Very frequently, a general drug-independent guidance is requested specifying that it is sufficient to investigate two or three times (or simply *x* times) the therapeutic dose of a compound. Unfortunately, such a question is based on misunderstanding.

The objective of TQTS is to evaluate the dose- and concentration-response relationship for QT/QTc interval prolongation, including exploration of concentrations higher than those achieved following the anticipated therapeutic dose. At a minimum, the investigated dose should cover the expected increase in steady-state drug and metabolite exposures due to intrinsic (e.g. renal, hepatic, age-related) and extrinsic (e.g. metabolic inhibition, food effects) factors in the patient population taking the labelled dose. These considerations are naturally drug dependent and, thus, a generally applicable ratio between the therapeutic dose and the appropriate TQTS dose cannot be provided.

The TQTS objective is best achieved by the maximum tolerated dose. In cases where the maximum tolerated dose has not been identified prior to conducting TQTS, the correct dose is the largest dose that can be safely administered and which is expected not to invalidate the ECG readings (e.g. because of tolerability and/or autonomic adverse effects).

It has also become customary to include the standard therapeutic dose as a separate part of TQTS (i.e. a separate crossover period or separate parallel arm). If TQTS is negative at the supratherapeutic dose, it can be assumed that lower doses will also not have a relevant effect on the QTc interval. Hence, if the sponsor has good reasons (e.g. from earlier studies) to believe that the TQTS will be negative at the supratherapeutic dose, investigation of the standard therapeutic dose can be omitted without any regulatory implications. If the TQTS is positive and the supratherapeutic dose level prolongs the QTc interval in a concentration-dependent manner, then exposure-response modelling can be used to compute the expected effect on the QTc interval at clinically relevant concentrations.[29] The accuracy of this estimate can be improved by collecting ECGs at concentrations corresponding to the therapeutic dose.

With the exception of the rare cases in which the therapeutic dose is also the maximum tolerated dose, investigating only the therapeutic dose in TQTS is not sufficient. A number of torsadogenic drugs prolong QTc interval only minimally when administered at standard therapeutic doses,[30,31] while showing very clear QTc signal when overdosed and/or metabolically multiplied.[11] (Note that by 'torsadogenic drugs' we do not mean the rare, exceptional cases mentioned in section 1 where drugs do not prolong QTc while having been reported to cause TdP in frequencies well below the 'regulatory awareness threshold'.) Therefore, QT studies of only therapeutic dose ranges can even produce false negative results and might thus be misleading.

4. Study Population

TQTS are usually conducted in populations of healthy subjects. Since TQTS are designed to rule out that the investigated drugs have the potential to delay cardiac repolarization, they address a pharmacology question for which the population of healthy subjects is appropriate. As already discussed in section 1, only if TQTS is positive is subsequent investigation of susceptible patients (e.g. those from the target population who also

have hepatic or renal impairment, heart failure, advanced age, etc.) needed to answer the clinical safety question of proarrhythmic liability.

The concept of enrolling highly susceptible patients into TQTS is also discussed from time to time. Such a concept can hardly be recommended. Administering overdoses of the drug in this population (in order to fulfill the goals of TQTS) would make such a study ethically problematic. Also, the necessary precision of ECG measurements is difficult to achieve in patients with renal or hepatic impairment or in heart failure since the underlying condition has variable ECG effects.[32] Some compounds (e.g. neurological agents) are not tolerated by healthy subjects when administered in high doses. TQTS of such compounds need to be investigated in target populations,[33] but in that case, susceptible patients with other complicating conditions (e.g. those with prolonged QTc interval at baseline) are usually excluded. For the same reasons, patients with advanced age, or children, are usually not enrolled into TQTS.

Hence, unless the drug cannot be administered to healthy subjects, conducting TQTS in healthy individuals is preferable irrespective of the target population and/or intended use of the drug. Compared with patients, a high level of ECG precision is more easily achievable in healthy subjects, and their autonomic conditions and responses to environment are easier to control.

