

The Use of iCell Cardiomyocytes with Cell-by-Cell Calcium Analysis Reveals Compound Induced Changes in Cardiac Repolarization and Generation of Pro-arrhythmia Signals

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Abstract: Cardiomyocytes derived from human stem cells (hSC-CM's) provide researchers with a model that facilitates direct assessment of compound effects on cardiac excitation regardless of whether the effects are mediated through single ion channel agonist/antagonist action, through simultaneous multichannel effects or through altered signaling. In this study we analyzed 90 compounds using an automated cell-by-cell analysis of calcium transients to detect compound induced changes in repolarization and the generation of proarrhythmia signals. Most compound effects were found to occur at concentrations that match clinical outcomes. Additionally, many of the compounds that are known to induce arrhythmia also demonstrated pro-arrhythmia signals.

Background: Compound induced alterations in the action potential which lead to arrhythmia can be caused by either direct agonist/antagonist effects on the ion channels which contribute the generation of the action potential or through other signaling cascades which regulate the activity or expression levels of these channels. The currently available high throughput assays used in early drug development focus on single ion channels and/or utilize models which do not reproduce the complexity of cardiac physiology. However, stem cell technology can now provide a renewable and scalable source of human cardiomyocytes which can recreate many of the features of cardiac physiology. These models allow for the development of high throughput, integrated assays which can query compound effects across a wide range of potential arrhythmogenic mechanisms in the context of a functional human cardiomyocyte.

The excitation-contraction coupling model describes how the electrochemical signals of the action potential are coupled to cardiomyocyte contraction using calcium as a second messenger (Fig. 1). Compound induced alterations in the shape or duration of the action potentials, due to changes in the activity or expression level of the component ion channels, is reflected in changes to the shape and duration of the resulting calcium transient. Therefore, calcium can be used as an integrating signal to detect compound effects which may lead to arrhythmia through a variety of mechanisms. To take advantage of these stem cell derived cardiomyocyte models we created the Kinetic Image Cytometer (KIC), which provides for high throughput, automated cell-by-cell analysis of intracellular calcium transients (Fig. 2).

