Should You Run a Dedicated TQT Study? Sponsor and Regulatory Considerations on Substitution Pathways to Assess QT Liability

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Cardiac safety regulatory guidance for drug development has undergone several monumental shifts over the past decade as technological advancements, analysis models and study best practices have transformed this landscape. Once, clinical proarrhythmic risk assessment of a new chemical entity (NCE) was nearly exclusively evaluated in a dedicated thorough QT (TQT) study. However, since the introduction of the International Council for Harmonisation (ICH) E14/S7B Q&A 5.1 and 6.1 TQT substitutions, drug developers are offered an alternative pathway to evaluate proarrhythmic risk during an ascending dose study in healthy volunteers or during a powered patient study, respectively. In addition, the findings as well as the manner in which nonclinical studies are conducted (i.e., utilizing best practices) can dictate the need for a positive control in the clinical study and/or affect the labeling outcome. Drug sponsors are now faced with the option of pursuing a dedicated TQT study or requesting a TQT substitution. Potential factors influencing the choice of pathway include the NCE mechanism of action, pharmacokinetic properties, and safety profile, as well as business considerations. This tutorial will highlight the regulatory framework for integrated arrhythmia risk prediction models to outline drug safety, delineate potential reasons why a TQT substitution request may be rejected and discuss when a standalone TQT is recommended.

HISTORICAL BACKGROUND

An undesirable property of certain drugs is their potential to delay ventricular repolarization, which is manifested on electrocardiograms (ECGs) as lengthening of the QT interval. This QT lengthening, although an imperfect surrogate marker for arrhythmia risk, has in turn been associated with life-threatening and lethal ventricular arrhythmias, most notably being Torsades de Pointes (TdP).[1]

It has been known for over 50 years that antiarrhythmic medications, beginning with quinidine, were linked to fatal ventricular arrhythmias due to prolongation of the QT interval. However, it was not until the early 1990s that the antihistamine terfenadine (Seldane) and subsequently multiple classes of non-antiarrhythmic drugs were also recognized to have the potential to prolong the QT interval resulting in serious cardiac arrhythmias and unexpected morbidity and mortality. As a result, between 1988 and 2001, 10 previously approved drugs were withdrawn from the market because of TdP-related events.[2]

In the ensuing years, the primary electrophysiologic substrate for QT prolongation was determined to be the downregulation of several potassium currents responsible for ventricular repolarization and less commonly upregulation of the late sodium current. Chief amongst the potassium currents was blockade of the delayed inward rectifier potassium channel (IKr) encoded by the human ether-a-go-go gene (hERG) channel (reviewed in Lester *et al.*[3]). This mechanistic understanding of TdP coupled with clinical reports of non-cardiac drugs causing sudden death prompted regulators to convene a multinational conference in 2005, the International Council for Harmonisation (ICH), for the purpose of harmonizing guidance regarding appropriate clinical (ICH E14) and nonclinical (ICH S7B) studies to profile a new chemical entity's (NCE) effects on ventricular repolarization.[4] This seminal guidance specifically required that all new small molecules that have systemic bioavailability undergo rigorous hERG and QT evaluation in both nonclinical and clinical cardiac safety studies, respectively. In addition, recent Food and Drug Administration (FDA) draft guidance on oligonucleotide drug development also recommends assessment for QT interval prolongation and proarrhythmic risk, given that this is a relatively novel class of therapeutic agents with limited cardiac liability data.[5] However, this recommendation has been recently called into question as a review of approved oligonucleotide drugs failed to demonstrate a significant impact on the QT interval. As such, full characterization beyond best practice involving *in vivo* non-rodent and first-in-human (FIH) studies was viewed as "inefficient" and probably unwarranted.[6]

There are exceptions to a NCE's QT evaluation. These include biological drugs (such as monoclonal antibodies) and large targeted proteins, which have little if any direct impact on myocardial cells via the hERG channel, topical agents with no appreciable systemic exposure or those with localized distribution, and combination drugs in which each of the components have been

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evaluated for safety without evidence of any QT prolonging effect.[7] Additionally, oncologic agents under development for patients with advanced cancer and life-threatening disease, per ICH S9 (Nonclinical Evaluation for Anticancer Pharmaceuticals), may not be required to undergo standalone nonclinical safety pharmacology assessments prior to patient trials.[8]

EVOLUTION OF ICH E14 SAFE DRUG DEVELOPMENT: THE EARLY YEARS

The initial guidance to ensure safe new drug discovery outlined in the ICH E14[7] and S7B[9] documents involved challenging and resource intensive undertakings with the clinical study and nonclinical assays viewed as complimentary rather than integrated investigations. ICH E14 recommended performance of a randomized, placebo and positive-controlled, 4-arm clinical trial termed a thorough QT (TQT) study, designed as the definitive investigation to assess a drug's effect on the QT interval using the Intersection Union Test (IUT) or by timepoint analysis as the primary analysis tool. The TQT has been viewed and accepted for almost two decades as the cornerstone study for assessing a pharmaceutical's effect on ventricular repolarization. The conventional TQT study design incorporates a large number of subjects and is powered to determine if the "upper bound of the 95% one-sided confidence interval for the largest time-matched mean maximal effect of the drug on the [corrected QT for heart rate] QTc interval excludes 10 milliseconds."[7] This result would be viewed as a negative study, while QTc values exceeding 10 milliseconds would be considered positive for delayed ventricular repolarization.[3,10] TQT studies can be performed either early or later in drug development. Some sponsors prefer to wait for pharmacokinetic (PK) and proof-of-concept (POC) studies to be completed, while others have chosen to conduct the study during the early stages of drug evaluation in an effort to de-risk the compound and leverage the information for commercial purposes.