Opinions occasionally differ about the sex composite of the TQTS population. Women are well known to have a longer QTc interval than men[34] and are also more susceptible to TdP, which has possible consequences for drug development.[35] Whether women are also more susceptible to drug-induced QTc interval prolongation is a matter of debate. Because of this debate, it can be presently recommended that the TQTS populations include both women and men (e.g. in equal numbers), although separate evaluations of the TQTS outcome criteria in women and men are not needed as long as randomization is stratified by sex. In other words, strict ICH E14 criteria (all one-sided upper bounds of 95% CI <10 ms) do not need to be tested separately in women and men. However, descriptive evaluation of QTc changes in women and men separately might be helpful in depicting different QTc response between sexes.

5. Study Duration

While the investigations of drug-induced QTc changes are often conducted after multiple drug doses, discussion of whether a single dose would be sufficient is not infrequent. The arguments range from fast drug clearance to (irrelevant) comparisons with a single dose of moxifloxacin used as a positive control. Several considerations need to be made before a single-dose TQTS is designed.

As already explained, TQTS needs to model the effects of the drug under almost the worst possible circumstances. This does not concern only the administered drug but also all metabolites, perhaps including secondary and tertiary metabolites, formed at >10% of parent drug systemic exposure at steady state.[36] Hence, with the exception of compounds that are clinically aimed at single administration only (e.g. contrast agents), single-dose TQTS are generally acceptable if the drug is not extensively metabolized, has a short half-life and has time-independent pharmacokinetics (e.g. the single-dose pharmacokinetic profile for the drug and metabolites can predict multiple-dose profiles). Most importantly, the investigated supratherapeutic dose must cover not only drug accumulation but also increases due to intrinsic and/or extrinsic factors for both the parent drug and the metabolites.

While in the past, assessment of proarrhythmic drug safety has occasionally been approximated from single-dose studies,[37] the described conditions can be difficult to prove for the majority of pharmaceutical compounds. Hence, unless the sponsor has a very good understanding of the metabolic profile to support a single-dose TQTS, reaching steady-state concentrations of the investigated drug and its metabolites should be the TQTS design of choice.

6. Positive Controls in Positive TQTS

Following the ICH E14 requirements, every TQTS needs to demonstrate ECG assay sensitivity. That is, it has to show that the study conduct

and ECG processing is capable of documenting small drug-induced QTc changes. At present, this is achieved by administering a drug with known QTc effects as a positive control. While other possibilities have been investigated,[38] it has become customary to use a single 400 mg dose of moxifloxacin.[39-41] Using a single 400 mg dose of moxifloxacin as a 'gold standard' for positive control can only be advocated, as experience with it has already accumulated, thus allowing careful judgement of the quality of TQTS conduct and evaluation. Therefore, unless there are good and well defined reasons to use a different positive control, a single 400 mg dose of moxifloxacin should be used to prove TQTS assay sensitivity.

Indeed, in carefully conducted studies, the population mean QTc changes after a single 400 mg dose of moxifloxacin not only closely follow the plasma concentration of the drug,[41] but their one-sided lower 95% CI also exceeds a 5 ms QTc prolongation in at least one of the study timepoints, which is the presently accepted test of moxifloxacin-based assay sensitivity. Regrettably, authors of TQTS sporadically claim to have achieved assay sensitivity while using much weaker, and thus less persuasive, criteria.[42]

The question occasionally arises whether it is necessary to include such an independent test of assay sensitivity when investigating a drug that has already been observed to prolong QTc interval and when the sole goal of TQTS is to characterize the ECG effects of the drug more accurately. The usual argument goes that if demonstrating QTc interval prolongation by the investigated compound, the study would have already demonstrated the capability of finding such drug effects. Nevertheless, no measurement can provide accuracy assessment for itself. Thus, if the goal of a TQTS is to characterize the QTc prolongation of a drug known to prolong QTc more accurately, and if such a characterization should play any role in regulatory benefit-risk discussions, an independent positive control is needed to show the validity and accuracy of the study. Indeed, even if the same ECG recordings are analyzed differently, markedly different observations can be made (figure 1).

7. Comparator and Positive Control

If the investigated compound belongs to a therapeutic class in which cardiac repolarization liability has previously been observed, it might be useful to include a comparator from the same drug class into the TQTS design. This allows estimating where the investigated drug falls in the spectrum of repolarization effects of the therapeutic class and may thus serve a useful role in benefit-risk evaluation of the new pharmaceutical. The historical study of ziprasidone is a good example of such an approach,[45] although it cannot be used as a model of contemporary TQTS design (among others, the ziprasidone study[45] was not placebo-controlled, contained too few ECG readings and did not cope accurately with drug-induced heart rate changes). Naturally, the doses and exposure of the comparator or comparators must be carefully selected so that no bias exists towards or against the investigated drug.