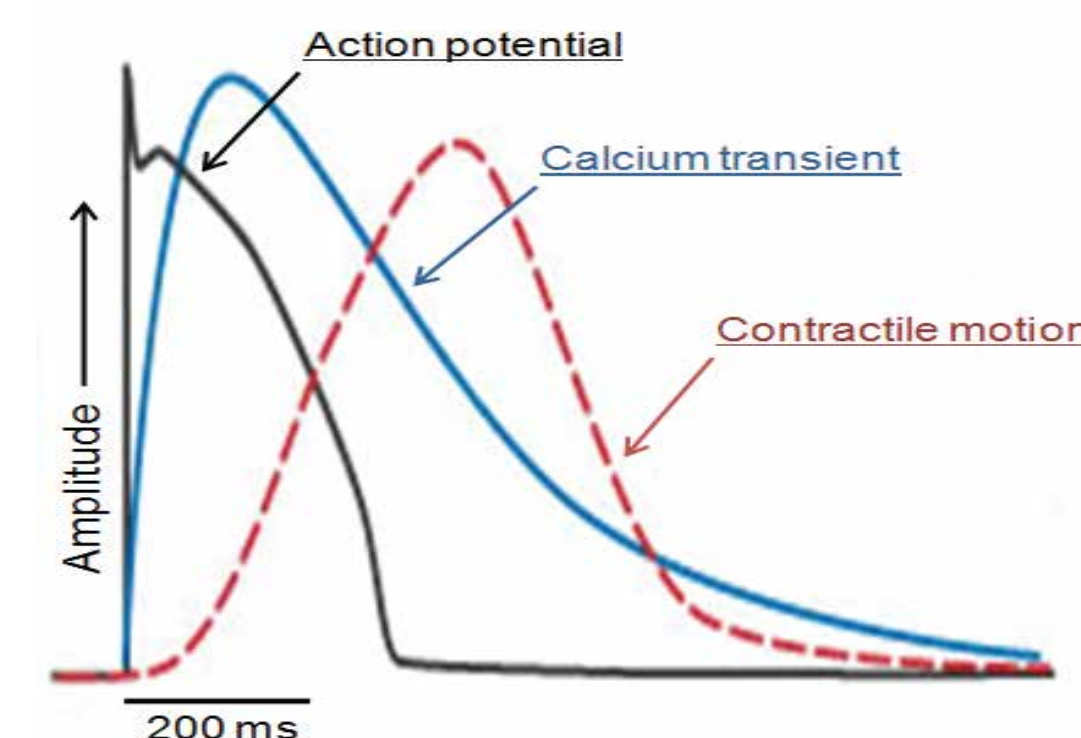
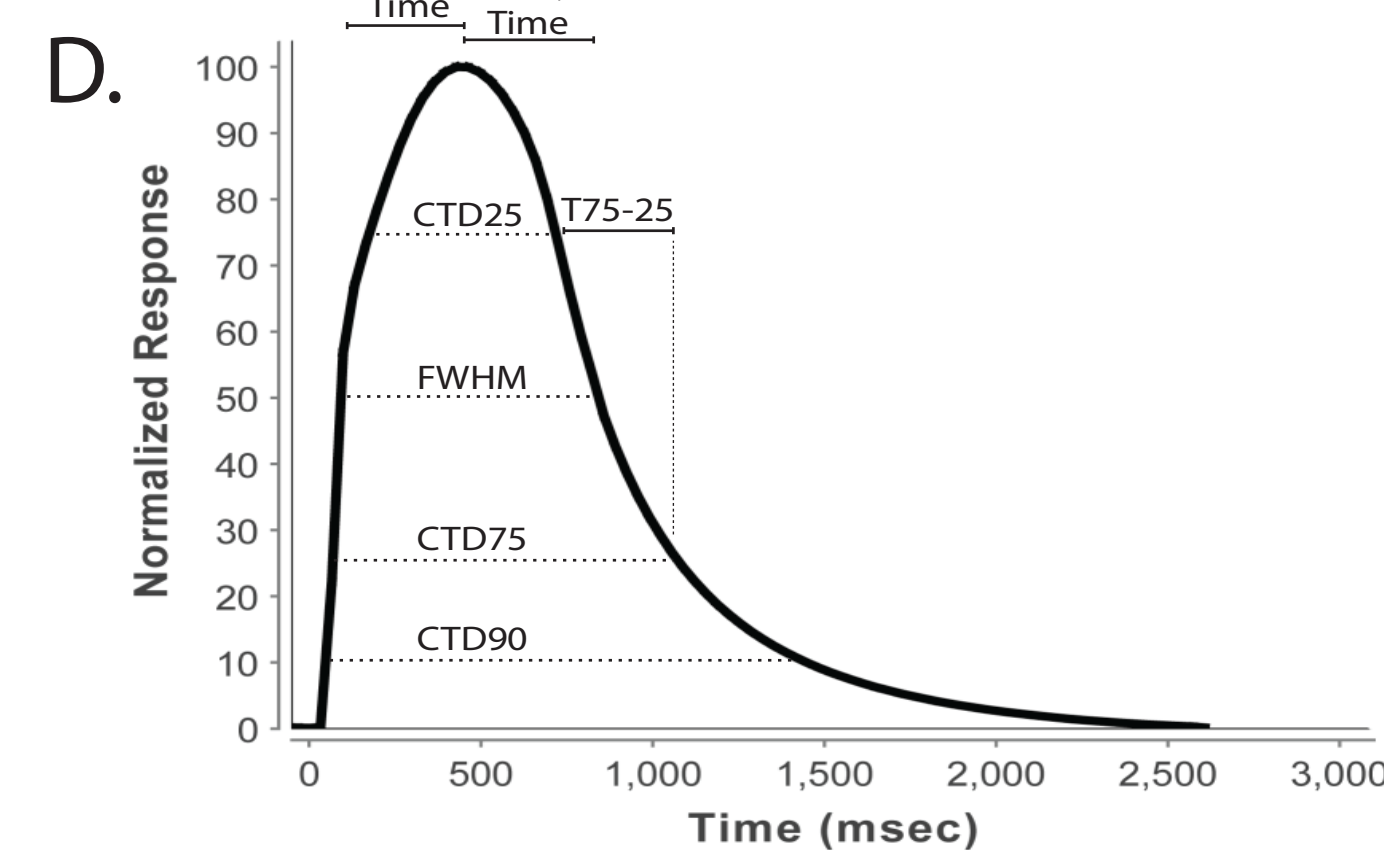