In a standard format TQT study, the drug levels to be assessed include therapeutic and supratherapeutic doses along with a positive control (usually oral moxifloxacin) and a placebo. The study design is dictated by the PK properties of the drug, for example a single dose 4-way crossover for short half-life drugs vs. a single dose parallel design for long half-life compounds. The crossover design with a predose baseline offers the advantages of smaller numbers of subjects, less variability of data and easier correction of heart rate as subjects serve as their own controls, thus accounting for diurnal QT variation. A single-dose parallel design allows a shorter duration, avoids potential aliasing or carryover effects, and mitigates against the risk of subject dropout. While not strictly necessary for the concentration QTc modeling (C-QTc) analysis in a parallel design, in our experience time-matched within baseline recordings in addition to placebo correction, can reduce variability since the subjects on active treatment will be corrected with a different set of subjects on placebo. The parallel design is also considered for a multiple dose study when the drug's PK is time-dependent, if there are active metabolites, or if accumulation is observed at steady state and a single-dose administration cannot produce a high enough exposure observed in a clinical setting to discern any potential QT effects.

Although they address different statistical hypotheses, either the IUT or C-QTc have been used and are acceptable to analyze the datasets from a TQT study, with the former historically representing the most commonly adopted primary statistical method.[11,12] On the other hand, the IUT is not appropriate for the smaller sample size of FIH study designs using C-QTc modeling as the primary analysis tool unless the magnitude and time course of QT effects at each timepoint are rigorously assessed. More recent TQT protocols are adopting C-QTc analysis as the primary analysis modality, as this permits a significant reduction in the number of study subjects and therefore considerable time and cost savings.

Finally, it should be underscored that the main purpose of the TQT study is to determine whether a threshold effect on the QT interval is present so as to guide the intensity and timing of ECG acquisitions in further drug development. It was and is not designed to provide risk assessment of the probability of TdP or other serious ventricular arrhythmias.

EVOLUTION OF ICH E14 AND SAFE DRUG DEVELOPMENT: THE LATER YEARS

In an effort to shorten the timelines and combat the high costs of executing a TQT study while still being able to accurately identify a QT safety signal, a novel study design was introduced in 2014 in which a smaller number of subjects was administered approved drugs in an ascending dose protocol.[13] QT liability was then determined utilizing C-QTc as the primary analysis tool as the sample size was too small to permit adequate QT evaluation using the IUT. The trial results demonstrated concordance between the QT effects of the test articles and their known QT effects based upon previously conducted TQT studies with each of the selected drugs. This innovative design involving a small number of subjects was instrumental in validating the utility of C-QTc in ascending dose protocols as a potential substitution for TQT studies. In the last decade, C-QTc has played an increasingly important and preferred role in evaluating the QT effects of candidate drugs on ventricular repolarization leading to an initial reduction in the number of TQT studies being completed, from 74% in 2016 to 46% the following year (**Figure 1a**). In this regard, the 2015 ICH E14 Q&A helped to expand and solidify the central role of C-QTc for QT assessment in FIH studies further contributing toward replacement of resource-intensive traditional TQT trials with less expensive and more efficient study designs.

To adequately characterize whether a pharmaceutical delays ventricular repolarization in healthy volunteers utilizing C-QTc in early-stage clinical trials that do not include a positive control, it was suggested that drug exposures should exceed twofold the worst-case clinical scenario accounting for both intrinsic (e.g., renal or hepatic disease and genetic polymorphisms) and extrinsic factors (e.g., food or drug–drug interactions) that may alter a drug's maximal exposure.[11] However, it is not always possible to achieve a twofold worst-case scenario due to factors such as saturation absorption, safety, or tolerability concerns. To address this shortcoming, the International Working Group (IWG) in August of 2020 drafted guidance entitled "Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential: Questions and Answers."[14] A final ICH E14/S7B Q&A guidance

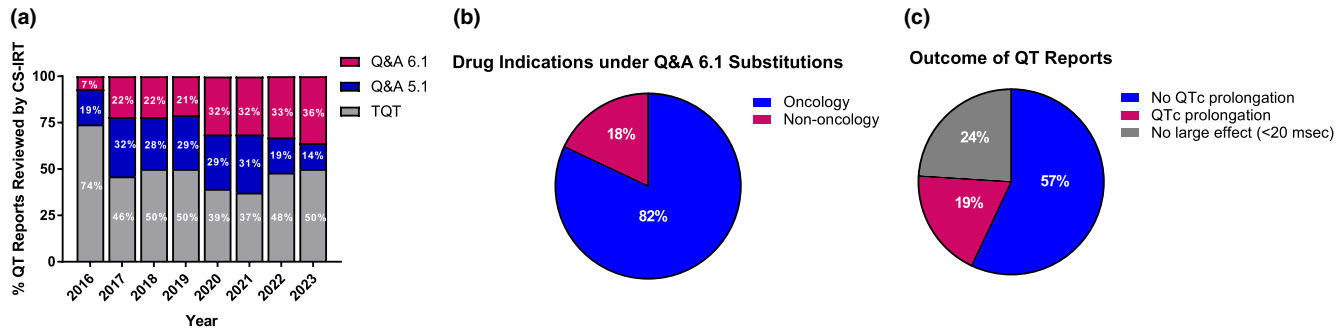

Figure 1 Finding from QT reports reviewed by CS-IRT. (**a**) Percent of QT reports reviewed by CS-IRT over 8-year period, 2016–2023, by pathway. Adapted from a conference proceeding presentation.[34] (**b**) Percent of Q&A 6.1 substitution requests submitted from 2016–2020 by drug indication. (**c**) Conclusions from QT study reports submitted from 2016–2020 reviewed by CS-IRT. Data from (**b**) and (**c**) was adapted from Strauss *et al*.[17]