The same-class comparator can only rarely be used also as a positive control. The class comparator has typically not been evaluated in TQTS. Consequently, the expected effect size of the comparator is not known beforehand, which precludes establishing assay sensitivity. To illustrate this point, even when considering TQTS of a new quinolone antibacterial, it would be difficult to use the same moxifloxacin administration for assay sensitivity and as a class comparator. A class comparator needs to be administered in the same or corresponding dosing regimen as the investigated drug, whilst the moxifloxacin-based assay sensitivity must use a single dose.

On the contrary, comparing the results of the investigated drug with the results obtained with the positive control compound of a different class serves no useful purpose. The positive control is used for the sole reason of establishing that the conduct of the study (including all the ECG processing and measurements) is capable of documenting small drug-induced QTc changes. It has no role in setting safety limits. Thus, suggestions that a positive TQTS should have different regulatory implications because the investigated drug prolonged QTc interval less than, say, the

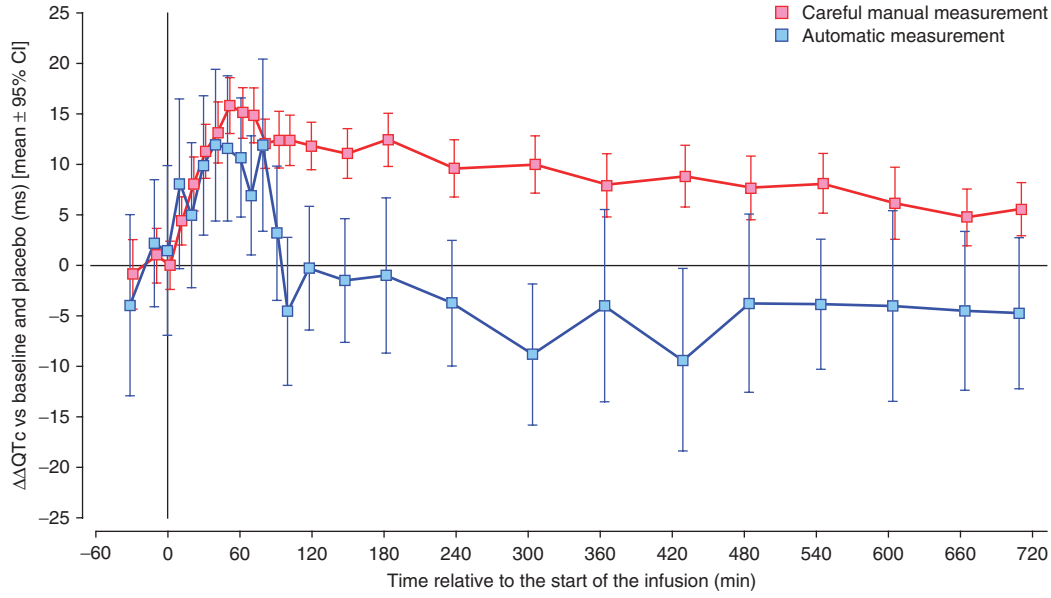

Fig. 1. Baseline and placebo-corrected changes in QTc interval during and after 1-hour moxifloxacin 400 mg infusion. The same ECG recordings were carefully measured manually (red graph) and processed with the standard QTc measurement algorithm implemented in an ECG machine (blue graph). Note that while the manual measurements led to clear QTc signal satisfying the assay sensitivity criteria, the effect estimated by the ECG machine algorithm failed the strict assay sensitivity test since the 95% CIs of all point estimates include 5 ms QTc prolongation. Nevertheless, this comparison shows only that different ECG processing techniques applied to the very same ECG recordings can lead to different results. It must not be interpreted as a suggestion that an automatic ECG reading is bound to be inferior to manual reading, which does not need to be the case,[43] especially if modern automatic algorithms are used.[44] Data extracted from a previously published study.[41]

single dose of moxifloxacin, are misplaced. For that reason, in general, the same class comparator should not replace the positive control which is used to establish assay sensitivity.