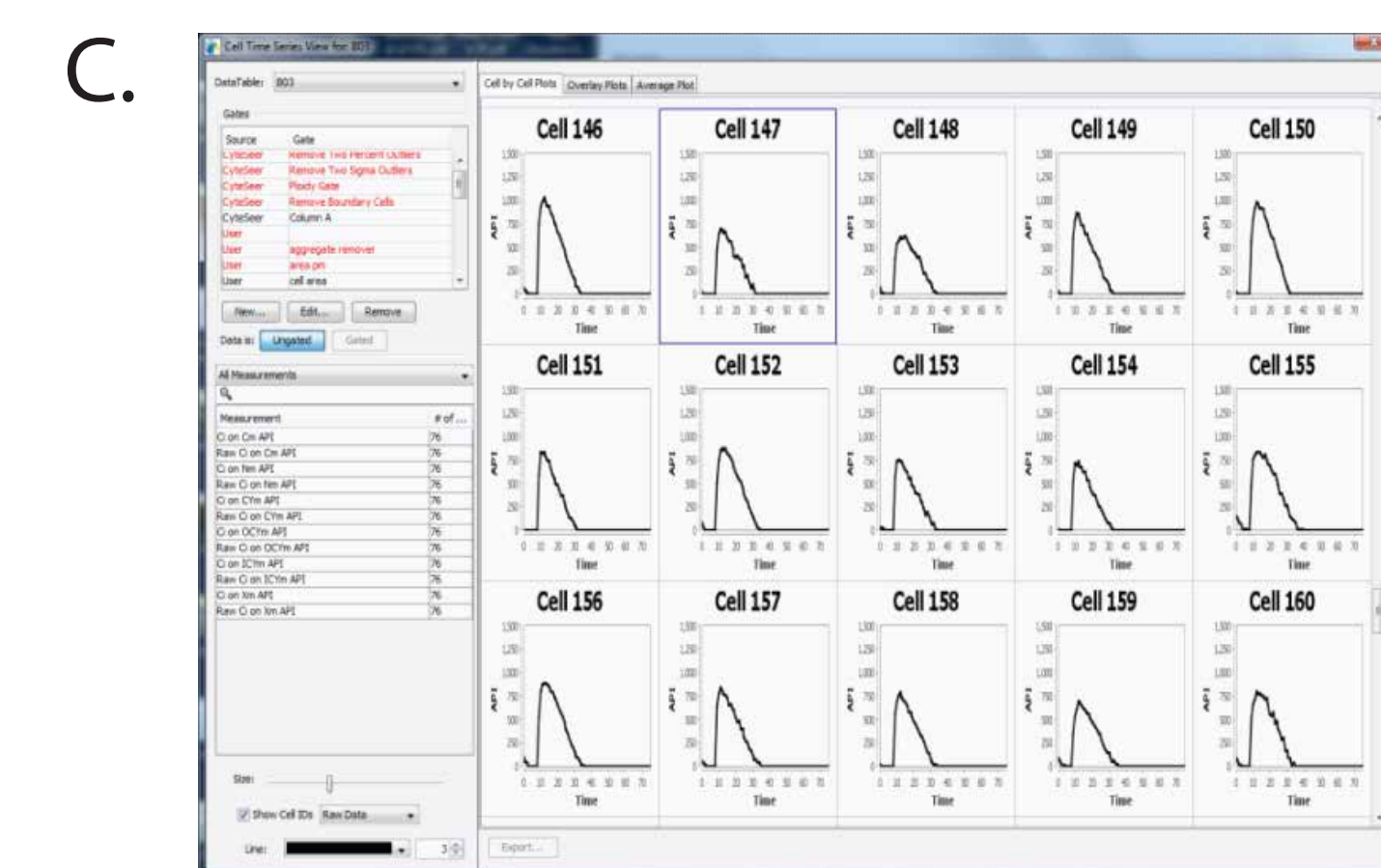
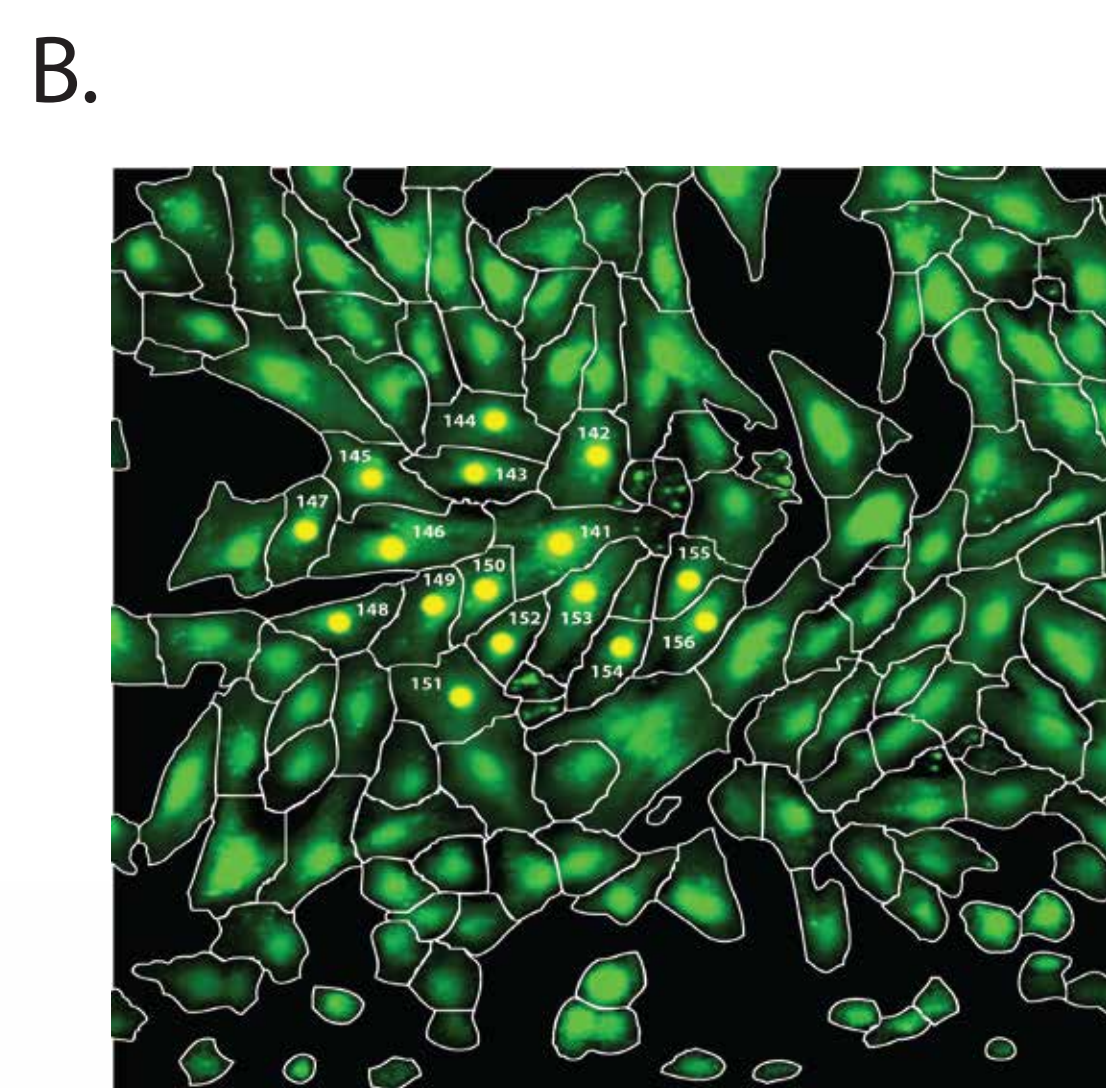