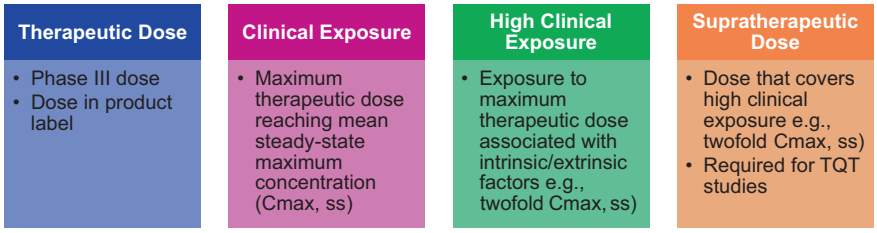

Figure 2 Key drug exposure definitions. Dose and exposure definitions for TQT studies and a TQT substitution request. Adapted from Lester.[16]

was published in February of 2022 that expanded the details of core nonclinical repolarization studies and described the role and performance of follow-up studies when indicated.[15] The goal of the updated Q&A document was to illustrate scenarios where nonclinical data could reduce the number of clinical studies and inform clinical regulatory decision making and product labeling.

Two pathways labeled ICH E14/S7B Q&A 5.1 and 6.1 were delineated placing increased emphasis and prescriptive detail on hERG and non-rodent *in vivo* animal studies. The concept of a "*double negative*" was introduced in which the aforementioned nonclinical assays were classified as negative if they did not demonstrate any proarrhythmic risk potential when tested at or above free drug concentrations corresponding to the anticipated high clinical exposure (HCE). In the setting of a "*double-negative*" scenario, alternative study designs would therefore be deemed acceptable to regulators *in lieu* of a formal standalone TQT study (reviewed in [16,17]). This nonbinding guidance also represented an important paradigm shift away from the previous QT centric focus toward introducing an integrated risk assessment strategy whereby nonclinical and clinical study results would be incorporated into a proarrhythmic risk prediction model.

SUBSTITUTION TQT STUDY CONSISTENT WITH ICH E14 Q&A 5.1: CONCENTRATION-RESPONSE ANALYSIS

An appreciation of alternative study designs and the role of C-QTc necessitates an understanding of drug exposure terms as detailed in **Figure 2**. In recent years, the pharmaceutical industry has witnessed the growing movement to utilize C-QTc in ascending dose escalation studies as the primary analysis tool to analyze a drug's PK-pharmacodynamic (PD) relationship. C-QTc has also proven beneficial in estimating effects of varied drug dosing regimens not directly studied or the influence of intrinsic and extrinsic factors on ventricular repolarization, such as the cytochrome P450 system. Furthermore, C-QTc may also play a role in TQT studies to clarify ambiguous QTc results obtained from IUT data analysis.

High-quality ECG collection and concentration–response data can be captured in early phase studies such as FIH single ascending dose (SAD) or multiple ascending dose (MAD) protocols. The FDA does not mandate cardiodynamic ECG collection during ascending dose trials and sponsors may elect to defer formal QT assessment to conserve costs. This may be the case if the sponsor's objectives are primarily tolerability, PK and generalized safety assessment or when they are waiting for POC information before proceeding with development, as an estimated 70% of candidate drugs do not progress beyond phase II.

For the Q&A 5.1 paradigm, 12-lead continuous ambulatory digital ECG recordings are recommended to discern the QT-PD effects of the test article. Triplicate cardiodynamic ECGs are obtained at multiple timepoints corresponding to the time of PK sampling so as to permit C-QTc modeling. This then becomes the primary analysis tool of SAD protocols that typically include three or more cohorts to explore a wide range of drug concentrations in an adequate number of healthy volunteers, where the highest clinical drug exposures are usually obtained. ECGs may also be secured in MAD studies, particularly if the candidate drug has a long half-life, shows time-dependent PK, has delayed effects or contains active metabolites. In MAD studies, ECG extractions with Holter devices are obtained after the initial study dose but are not required if a full spectrum of drug exposures were previously evaluated in a SAD protocol. They are indicated at the end of the trial when steady-state drug exposures are achieved with C-QTc applied in these cases as well. Moreover, when there are significant

metabolites representing at least 10% of the total drug related exposure at steady-state of the parent compound, these should be monitored for safety.[18] They should also be considered for C-QTc analysis, particularly in cases when hysteresis is explained by a metabolite or when the QTc time course is different from that of the parent. Lastly, with either SAD or MAD studies, sponsors have the opportunity to capture ECG data and store it for later review and analysis although most sponsors do not opt for this approach.