8. Placebo Control and Subject Conditioning

Another topic that is frequently discussed is whether placebo control is truly needed in TQTS or whether it might realistically be replaced with comparisons to drug-free baseline. Since it has been documented that the unprovoked QT/RR relationship is fairly stable in each healthy subject,[46] it is argued that placebo control is unnecessary since baseline recording may replace placebo data, thus saving one crossover period or one parallel arm of the study.

Unfortunately these arguments are misplaced. While it is true that repeated recordings made under the same conditions in healthy subjects result in a fairly reproducible individual QT/RR relationship, it is not true that the baseline and on-treatment recordings in TQTS can safely be regarded as being made under the same conditions. Although investigations into the exact mechanisms are ongoing, it is clear that the QTc interval is under the influence of the autonomic nervous system, which is in turn affected by the TQTS procedures. Although such an influence can be eliminated or at least suppressed by very careful clinical handling of study subjects, the placebo (i.e. just the conduct of the study on its own) is frequently found to shorten the QTc interval. Indeed, the expected effect of moxifloxacin-based positive control is frequently established only after placebo correction, since, in some studies, a single 400 mg dose of moxifloxacin may not prolong QTc intervals enough compared with drug-free baseline (figure 2).

Therefore, drug-free baselines and on-placebo recordings cannot be considered equivalent.

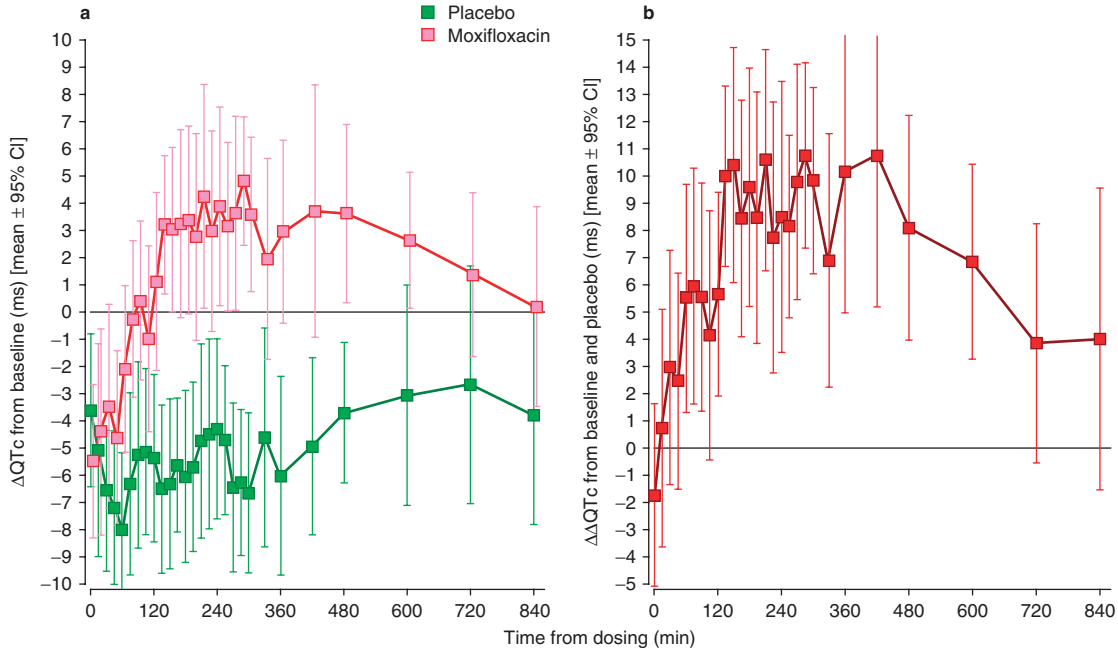

Fig. 2. Example of placebo and moxifloxacin QTc effects from an unpublished crossover thorough QT/QTc study (TQTS) in which an encapsulated 400 mg moxifloxacin was used as a positive control. (**a**) Baseline-corrected QTc changes after moxifloxacin and placebo administration. (**b**) Baseline- and placebo-corrected QTc changes after moxifloxacin administration. Each curve shows the result in the study subpopulation in whom the data were available (the placebo and baseline corrected QTc changes on moxifloxacin were assessed in a subpopulation of study subjects in whom both moxifloxacin and placebo periods were available, i.e. the graph in figure 2b is not a simple subtraction of graphs in figure 2a).