Fig 1. The Excitation-Contraction Coupling Model
Bers, Nature 2002, Jan 10; 415(6868):198-205

Fig. 2 Overview of the KIC platform and assay

A.) The Kinetic Image Cytometer (KIC) is a high content based system that has been designed to produce and analyze recordings of intracellular calcium transients in an automated fashion at up to 100fps providing excellent temporal resolution for analyzing compound effects on the transients. During recording the cells are placed in an environmental control chamber which maintains temperature and CO2 levels. B.) Cells, in the case of this study iCell cardiomyocytes, are cultured in 96 or 384 well plates. For calcium transients the cells are loaded with the calcium sensitive fluorescent indicator, fluo-4 and pretreated with test compounds. Recordings of either spontaneous beating activity or electrically paced activity are made and those recordings are then analyzed using the KIC software. Each cell in the field of view is identified, segmented and indexed. C.) This allows for a cell-by-cell analysis of the calcium transients to be performed. From a typical well transients from 200-400 cells are recorded and analyzed. D.) The KIC software performs a number of measurements which describe the shape and duration of the transient.



Methods: iCell Cardiomyocytes (CDI, Madison, WI) were thawed and plated onto 96 well plates at density of 18,000 cells per well. Cardiomyocytes were maintained in culture according to the manufacturers recommendations for 10 days prior to KIC experiments being performed. Prior to treatment with the test compounds cardiomyocytes were loaded with Hoescht 33342 and Fluo-4 no-wash (Fluo-4NW, Invitrogen) for 1 hour at 37C. Following dye loading the test compounds were diluted to their final concentration in Tyrode's solution and added to the appropriate wells. The final DMSO concentration for all test compounds and controls was 0.1%.

Results and Conclusions:
Overall the KIC assay correctly classified 89% of the compounds in the training set (Figure 3)

Using the CTD 75 measurement and a threshold of 140% of control values, the KIC assay correctly classified 98% (45/46 compounds) of the compounds which do not prolong the QT interval. Only two compounds, silodosin and paroxetine, exceeded the 140% threshold. Silodosin did not exceed the 140% threshold until a dose of 2,700 times expected free Cmax was reached and therefore it was not scored as a false positive. Paroxetine on the other hand has a free Cmax of approximately 8.25nM at therapeutic doses and exceeded the 140% threshold at 1uM. We took a conservative approach and scored this result as a false positive however it exceeds the 100 fold therapeutic window that is often used to define a safety margin.

