Human Cardiomyocytes Derived from Induced Pluripotent Stem Cells: High Throughput and High Content Assessment of Cardiac Toxicity and Drug Efficacy by Monitoring Cytosolic Free Calcium Transients

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Overview

- **Purpose:**
  - Introduction of selected, pure human cardiomyocytes derived from induced pluripotent stem cell (hiPSCM) into a calcium transient imaging high throuput screening (HTS) assay to assess cardiotoxicity and drug efficacies.
- **Methode:**
  - Monitoring of intracellular free calcium $[Ca^{2+}]_i$ with a Ca^{2+} sensitive fluorescent dye under serum-free conditions
  - Recording of $[Ca^{2+}]_i$ with the high speed fluorescence imaging camera using the Hamamatsu FDSS/μCell (Hamamatsu Photonics K.K., see fig 1)
  - Monitoring of $[Ca^{2+}]_i$ modulation in the presence of reference compounds.
  - Offline raw data analysis with the Hamamatsu CalDio software
- **Results:**
  - 39 compounds were successfully tested in triplicates and 10 concentrations each
  - hiPSCM responded as expected to the references compounds under serum-free condition in BMCC Medium

Introduction

For high throughput screening (HTS) assays mainly recombinant cell lines expressing the target of interest are used due to the specificity, robustness, sensitivity, and the low costs of the assays. As is to be expected, these assays frequently lead to a high number of false positive hits.

Complex physiological processes like the excitation-contraction coupling of cardiac myocytes and the efficacy of drugs or toxic drug action on such a system cannot be represented correctly with such simple cell models.

Human iPSCM display a primary-like phenotype, reveal a regular spontaneous beating pattern and a functional calcium induced calcium release. Furthermore, these cells can be produced in large quantities in a quality controlled environment and are therefore an ideal model to be introduced into a standard calcium imaging HTS assay using Ca^{2+} sensitive fluorescent dyes in combination with a fluorescence plate reader system.

The Hamamatsu FDSS kinetic plate readers are equipped with a high-speed camera, an integrated dispenser head and temperature control, allowing for detection of fast calcium signals under physiological temperatures.

We have used cryopreserved hiPS-derived Cor.4U® cardiomyocytes in 384 well plates to optimise assay conditions and to detect changes in calcium transients induced by cardiac ion channel modulators.

Using hiPS-derived Cor.4U® cardiomyocytes precultured for 3 - 5 days in 384 well plates, stable $[Ca^{2+}]_i$ signals were measured over 35 min and the effect of more than 30 compounds on $[Ca^{2+}]_i$ transients in human iPS-derived cardiomyocytes were detected and analyzed.

Material and Methods

Cryopreserved Cor.4U hiPSCM (Axiogenesis AG) were thawed and seeded in 384-well microplates (Greiner Bio-one) coated with a 1:100 diluted fibronectin solution (Sigma). Three days after thawing and seeding the hiPSCM revealed stable synchronous beating and were ready to be introduced into the assay. Two hours before the experiment the medium was changed to the serum-free BMCC Medium (Axiogenesis) to a total volume of 25 μl. A 0.5x dilution of the FLIPR Calcium 5 Assay dye (Molecular Devices) was prepared according to the manufacturers instructions and 25 μl were loaded onto the cells for a 20 min incubation at 37˚C and 5% CO_2. The assay chamber of the FDSS/μCELL was heated to 37°C before the hiPSCM were transfer into the device. After an additional 20 min equilibration time, the baseline $[Ca^{2+}]_i$ was recorded of all wells. Subsequently, 25 μl of 3x concentrated compound dilutions or DMSO vehicle control were added to each well at the same time with the 384 dispenser head of the FDSS/μCell and recordings were taken after 5 min each for a total time of 35 min. Fluorescence images of all wells of the microplate were recorded every 0.016 s to capture changes of $[Ca^{2+}]_i$. The raw data were analyzed offline for a total of 16 parameter of the $[Ca^{2+}]_i$ waveform with the CalDio software (see Fig. 2 and 3).