Consequently, the placebo control of TQTS is a crucial part of their design. Without placebo adjustment, the studies might lead to false negative conclusions if the placebo effect (i.e. the study conduct) shortens QTc or might lead to false positive conclusions if the placebo effect prolongs QTc.

This also means that the conditions (environmental, clinical and otherwise) at which the ECG recordings are obtained in subjects receiving placebo and active treatment (as well as those receiving the positive control) should closely replicate each other. Even when studying a drug that requires a prolonged titration period, placebo must be 'titrated' over an equally prolonged period. Serial administration with a short placebo period followed by a prolonged active treatment period is clearly a poor and unacceptable TQTS design.

From a design point of view, placebo also serves as a negative control group to fulfill the randomization requirement for a TQTS. Baseline data are needed to reflect the true condition of the participants, and a placebo arm is needed to guarantee the validity of statistical comparisons between the different treatment groups. Because of the same considerations, all clinical and environmental provocations should be carefully standardized during the study. For instance, while blood sampling is needed synchronous with either selected or all ECG timepoints, ECG readings should be made before blood is drawn so that autonomic and/or psychological influences on the ECG are avoided.

Subject conditioning is frequently overlooked or largely neglected in TQTS designs. For instance, while it is known that a simple supine position does not guarantee heart-rate stability,[47] this unsubstantiated belief (figure 3) is repeated in TQTS designs over and over again.[48] If a continuous ECG recording is made, the stability of heart rate preceding the QT interval measurement

should be verified rather than only assumed, based on simple manoeuvres.

For these reasons, clinical procedures and environmental factors of the study are also important for the positive control. Although preference should be given to administering moxifloxacin under double-blind conditions with the same medication appearance as for active treatment and placebo, even when it is administered open-label, the study procedures involved in assay sensitivity should closely mimic those of placebo and active treatment arms or periods. The very same schedule of ECG collection and evaluation must be used not only for the investigated drug and placebo but also for the positive control. Even when open-label positive control is used (instead of the preferable double-blind administration) it needs to be carefully assured that the procedures involved in ECG measurement of all the recordings are the same (e.g. the ECG laboratory must be carefully blinded as to which recordings were obtained on positive control, and/or the very same computer algorithm for ECG measurement must be used).

9. Frequency of ECG Acquisition

It is usually believed that the number of datapoints at which the drug-induced QTc change is tested has an influence on the study power. However, the truth is that the study power is determined by the shape of the true mean difference between the study drug and placebo profile after baseline adjustment, supposing that the study variability and the data correlation stay the same;[49] therefore, it is not very helpful for pharmaceutical sponsors aiming at reducing the number of ECG datapoints as much as