The *initial* version[19] of Q&A 5.1 from 2014 primarily focused on the expanded role of concentration–response modeling and did not provide prescriptive details about best practices for nonclinical assays, clear definitions of exposures and margins or how the results of these studies might be incorporated into the totality of evidence regarding proarrhythmic risk in support of a TQT "waiver." The original Q&A 5.1 pathway did provide commentary for drugs that could be administered to healthy volunteers in doses that would achieve sufficiently high exposures. The guidance required that the candidate drug reached an exposure margin that was equal to or exceeded twofold the HCE in order for a positive control to be waived. In this regard, it is of note that only 42% of submitted reports to the FDA between 2016 and 2020 achieved this exposure threshold.[17]

The *updated* 2022 Q&A 5.1 guidance[15] to the contrary, provided considerable detail about the role of nonclinical assays and reduced the target clinical drug exposure to simply the HCE rather than a sufficient multiple of the HCE. For a detailed summary of nonclinical best practice studies, which is beyond the scope of this tutorial, the reader is referred to recent reviews by Rossman *et al.*[20]; Darpo and Leishman[21]; and Vargas *et al.*[22] The Q&A 5.1 guidance document also introduced the concept of a "*double negative*," which refers to the nonclinical hERG and *in vivo* non-rodent animal studies both being negative for evidence of delayed ventricular repolarization when best practices are present. Coupling this information with a well-designed negative clinical study in healthy volunteers might then be categorized as a "*triple negative*", which would represent the ultimate dataset to support a TQT "waiver" or substitution (**Figure 3**).

In order for a Q&A 5.1 substitution request to have the highest likelihood of being granted, there are two different scenarios either of which would support a TQT substitution when specific criteria are satisfied (**Figure 3**). The first scenario reflects the *original* regulatory guidance and focuses primarily on exposure margins of the candidate pharmaceutical and involves the following criterion:

1. A clinical healthy volunteer ECG study attains a drug level of at least twofold the HCE and excludes QTc increase > 10 milliseconds at the geometric mean steady-state maximum exposure.

The second *updated* guidance scenario to support a substitution consists of the following three conditions:

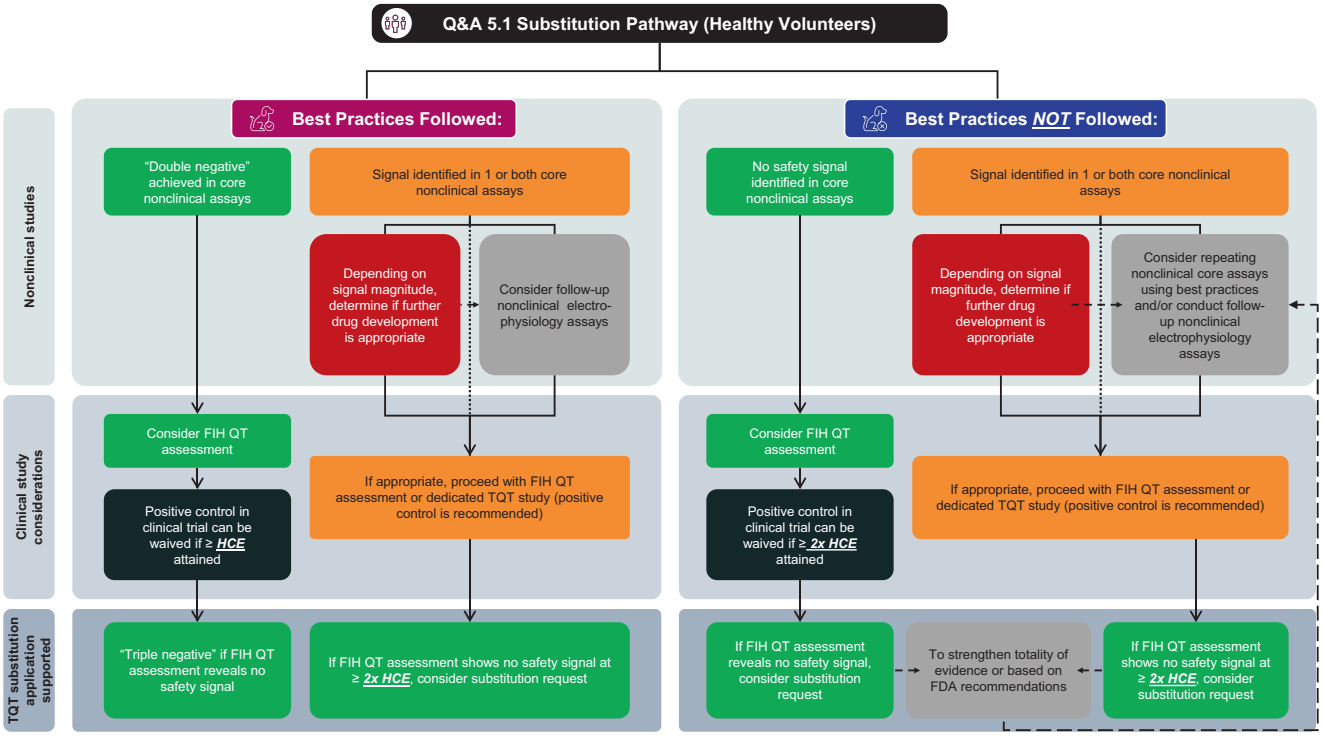

Figure 3 Examples of integrated cardiac liability assessments under Q&A 5.1. Drug developers have the option to run a dedicated TQT study or seek a TQT substitution as part of cardiac liability assessment for their new chemical entity. While not an all-encompassing list of options and outcomes, the figure depicts key inflection points in the decision making process. Under the Q&A 5.1 paradigm, core nonclinical assay results, the use of best practices, and the exposure margins attained contribute to the clinical study design and govern the need for a positive control in the healthy volunteer study. A "triple negative" refers to when both nonclinical and clinical data do not reveal a safety signal and the cardiovascular database does not document any arrhythmic events of concern. If positive signal(s) in the core nonclinical studies are detected when best practices are not followed, drug developers may repeat these investigations and/or consider additional follow-up electrophysiologic studies. The colored bubbles reflect key steps along the development pathway: Green—move drug development forward; Orange—proceed drug development with caution; Red—pause drug development and assess the situation.

