



# Assay Sensitivity in QT Assessment

The ability to detect a study endpoint of interest should be reliably detected by the positive control. If the positive control does not produce the expected result, validation of the experimental procedure is not possible. A dedicated study to determine if a drug has the potential to prolong the QT interval has been identified by the E14 guideline of the International Conference on Harmonization as an essential element to assess potential cardiac risks<sup>1</sup>. This guidance emphasises the importance of the use of a positive control in order to access the assay sensitivity. Thorough QT/QTc studies (TQT) assay sensitivity is confirmed by the ability of the positive control to show an effect of approximately 5 ms, meaning that the lower-bound of the 90% confidence interval (CI) should be above 5 ms.

Typically in a TQT study, the fluoroquinolone antibiotic moxifloxacin is used as a positive control to confirm assay sensitivity. Following a 400 mg single dose moxifloxacin is well established to produce a peak of usually 10 ms or more in the time window between 1 and 4 h post-dose<sup>2</sup>. Although moxifloxacin is the most commonly used reference to confirm assay sensitivity, the use outside its indication, its larger QTc effect than the anticipated by ICH E14 guidelines and the difficulties with over-encapsulation and blinding have been recognised as some of the limitations of the use of moxifloxacin. Furthermore, failure in demonstrating a >5 ms increase in QTc on moxifloxacin has been reported<sup>3</sup>.

Other pharmacological compounds such as levofloxacin with a smaller increase in QTc have been proposed as an alternative method of confirming assay sensitivity but have not been generally adopted. Nevertheless, a non-pharmacological approach may be desirable in studies where the use of pharmacological compounds to confirm assay sensitivity is not appropriate.

## Discussion in the Regulatory Environment

The TQT study, since its implementation in 2005, has been a target for criticism due to the increased cost of drug development<sup>4</sup>. For the last 10 years, more efficient approaches to reducing the associated costs and alternatives to a conventional TQT study have been extensively discussed. It has been proposed that QTc data obtained in Phase I studies and exposure-response (ER) analysis are potential alternatives to TQT<sup>5</sup>. The results from the IQ-CSRC study seek to provide support for the transition from TQT to early QT assessment<sup>6</sup>. A key question still raising some concern is whether the same high level of confidence can be obtained through Phase I studies due to the small sample size and therefore their ability to show small QTc changes. This has been discussed by Ferber *et al.*<sup>7,8</sup> concluding that a small sample size may be suitable. The main limitation of these studies is the use of data from formal TQT studies or in the case of the IQ-CRS study, the design is solely focused on the ECG assessment, differing from the primary objectives of normal SAD and MAD studies.

Phase I studies do not typically include a positive control

which can constitute a limitation when excluding an effect of regulatory concern. Systematic errors can occur that cannot be reliably detected without a positive control and therefore it is our opinion that a positive control in ECG assessment has an important role in reducing the likelihood of false negatives.

Recently, moxifloxacin was used as a positive control in a SAD and a TQT study and the results were compared<sup>9</sup>. In this same study the cost-effectiveness of integrating a positive control in SAD studies was also evaluated. Assay sensitivity was established by the 90% lower bound exceeding 5 ms for both SAD and TQT moxifloxacin arms as per ICH E14 requirements. Even though not considered to be relevant, the moxifloxacin ER slope value from the SAD study was twice the value in the TQT study and the 90% CI were 30-40% greater in the SAD study. The authors also estimate an additional €60K to be added to the final cost of typical SAD study if 24 subjects are to be included in a moxifloxacin arm. While this represents a more favourable cost compared with the traditional TQT study, in fact the addition of a moxifloxacin arm still translates into unnecessary additional costs.

Several paths to overcome the lack of a cost-effective approach for assay sensitivity evaluation are being investigated, but no solution has been generally accepted. A widely acceptable method should be robust and reproducible, simple to implement, able to detect small QTc changes and should present advantages compared to the current method of moxifloxacin. Other desirable benefits that must not be neglected when choosing an adequate and cost-effective positive control include the possibility of being used in small groups and be applicable across different ethnicities, avoidance of unnecessary drug exposure, absence of anticipated adverse events and no hindrance for ethical approval. Taking into account all the desirable features of a positive control, it seems reasonable to consider non-pharmacological approaches as candidates to alternatives methods of confirming assay sensitivity when assessing the effect of a drug on the QT interval.

## Non-pharmacological Approaches

Alternative methodologies to demonstrate assay sensitivity have been considered and discussed by the Cardiac Safety Research Consortium in an attempt to improve the confidence in QT assessment in early-phase studies.

The ability to detect postural changes on QTc assessments has been proposed as an alternative method for assay sensitivity evaluation<sup>10</sup>. Postural changes were shown to produce 5-7 ms change in QTcF but the adoption of this method has not been pursued, probably due to the possibility of ECG methods being affected by hysteresis. On the other hand, the effect of a meal on QT interval has been demonstrated in different dedicated studies<sup>11-15</sup>. Adoption of food effect as the positive control in QT assessment studies would eliminate the need for a separate arm/period to determine assay sensitivity, thereby resulting in much smaller studies and significant cost savings. A "food day" can easily be added into almost any type of clinical trial