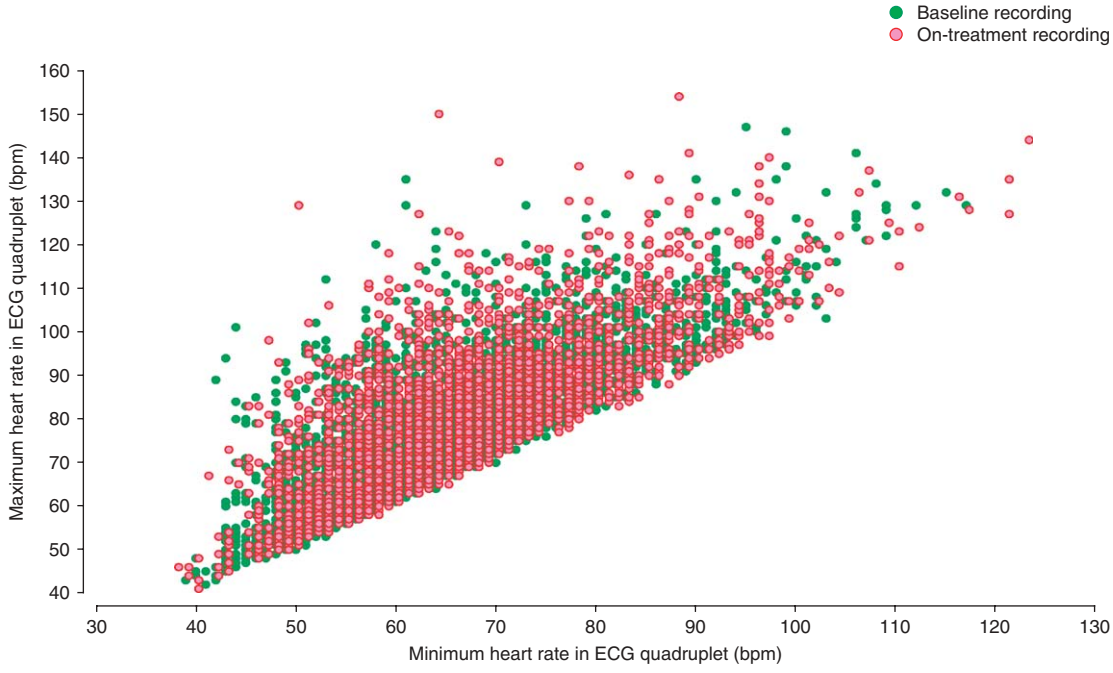

Fig. 3. Heart rate instability in a thorough QT/QTc study (TQTS) that relied on simple subject positioning. Per protocol, each subject was in a supine position for 5 minutes before four replicated ECG readings were extracted from a continuous 12-lead Holter recording. In each extracted ECG, a commercial central laboratory measured heart rate based on three RR intervals. The graph shows a scatter diagram between the slowest and fastest heart rate measured in each quadruplet of extracted ECGs. The readings at baseline and on-treatment are shown in green and red, respectively. Note that despite the protocol assumption, the measured heart rate frequently ranged substantially – more than 15 beats/minute (bpm) in 34% of the ECG quadruplets. (To avoid overlay, the red scatter is shifted 0.3 bpm to the right.)

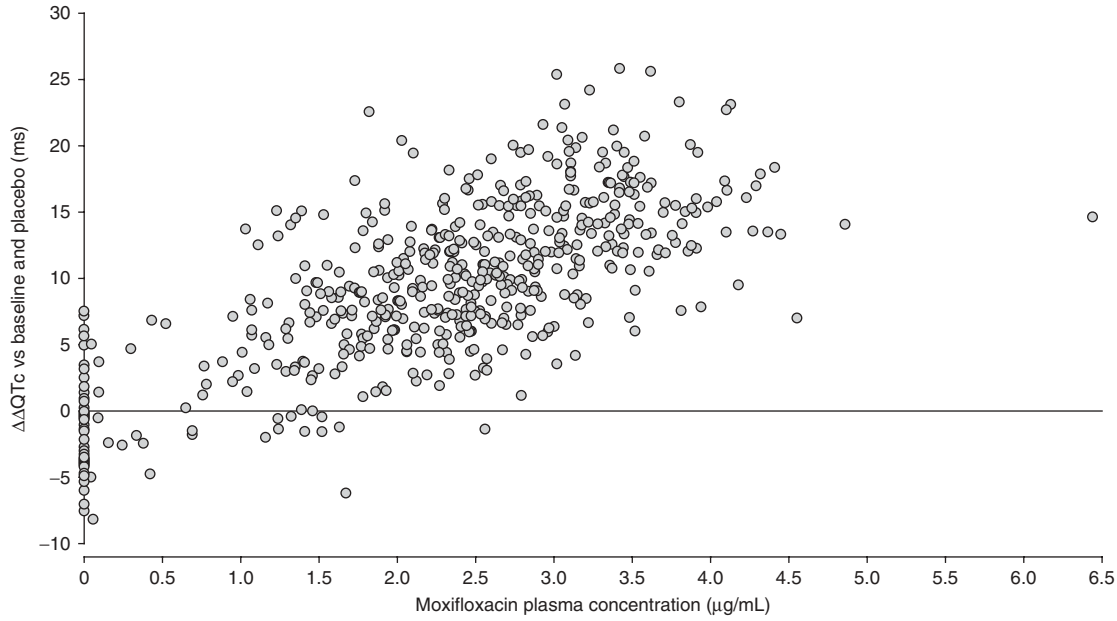

Fig. 4. Example of outlier analysis of moxifloxacin QTc effects in an unpublished thorough QT/QTc study (TQTS). Note that while the scatter diagram shows clear QTc increases with increasing moxifloxacin plasma levels, all the values are below the conventional +30 ms limit of QTc prolongation.

possible. It is frequently proposed that recording the ECGs only around the expected time to reach the maximum plasma concentration of the investigated compound is sufficient. Unfortunately, this is a poor practice that should be avoided.