1. The hERG study *employing best practices* fails to show any significant block by the test article with a robust safety margin of at least 30-fold, and possibly 50-fold as suggested by Ridder *et al*.[23] or as high as 100-fold as proposed by Abernathy and Leishman.[6]
2. The *in vivo* animal study, *employing best practices with a positive control*, fails to demonstrate any QTc prolongation at steady-state exposures comparable to the HCE achieved in human subjects.
3. When a multiple of the HCE is not achieved in a healthy volunteer ascending dose study due to safety, saturation absorption or tolerability concerns and C-QTc modeling excludes a geometric mean steady-state QTc effect at peak exposure > 10 milliseconds at the HCE.

In instances where the above criteria for a negative clinical study are satisfied *but* the nonclinical studies are not performed in accordance with best practices or demonstrate conflicting results, a positive control should be introduced. Alternatively, to strengthen the totality of evidence in support of a TQT substitution, the sponsor has the option to repeat either the hERG or *in vivo* animal studies employing best practices (**Figure 3**). They might also consider additional follow-up nonclinical electrophysiologic and biomarker assays as per the Comprehensive *in vitro* Proarrhythmic Assay (CiPA) program developed in 2013 (https://cipaproject.org/). This initiative was designed to assess *in vitro*, *in vivo*, *ex vivo*, and ECG mixed ion channel effects (e.g., qNET and J-T peak metrics) of a compound on proarrhythmic risk, although its regulatory value is uncertain as all of these assays have not been standardized and routinely adopted.[24–26] Finally, in all cases where there is a safety signal in nonclinical studies and there is a concern about reaching an adequate exposure margin (i.e., twofold HCE) in the healthy volunteer study, a positive control arm is recommended (**Figure 3**).

TQT ALTERNATIVE STUDY CONSISTENT WITH ICH E14 Q&A 6.1: NON-FEASIBILITY

The Q&A 6.1 pathway was developed to inform product labeling and regulatory decision making for compounds that could not be safely administered to healthy volunteers in supratherapeutic doses due to tolerability or other factors. This pathway predominantly involves oncology compounds as 82% of submitted reports to the FDA between 2016 and 2020 were designated oncologic agents while 18% were described as "other"[17] (**Figure 1b**). Moreover, there has been a steady increase in this TQT substitution option, from 7% in 2016 to 36% in 2023 (**Figure 1a**). The Q&A 6.1 paradigm is also appropriate for agents where a placebo control comparison is not possible and in cases where the use of a positive control is precluded or where substantial heart rate effects might confound the interpretation of QTc results. In these situations, the clinical studies still need to be powered with an appropriate sample size to characterize the QT liability of the candidate drug.[15] The study should also plan to incorporate as many ECG design and analysis elements as would be contained in a traditional TQT protocol. The *initial* Q&A 6.1 pathway[19] outlined three key components:

1. No evidence of delayed ventricular repolarization in nonclinical hERG and non-rodent animal studies.
2. A negative ECG study in patients where > 10 milliseconds QTc prolongation has been excluded
3. A cardiovascular safety database devoid of an increase in the incidence of proarrhythmic events.

When these criteria were satisfied, even in the absence of placebo administration or a positive control, the drug was designated as "unlikely to have a substantial QT effect" (typically < 20 milliseconds increase) and would presumably move forward in development without further in-depth QT surveillance.

The *updated* Q&A 6.1 guidance[15] has several important modifications where there is a pivoting away from the previous QT-centric focus toward integrating core assays and human clinical data into a proarrhythmic risk prediction model:

1. The hERG study should be undertaken employing best practice guidelines which are reviewed in detail by Darpo and Leishman.[21]
2. The *in vivo* animal study should also follow best practice guidance; (i) with exposures reaching the free HCE concentration and (ii) the assay employed should have a sensitivity to detect a QTc effect of a magnitude corresponding to that which would be found in human subjects accounting for interspecies differences in sensitivity (e.g. dog QT intervals are shorter than human and an exposure of at least threefold relative to the human exposure is proposed). Moreover, the study should include a positive control, be sufficiently powered, and employ C-QTc analysis to evaluate the results.
3. When there are no safety signals identified in these evaluations and the clinical ECG study shows <10 milliseconds QTc lengthening in conjunction with a cardiovascular safety database that does not reveal an increased incidence of adverse arrhythmic events, then the phrase "low likelihood of proarrhythmic effects due to delayed ventricular repolarization" can be applied (**Figure 4**).

In this scenario, the compound could progress in development without major reservation about proarrhythmic risk. It is of interest that even in the setting where nonclinical studies demonstrate low risk findings, as long as the clinical study reveals a maximal mean effect of the QTc < 10 milliseconds, the pharmaceutical would be unlikely to have a mean effect as large as 20 milliseconds. In this example, an argument could be made that the drug is unlikely to possess an unacceptable proarrhythmic risk when submitting a marketing application and may deserve approval due to an unmet patient need.