The KIC assay correctly classified 80% of the compounds (35/44 compounds) which are known to prolong the QT interval. There were 9 compounds which did not exceed the 140% threshold for the CTD75 measurement and were classified as false negatives. Two thirds of the false negative compounds belonged to two classes of drugs. Four of the compounds, chlorpromazine, amitryptiline, desipramine, and imipramine, which were not detected by the KIC assay all belong to the same drug class, tricyclic antidepressants, more work is being done to understand this result. Toxicity issues were encountered with compounds which required a longer term incubation such as nilotinib, lapatanib and arsenic trioxide. Terfenadine did not cause a prolongation of the transient beyond the 140% threshold. Terfenadine caused a prolongation of 134.4% of control at 625nM and at 1.25uM the transient had a shorter duration with a triangulated shape. At 2.5uM all beating stopped, this is consistent with previous KIC experiments with terfenadine. Recent results from other groups have suggested that the effects of terfenadine are time dependent and occur after exposure for several hours, in this study terfenadine was only tested with an acute exposure.

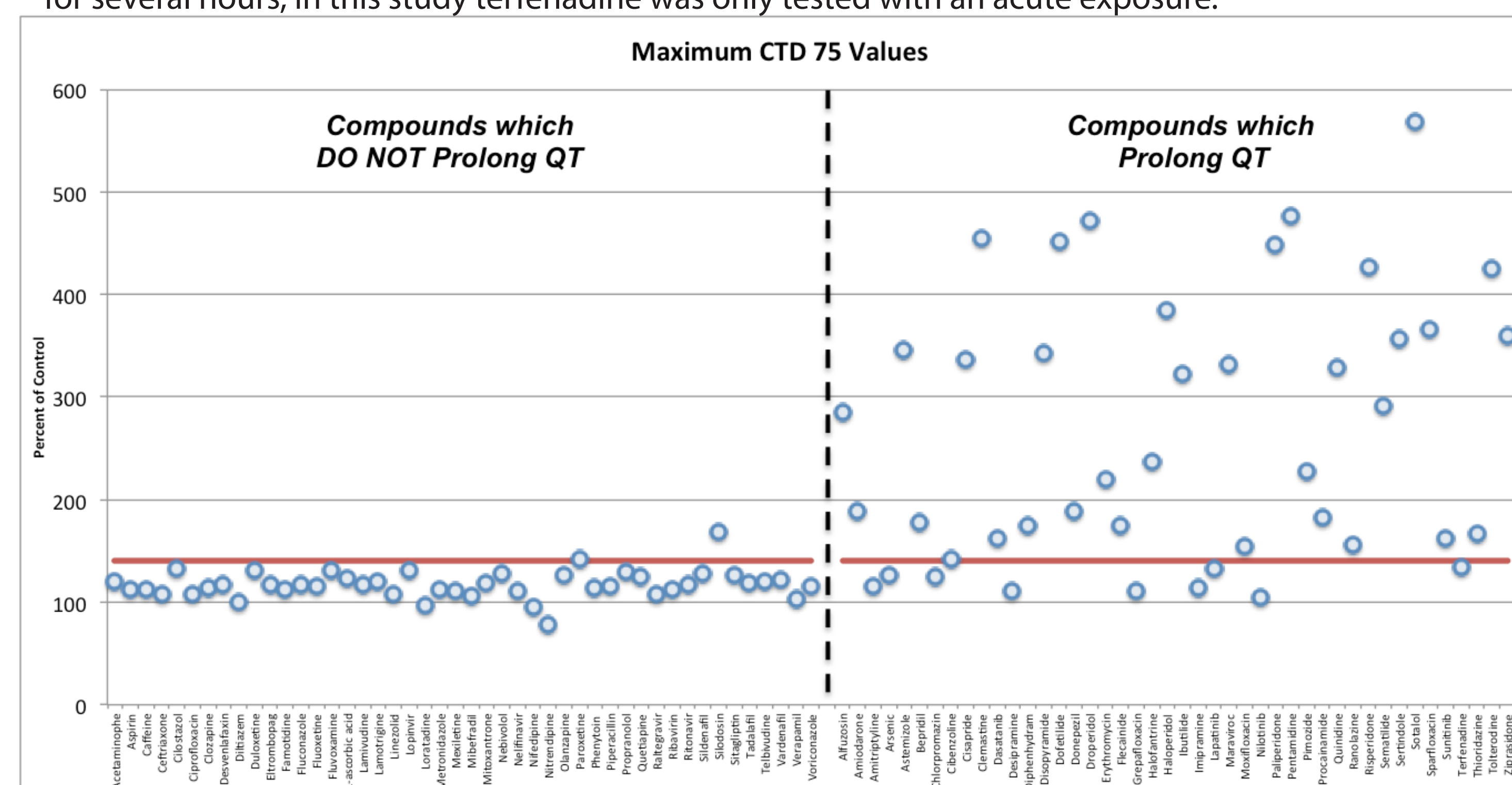


Fig. 3 Summary of the results from the 90 compound screen
The maximum effect on the CTD75 measurement is shown for each of the 90 compounds in the set. Compounds which are not known to prolong the QT interval are shown on the left side of the chart and compounds which are known to prolong the QT interval are shown on the right. There is a clear difference in the signal between two classes of compounds. The red line represents the 140% threshold that is used to classify compounds. Setting the threshold at this level resulted in a minimal number of false positive identifications. There were 9 compounds which were classified as false positives using this threshold level. Interestingly 4 of the 9 compounds which came up as false positives were tricyclic antidepressants. In addition 3 of the 9 compounds which came up as false positives required extended incubation times at which point compounds demonstrated a toxic effect and significant cell loss was observed.