Collected ECGs should cover peak concentrations of both the parent drug and of the metabolites. Moreover, later timepoints are needed to assess the potential for delayed effects (e.g. the human ether-à-go-go-related gene [hERG] trafficking,[50] concentration/QTc hysteresis [that is, the possible delay between QTc changes and plasma concentration] due to the difference between plasma and tissue levels, or metabolite formation). No general rule can be given that *x* number of half-lives of the parent drug is sufficient for ECG data collection. If a single dose is administered (even for drugs with short half-lives), ECGs should be obtained for at least 24 hours. If multiple doses are administered over several days and steady state is achieved, ECGs over the full dosing interval are expected.

10. Central Tendency and Outlier Analysis for Drug Effect

Based on historical experience,[51] the ICH E14 document requires that the data of each TQTS are evaluated in terms of the central tendency (or investigational drug and placebo time-matched analysis) of QTc changes as well as in terms of outlier analysis, i.e. listing the number of ECGs in which the drug-related QTc prolongation exceeds pre-specified thresholds, as well as a number of subjects in whom such above threshold QTc increases are observed. When the maximum central tendency effect is below the ICH E14 limit, this dual evaluation usually does not cause any problems. Recent advances in ECG technologies led to marked improvement in the precision of ECG measurements and, thus, substantial ECG outliers are hardly ever observed in negative studies.

When the central tendency and exposure-response analysis documents drug-related QTc prolongation at clinically relevant levels of exposure, the outlier analysis might potentially be

misleading. Not infrequently, pharmaceutical sponsors of these TQTS try to rely on the outlier analysis to lessen the central tendency finding. However, such argument based on entirely or nearly negative findings of an outlier analysis from a relatively small-sized TQTS is misguided.

If the ECG measurements are organized very diligently and if the modern technologies of pattern classifications are used,[52] excesses of QTc interval prolongation above the standard limit of 60 ms are found only with compounds that lead to massive repolarization changes. Even with the standard moxifloxacin positive control, which is an example of a clearly positive TQTS of a drug that causes TdP with a frequency that should play a role during regulatory benefit-risk evaluation (i.e. above the 'regulatory awareness threshold'),[53,54] outlying QTc values might be entirely absent (figure 4). Therefore, it seems reasonable to propose that while a clearly positive outlier analysis of TQTS, i.e. a statistically significant difference in the number of outliers on active treatment compared with placebo, is indicative of repolarization liability of the investigated drug, negative outlier analysis of TQTS does not have much informative value.

On the contrary, the outlier analysis is crucial in safety ECG investigations in susceptible patients for drugs found to prolong the QTc interval. Since, in these patients, the ECG measurements are frequently variable because of their underlying clinical conditions, the precision needed for an accurate central tendency analysis is difficult to achieve and the CIs of the mean QTc effects can be very wide. Thus, in these safety studies, the outlier analysis has a more important role in identifying patients at increased risk for arrhythmia. Therefore, the timing of safety ECGs is essential for an informative outlier analysis.

11. Baseline Definition

The baseline correction in TQTS is needed because, even with the same environmental conditions, QTc intervals vary substantially from subject to subject. Since the primary objective of