WHEN A DEDICATED TQT STUDY IS RECOMMENDED

A standalone TQT study may be indicated when cardiac liability assessment via either substitution pathways (ICH E14 Q&A 5.1 or 6.1) are not feasible or the sponsor chooses to proceed directly to a TQT study for a variety of reasons. As noted in **Figure 1a**, the percentage of TQT studies performed in aggregate is roughly the same as studies involving the Q&A 5.1 and

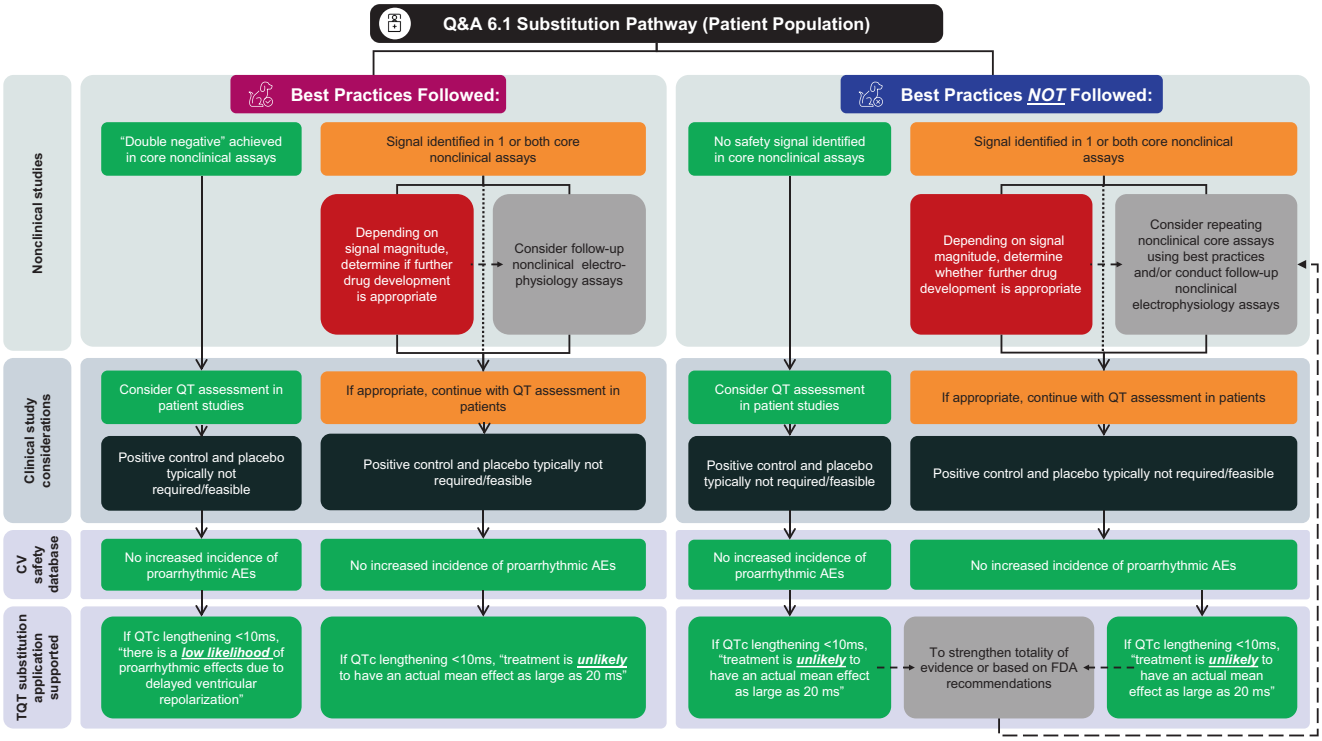

Figure 4 Examples of integrated cardiac liability assessments under Q&A 6.1. For drugs that are not feasible to dose in healthy volunteers, the Q&A 6.1 pathway offers a schema to evaluate the proarrhythmic risk in the targeted patient population. In this case, placebo and positive control arms are not required. The TQT substitution outcome and ultimately the drug label claim regarding QT liability will depend on whether nonclinical best practices were followed, whether QTc prolongation was observed in the clinical study, and were any adverse events noted in an associated cardiovascular safety database. If positive signal(s) in the core nonclinical studies are detected when best practices are not followed, drug developers may repeat these investigations and/or consider additional follow-up electrophysiologic studies. The colored bubbles reflect key steps along the development pathway: Green—move drug development forward; Orange—proceed drug development with caution; Red—pause drug development and assess the situation.

Table 1 Situations when a dedicated TQT may be recommended

Key factor	Conditions/Example
Substitution pathways deemed not appropriate	Sufficient nonclinical data not collected and/or cardiodynamic data not collected in phase I study
Mechanism of action	Drug interacts with multiple cardiac ion channels
Previous experience/results	Drug class suspected to prolong QTc
	Parent drug or metabolites likely to be associated with QTc prolongation
	Drug associated with large changes in heart rate
	Drug displays definite nonclinical proarrhythmic signals
PK/safety profile	Drug has a very long $t_{1/2}$
	Drugs associated with significant hysteresis
	Sample size is too small to cover range of exposures needed for C-QTc evaluation

6.1 pathways combined. While it is anticipated that this number will decrease as drug developers become more familiar with the substitution pathways, there are conditions when a dedicated TQT may still be warranted. For instance, a conventional TQT study may be recommended if the investigational drug interacts with multiple cardiac channels, when the parent drug and one or more metabolites are likely to be associated with QTc prolongation, or with drugs associated with large changes in heart rate (Table 1).

