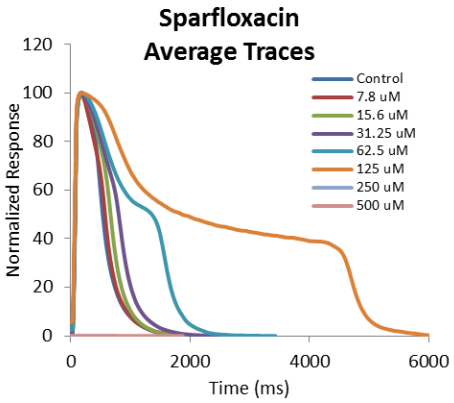
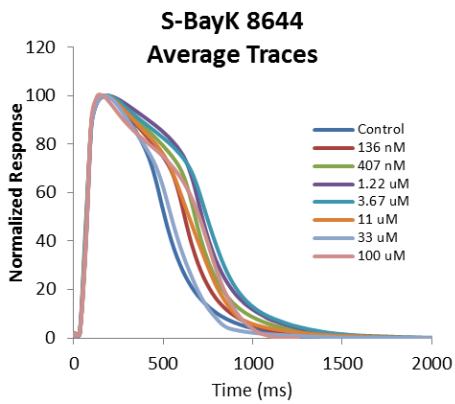
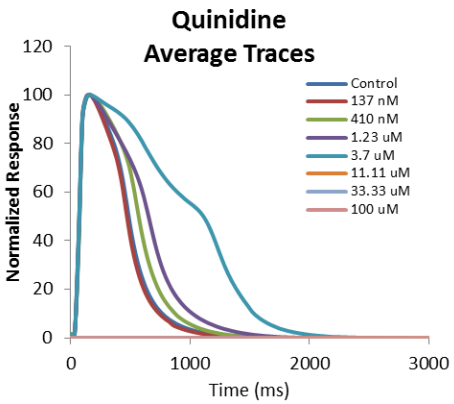
Front Line Cardiac Safety Assessment Using Vala Sciences' Arrhythmogenic Liability Assay and Axiogenesis Cor.4U Human iPSC Derived Cardiomyocytes

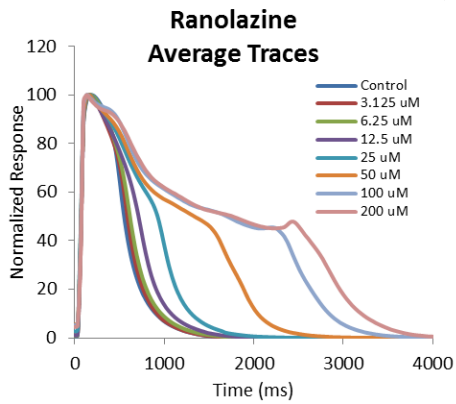
Introduction: Arrhythmogenic liability remains a key safety concern for the pharmaceutical industry. The emergence of human stem cell derived cardiomyocyte models, such as the Axiogenesis Cor.4U® human iPSC derived cardiomyocytes, has provided a scalable human cardiomyocyte model which allows researchers to assess compound effects across multiple ion channels and signaling pathways simultaneously. Vala Sciences' Arrhythmogenic Liability Assay uses intracellular calcium transients as an integrated signal to provide a high throughput cell-by-cell readout of compound effects in order to stratify compounds by arrhythmogenic potential early in the drug development process. By analyzing the alterations in the calcium transients of human iPSC derived cardiomyocytes, this method provides a single assay which can be used to survey for effects across the full complement of ion channels. The assay can also accurately assess the action of compounds which affect multiple channels simultaneously, or have an effect on other signaling pathways relevant to the generation of arrhythmia. Here we demonstrate the use of the Axiogenesis Cor.4U cardiomyocytes with Vala Sciences' Arrhythmogenic Liability Assay to detect changes to the action potential duration as well as the ability to detect pro-arrhythmia signals such as early after depolarization (EADs) and irregular beats.

Methods: Cor.4U® which had been grown in culture for 6 days prior to testing were loaded with Fluo-4 and Hoechst dyes and then exposed to different compounds for 15 minutes before being run on Vala Sciences' Arrhythmogenic Liability Assay. All analyses was done on a cell-by-cell basis with an average of ~ 200 cells per well analyzed. Concentrations were tested in triplicate and cells were maintained at 37°C for the duration of the experiment. Compound effects were scored according to changes in the duration of the calcium transient. Compounds were scored as prolongers when transients exceeded 140% of control and as shorteners when transient durations were less than 85% of controls.









QT prolonging compounds are of particular interest in safety pharmacology given that prolongation of the QT interval can lead to the fatal arrhythmia such as Torsades de Pointes (TdP). In the present study the effects of 4 QT prolonging compounds with various mechanisms of action, including primary hERG blockade (Sparfloxacin, Ranolazine), hERG blockade and simultaneous sodium channel blockade (Quinidine), as week as L-type calcium channel agonist activity (S-BayK 8644), are shown. Throughout the study, a prolongation beyond the 140% threshold was observed.