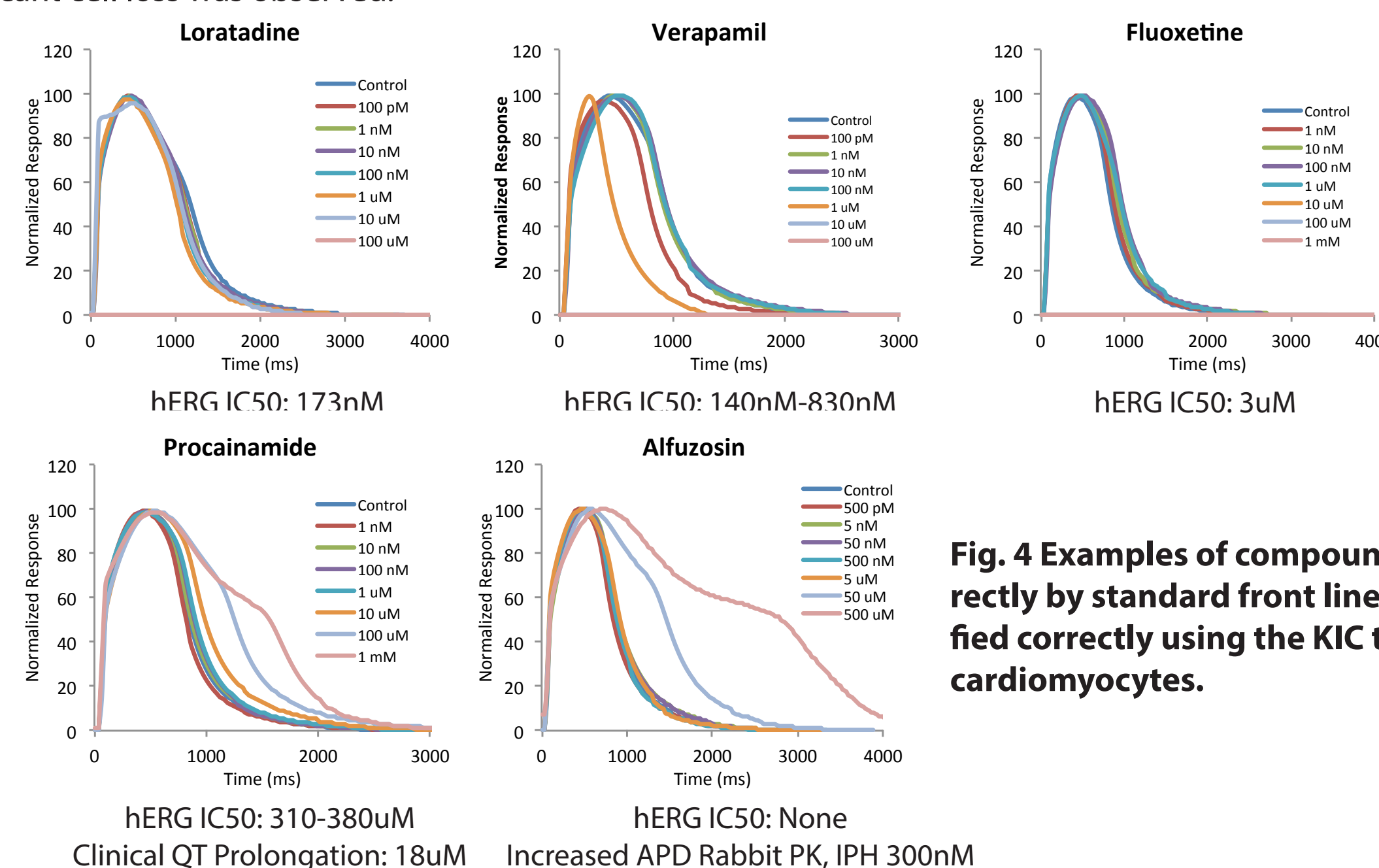


Fig. 4 Examples of compounds which may be classified incorrectly by standard front line hERG screens which are classified correctly using the KIC technology and hiPSC derived cardiomyocytes.