SPONSOR CONSIDERATIONS: CHOOSING A STUDY DESGIN

Sponsors are currently confronted with a number of options when considering study designs to profile a drug's proarrhythmic risk. The choice of study design involves a multitude of factors including but not limited to either clinical or nonclinical absorption, distribution, metabolism, and elimination (ADME) characteristics of the pharmaceutical, pharmacokinetic data, safety pharmacology information (see Table 1), as well as development timelines and budgetary constraints. However, the specific design chosen

does not reduce the likelihood of identifying a QT signal as 19% of all datasets recently reviewed by the FDA have been positive for QTc prolongation[17] (**Figure 1c**).

In the past, the decision to forego a TQT substitution was in part governed by relying on the predictive value and the specificity and sensitivity of nonclinical assays for delayed repolarization. Park *et al.*[27] retrospectively examined 150 Health and Environmental Science Institute (HESI)/FDA anonymized drug applications and analyzed hERG, action potential duration (APD) and *in vivo* animal QT studies contained in these applications. They found that the specificity was high while the sensitivity was low for the assays as a group in identifying QT prolongation. Additionally, individual *in vivo* studies, when attaining a clinical exposures of 30-fold or greater, offered the best specificity and predictive value for human QT effects. As such, the absence of a safety signal in the core nonclinical assays has been used to support a FIH ascending dose design rather than proceeding directly to a TQT study. To further validate this approach, Vargas *et al.*[28] examined published data spanning 51 years and found that drugs which demonstrated a QT signal in humans also did so in animals 91% of the time. Similarly, 88% of cases where drugs showed no QT change in humans there were concordant results in the non-rodent animal studies. In approximately 10% of cases there was a lack of concordance between the human and animal QT results.[28] More recently, using the same dataset of 150 drug candidates compiled by Park, Valentin *et al.*[29] evaluated the concordance between the human and animal studies for detecting a QT signal and explored the possible mechanisms of discordant results. Overall, 31% were found to be discordant which included 28 TQT-positive drugs. This discordance between clinical and nonclinical repolarization assays was thought to be related to factors such as major heart rate changes, incomplete nonclinical data, and the direct and indirect impact of the drug on additional cardiac ion channels.[29]

Discordance between human and animal results can confound the interpretation of proarrhythmic risk and sponsors are faced with the conundrum as to whether to repeat the core nonclinical assays adhering to best practice guidance or consider follow-up nonclinical studies designed to more fully characterize effects of their NCE on ventricular repolarization and enhance the totality of evidence for arrhythmia risk. During this transition period, as sponsors become more familiar with the requirements for executing nonclinical studies employing best practices designed to support a TQT substitution, it is anticipated that additional nonclinical and biomarker cardiovascular safety assessments as performed prior to the updated guidelines will likely not be necessary or appropriate. However, inherent in the sponsor's decision making process about study design and add-on investigations is the recommendation to have their protocol with supporting documents submitted to the review division of the FDA for their comments and feedback. This typically includes engaging the consultant services of the Cardiac Safety Interdisciplinary Review Team (CS-IRT), which consists of clinical analysts, clinical pharmacology reviewers, statistical reviewers, nonclinical reviewers and data specialists whose function is to review QT protocols and datasets for the Division of CardioNephrology (DCN). Sponsors need to ensure that they allocate sufficient time in their development program to complete this review. While not a comprehensive list, the following is a selection of considerations that may impact a sponsor's choice of development pathways and whether to seek a TQT substitution (**Table 2**). Much of this decision relies on identifying the HCE, which can often be a rate limiting step when building out a drug development plan to include a TQT substitution. One potential suggestion when safety concerns are not operant is to dose escalate to the maximum tolerated dose in the SAD study with the goal of reaching the HCE or even higher exposure levels.

Table 2 Selected considerations that may impact the decision to seek a TQT substitution

What are the timelines for the drug development program?
Are there budgetary constraints?
Were best practices followed for the core battery of nonclinical studies?
Does the sponsor plan to commercialize and de-risk the drug?
Is the sponsor risk averse if a QT signal is present in the nonclinical assays prompting premature termination of their candidate drug?
Are there similar compounds under development or those that have been approved and what pathway did they follow?
Are there potential concerns suggested by the safety pharmacology toxicity data and the PK properties of the candidate drug?
What is the perceived positive and negative predictive value of the nonclinical assays for delayed ventricular repolarization in humans?
Has the HCE been determined? Are there any safety concerns dosing at this level or higher?
Are there discordant QT findings between the nonclinical and clinical assays that prejudice in favor of a dedicated TQT?

As an adjunct to assessing a NCE's impact on ventricular repolarization and to strengthen clinical ECG data submission in support of a TQT substitution via either the Q&A 5.1 or 6.1 pathway, undertaking ECG bias sensitivity analysis has been described.[30] In this analysis, Bland–Altman plots are formulated to profile the difference in the Fridericia-corrected QTc (QTcF) measurement between machine-generated values and those of skilled readers as originally proposed by Ferber *et al.*[30] A value of < 20 milliseconds difference would be interpreted as demonstrating the absence of bias. Moreover, when the bias severity difference is < 10 milliseconds, the probability of a false-negative QT signal is reduced to under 5%.