Application Note

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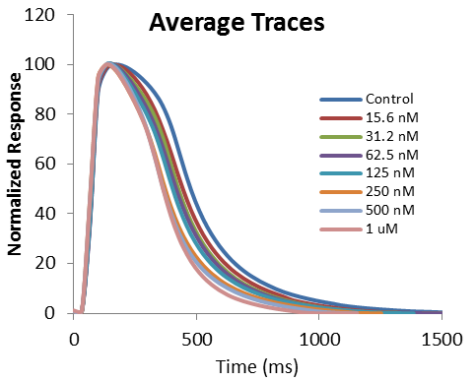
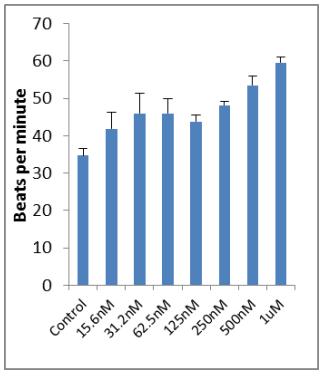
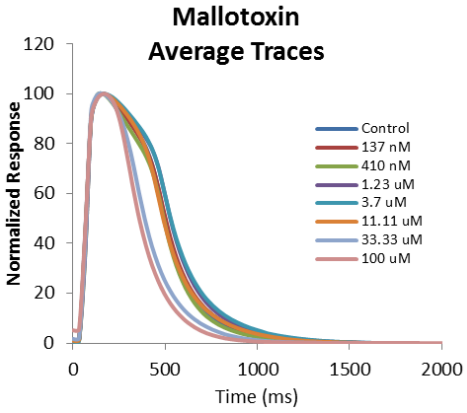
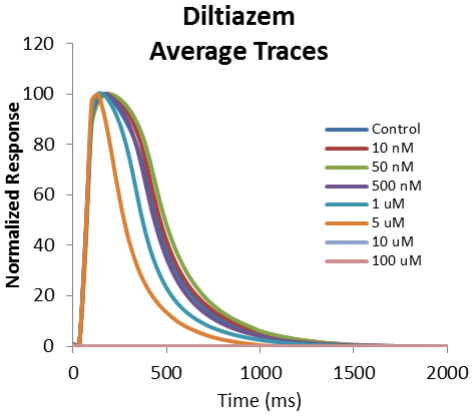


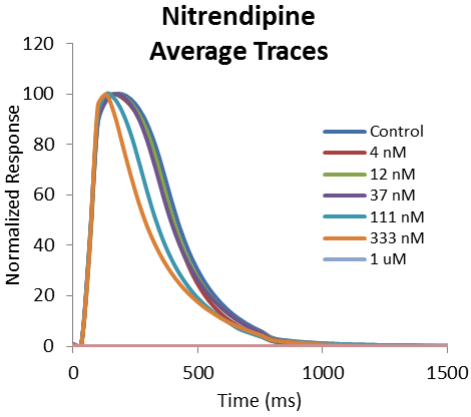


Positive chronotropes increase beat rate through a variety of mechanisms. Here the effects of Forskolin on Cor.4U® cells are shown.

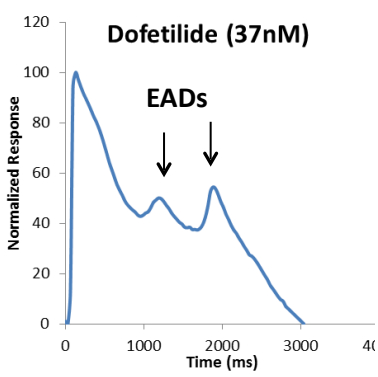


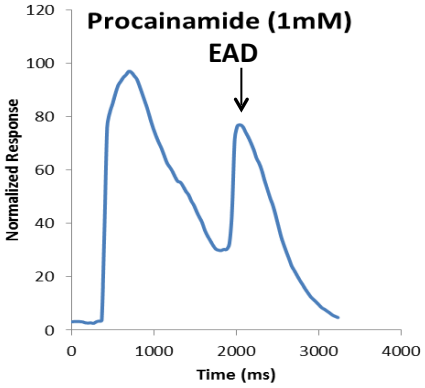
QT shortening compounds have the potential to induce arrhythmia such as ventricular tachycardia and ventricular fibrillation. Here, the effects of Mallotoxin (hERG activator) are shown.





Ca^{2+}channel blockers such as Diltiazem and Nitrendipine demonstrate a dose dependent reduction in the calcium transient duration. In both cases threshold concentrations are reached where all beating activity stops.





Vala Sciences' Arrhythmogenic Liability Assay and Axiogenesis Cor.4U® allow researchers to move beyond relying upon QT interval changes to predict arrhythmogenic potential and to directly observe pro-arrhythmia signals such as EADs and arrhythmic beats. Here, example traces from individual Cor.4U cardiomyocytes displaying EADs due to exposure to Dofetilide (37nM) or Procainamide (1mM) are shown.

<u>Conclusion:</u> The combination of the Axiogenesis Cor.4U® and Vala Sciences Arrhythmogenic Liability Assay provides a method for rapid assessment of a compound's arrhythmogenic potential. With the ability to screen up to 1,000 wells per day this assay can improve pipeline efficiency by providing a means for stratifying arrhythmogenic risk within a compound series early in the development process.

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For more information regarding
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For more information regarding Axiogenesis'
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