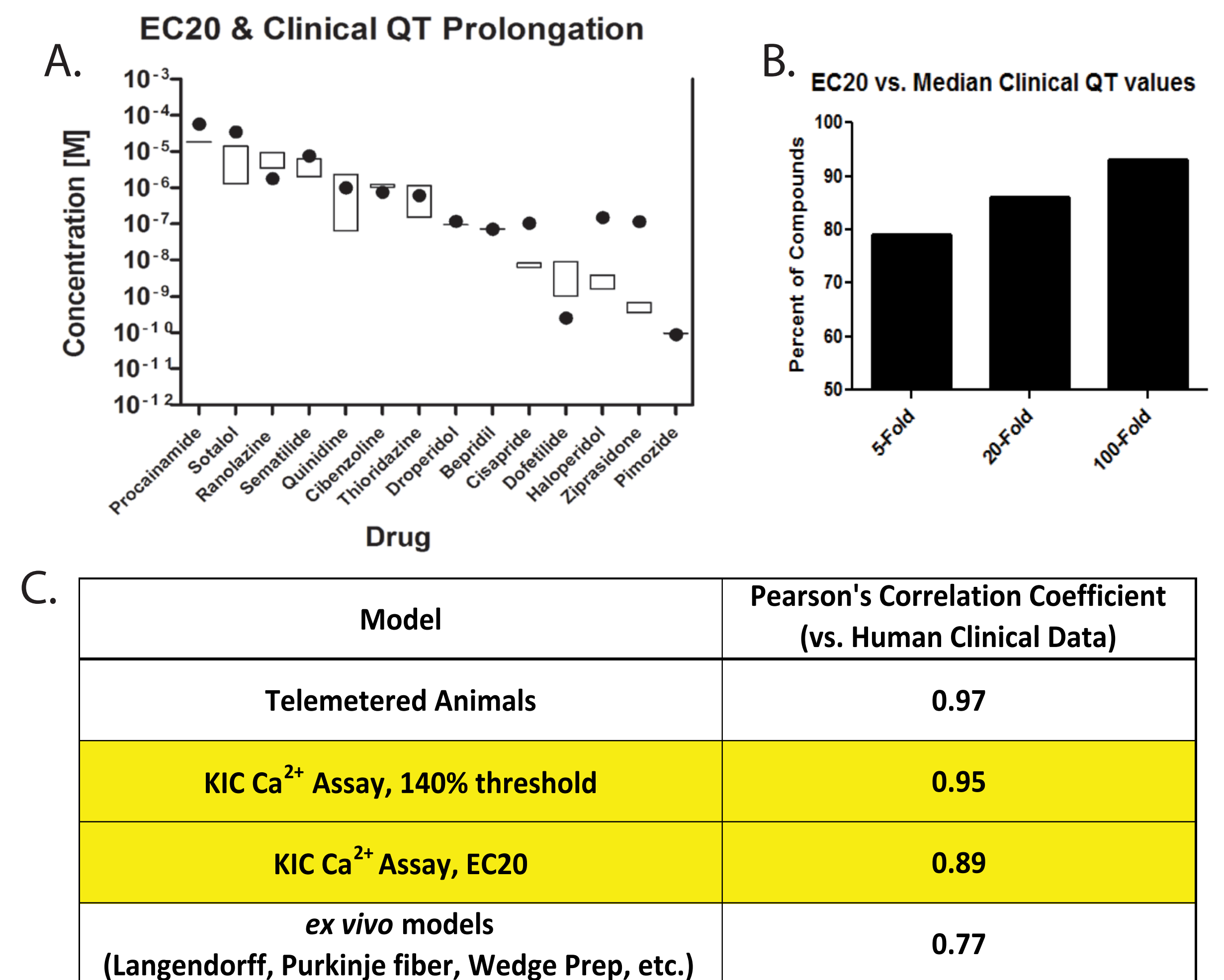


Fig. 5 Analysis of the sensitivity of the Arrhythmogenic Liability assay results compared to reported clinical outcomes. An extensive literature search was performed to identify clinical studies that reported both the free plasma concentration of the test compound and the effects on QT interval prolongation. A.) Of the 35 QT prolonging compounds identified correctly by the assay we were able to collect data from studies performed on 14 compounds with matching clinical EKG and Plasma concentration data. In these studies QT prolongation of between 10 and 80 ms was observed. Therefore, to perform a comparison with the results from the KIC study we compared the EC20 result (shown as -) with the results described in the literature (shown as bars to represent the range of reported concentrations). B.) Comparison of the results shows that almost 80% of the predictions made by the KIC technology were within 5 fold of the clinical values and over 90% were within 100 fold of clinical values. C.) There were 10 compounds where we were able to find comparable data for human clinical trials, telemetered animals, and ex vivo animal models (bepridil, cisapride, dofetilide, droperidol, haloperidol, pimozone, quinidine, sematilde, sotalol, and thioridazine). Using the median reported concentration for each model as well as the 140% threshold and the EC20 numbers from the KIC assay we compared the performance of each model type to clinical outcomes. We find that the prediction value of the Arrhythmogenic liability assay was much higher than ex vivo models and was on par with telemetered dog and monkey studies.