Finally, denial for a TQT substitution or QTc increases exceeding 10 milliseconds in a human study should not deter sponsors from proceeding in their development program as there may be a plateauing of effect as exposures are increased or the mechanism of altered ventricular repolarization may not be discerned by the nonclinical assays. Additionally as previously referenced, there are multiple avenues that can be pursued to further evaluate potential cardiac liability before electing to perform a conventional TQT study. These avenues include a supplemental battery of ion channel assays, *in silico* modeling of proarrhythmia, *ex vivo* Purkinje fiber action potential studies, human induced pluripotent cardiomyocyte stem cell preparations and ECG biomarker metrics, all of which are designed to create a human arrhythmia risk prediction model.[25,26,31]

REGULATORY CONSIDERATIONS: REASONS FOR TQT SUBSTITUTION REQUESTS BEING DENIED

The specific reasons and metrics for rejection of requests for a TQT substitution pertaining to the Q&A 5.1 pathway are not readily available in an open source database although the following justifications, gleaned from literature review, direct regulatory feedback and oral presentations by stakeholders, have been promulgated (**Table 3**).

The mechanics of how regulators view the aforementioned factors in their decision to accept or deny a TQT substitution request are unclear as is the weight given to different criteria, although consensus amongst the CS-IRT members is almost universally achieved and detailed in a "best advice" report issued by the DCN, which typically takes approximately 45 days to be issued (personal communication, FDA). In addition to the above, there are various modulating factors that may be considered in the totality of evidence such as the role of mixed ion channel block in modifying arrhythmia risk, ADME data, safety pharmacology and PK characteristics of the candidate drug and the perceived unmet medical need and benefit of the test article for society.

CONCLUSION

Early drug development of NCEs is a complex and resource-intensive undertaking as it applies to QT liability and overall cardiac safety. It is gratifying to witness the evolution away from dedicated TQT studies to smaller trials which have also been successful in preventing torsadogenic drugs from reaching the market. Moreover, the recent regulatory guidance involving prescriptive detail of best practices pertaining to nonclinical assays is most welcome. This harmonization of assay methodology reduces variability and permits more meaningful interpretation of findings, thereby mitigating the likelihood of both false positive and false negative results while simultaneously facilitating the choice of clinical study pathways. Furthermore, given the low prevalence of serious ventricular arrhythmias, the "*double-negative*" nonclinical scenario as part of an integrated risk assessment strategy along with future FDA initiatives, should help to streamline the development process and eventuate in fewer dedicated TQT studies being undertaken.

Table 3 Potential reasons associated with TQT substitution denial

Incomplete dataset submission or flawed statistical analysis
Inability to reach sufficiently high drug exposures
Quality and results of the nonclinical assays are concerning
Discordant data regarding nonclinical and clinical proarrhythmic assessment
Failure to comprehensively characterize active metabolites and accumulation
Failure to document assay sensitivity with a positive control
Heterogeneity in collection of cardiodynamic ECGs and non-centralized reading
Inappropriate modeling of QT effects of the test article due to hysteresis or substantial heart rate changes and inappropriate analysis methodology
There is a modest safety signal in the nonclinical and/or clinical studies and the risk to patients needs to be further evaluated in a dedicated TQT protocol
The cardiovascular database shows adverse events of concern
HCE is not well defined at the time of request

Despite the considerable effort and inroads to develop more efficient and cost-effective paradigms for proarrhythmic assessment of NCE, it is somewhat discouraging that standalone TQT studies still represent approximately 50% of all data reports reviewed by the FDA and this percentage has been stable in recent years (**Figure 1a**). Moreover, the decline in the Q&A 5.1 pathway percentages over the same timeframe is puzzling and brings into question what is the basis for this apparent discrepancy. Are sponsors not fully aware of the latest regulatory guidance or has there been a lag in their submitting results until POC was established; were nonclinical studies performed prior to the current Q&A guidance prompting sponsors to follow traditional testing procedures while concomitantly placing increased emphasis on clinical trial results rather than electing, at additional time and cost, to repeat nonclinical studies conforming to the updated best practices; are they risk averse and would prefer performing a definitive QT study from the outset; did the sponsors not reach the HCE in FIH studies; has the reduced sample size and scope of a dedicated TQT made these study designs more feasible and less costly; are the compounds being evaluated more complex and nuanced such that a TQT is the preferred design?

In order to address this apparent discrepancy and hopefully shift the balance away from dedicated TQT studies toward the Q&A 5.1 scenario, the development of an anonymized open source database of studies submitted to the FDA requesting a TQT substitution would be informative for all stakeholders along with metrics on how many were granted substitutions; and for submissions denied substitutions, what was the basis for denial?

Finally, when considering the future of cardiac safety and regulatory science, the role of technology holds much promise for faster and more efficient drug development. In this regard, Tang *et al.*[32] have opined that the creation of a multiomics database consisting of proteomics, genomics, epigenomics, and metabolomics integrated with machine learning might accelerate the development of safe new drugs. As a correlate, although in its infancy, would the regulatory bodies look favorably and embrace predictive artificial intelligence applied to 12-lead ECGs as a tool to assess proarrhythmic risk across the spectrum of NCEs? Would further refinement in best practice animal studies using the "one-step" approach enumerated by Leishman *et al.*[33] improve sensitivity in QT assessment and reduce the number of animals needed for detection of ventricular repolarization changes? These and other emerging and novel initiatives are exciting avenues to observe in the ongoing evolution of safe drug development.

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