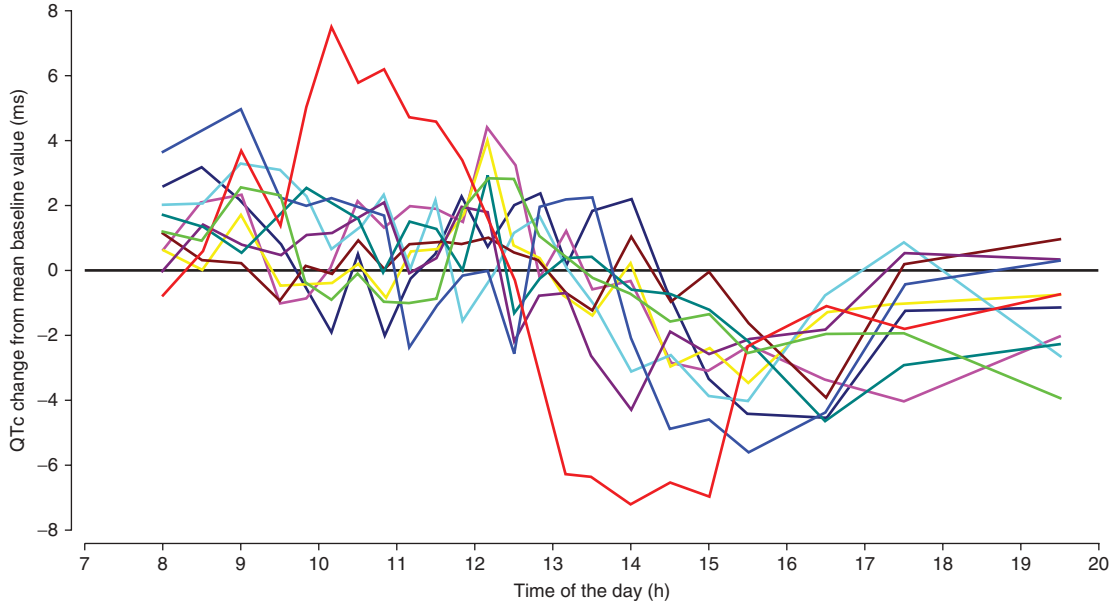

Fig. 5. Examples of daytime baseline intrasubject changes in QTc. The graphs show differences from an averaged subject-specific QTc baseline value (individual correction) calculated in ten subjects enrolled into an unpublished thorough QT/QTc study (TQTS) [different colours correspond to different individuals]. Note that whilst a common pattern can be seen with QTc decreases in the early afternoon hours, the profiles are not synchronized in individual subjects.

a TQTS is to compare the mean difference of the study drug and placebo over the entire time course, adjustment for baselines is necessary.

The simplest solution is to collect a full day, time-matched baseline on the day preceding the (first) dosing day.[55] Since the QTc values also show intra-subject stability, it is unnecessary to have a full day, time-matched baseline for each period of crossover studies. Baseline measurement at each period, just before treatment, on the same day as dosing to account for possible period effect, might be enough. Using only one baseline, or averaging the baselines of all periods where they exist, to design individual correction of QT interval for heart rate is sufficient if individual correction is needed.

On the other hand, for a parallel study the usual practice is to collect a whole day of time-matched baseline values on the day (or days) prior to dosing to help adjust for within-subject diurnal and inter-subject variability. Recently, proposals were made to use the average of all baseline readings in parallel studies to reduce the variability of the measurements and increase analysis efficiency. Mathematically, it is easy to prove that the variance of the difference between the post-dose and the time-matched baseline is larger than that between the post-dose and the whole day averaged baseline. However, this practice cannot be recommended since it can change the primary endpoint. Since the circadian pattern of QTc is not the same in each subject, (figure 5) and since the clinical conduct and environmental conditioning of the study can change the circadian profile even on placebo (as already shown in figure 2), both false positive and false negative results might be obtained if time-matched baseline adjustment is not used in parallel studies.

12. Conclusion

The design and conduct of TQTS need to reflect the goal of these investigations. It is wrong to approach the ICH E14 requirements merely as a red-tape exercise. Poor TQTS design risks not only the rejection by regulatory authorities but also, and much more importantly, risks deceiving the sponsor of the new compound in terms of its safety. The design of each TQTS must carefully reflect the character of the investigated pharmaceutical. Relying on stereotyped design templates in which only the name of the drug is changed is bound to backfire sooner or later.

Moreover, the criterion for assessing whether a drug prolongs QT/QTc as described in the ICH E14 guideline does not explicitly account for individual drug plasma concentration. The existing experience with reviewing TQTS indicates that understanding the relationship, if any, between individual drug concentration and QT/QTc change may provide important additional information to support regulatory decision making.[29] A consideration of the totality of evidence, i.e. results from the analysis specified in the ICH E14 guideline and from concentration-response analysis, may allow a more informed decision regarding whether a drug prolongs QTc or not, as well as the need for dose adjustment in special populations for benefit-risk based assessments. It can only be recommended that sponsors conduct analyses to explore these relationships, in addition to performing the analyses specified in the ICH E14 guideline.

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Correspondence: Professor *Marek Malik*, 16 Verulam Avenue, Purley, Surrey CR8 3NQ, UK.
E-mail: marek.malik@btinternet.com