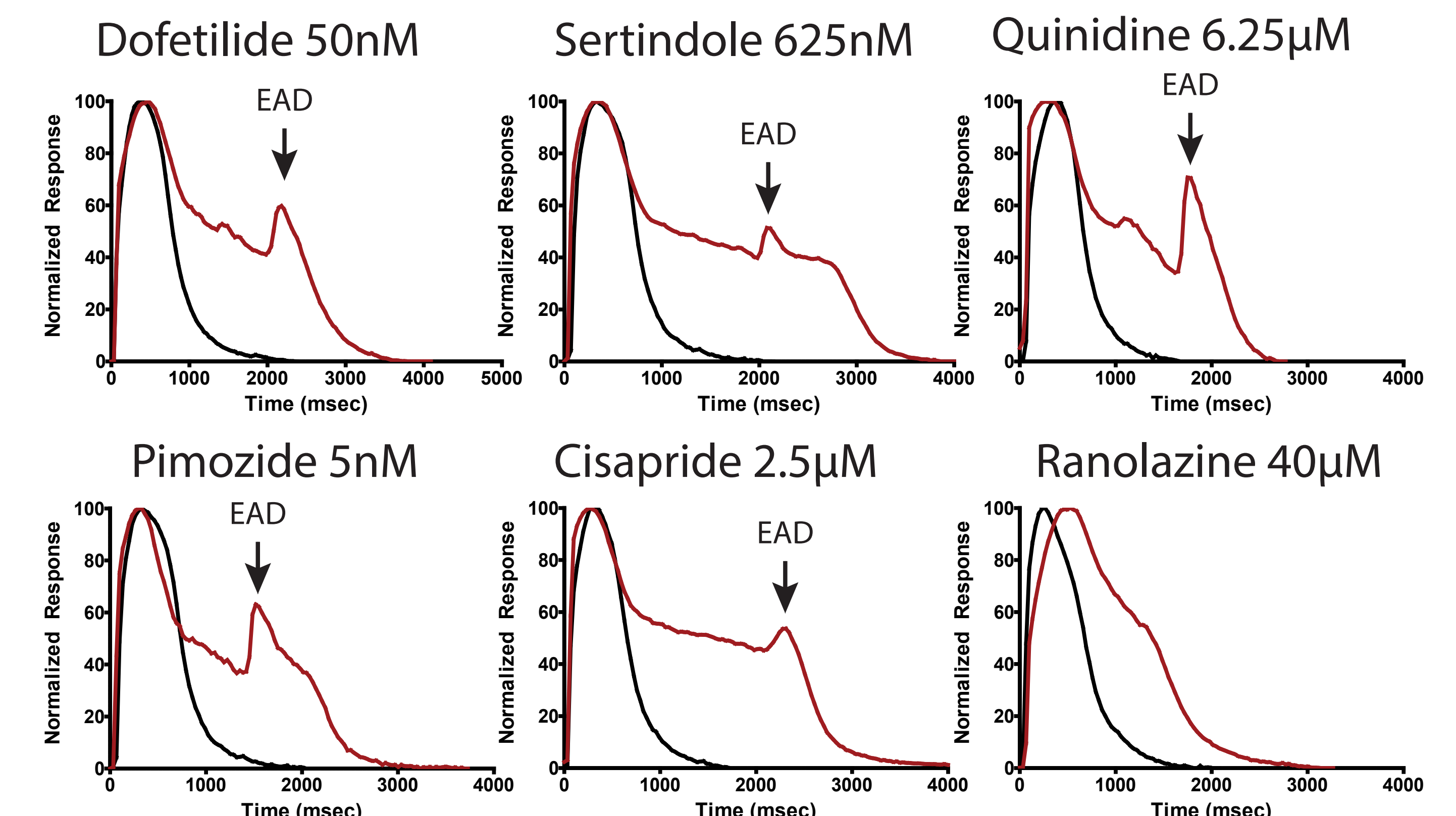


Fig. 6 Detection of pro-arrhythmia signals using the iCell cardiomyocytes and KIC technology. In addition to accurate QT prediction the assay is also able to reliably detect pro-arrhythmia signals when cells are exposed to known arrhythmogenic compounds. Dofetilide, Sertindole, Quinidine, Pimozone, Cisapride, each elicit EADs in the cells which are detected by the analysis software. In contrast, Ranolazine a compound known to cause minor, non-threatening prolongation of the QT interval and has no evidence of eliciting arrhythmias does not demonstrate EAD's or other arrhythmia signals even at supra-physiological exposure levels.