Fig: 1: Hamamatsu FDSS/μCell

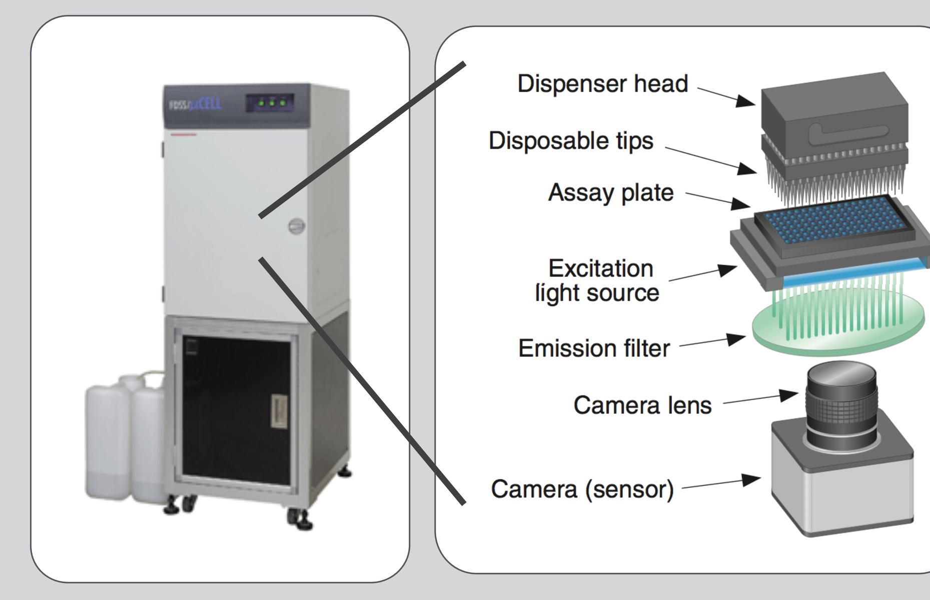


Fig. 2: Parameters calculated from raw data of Ca^{2+} transients

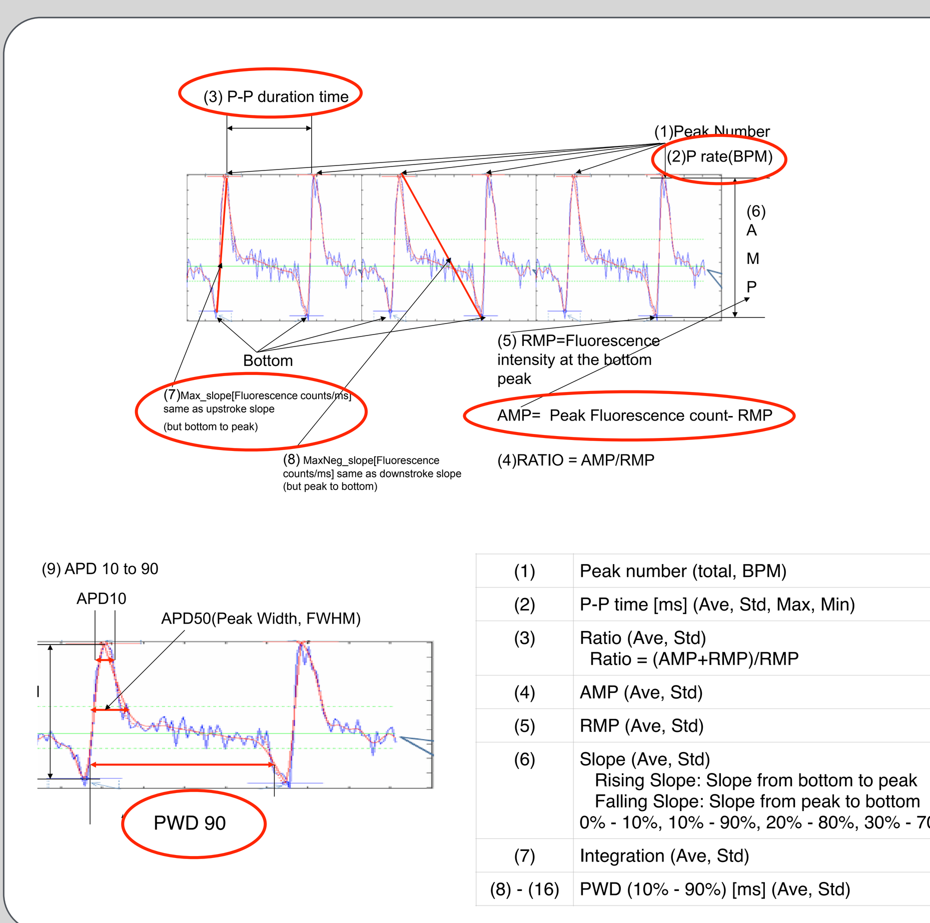


Fig. 3: Clustering of compounds due to their action on parameters of the calcium transient waveforms

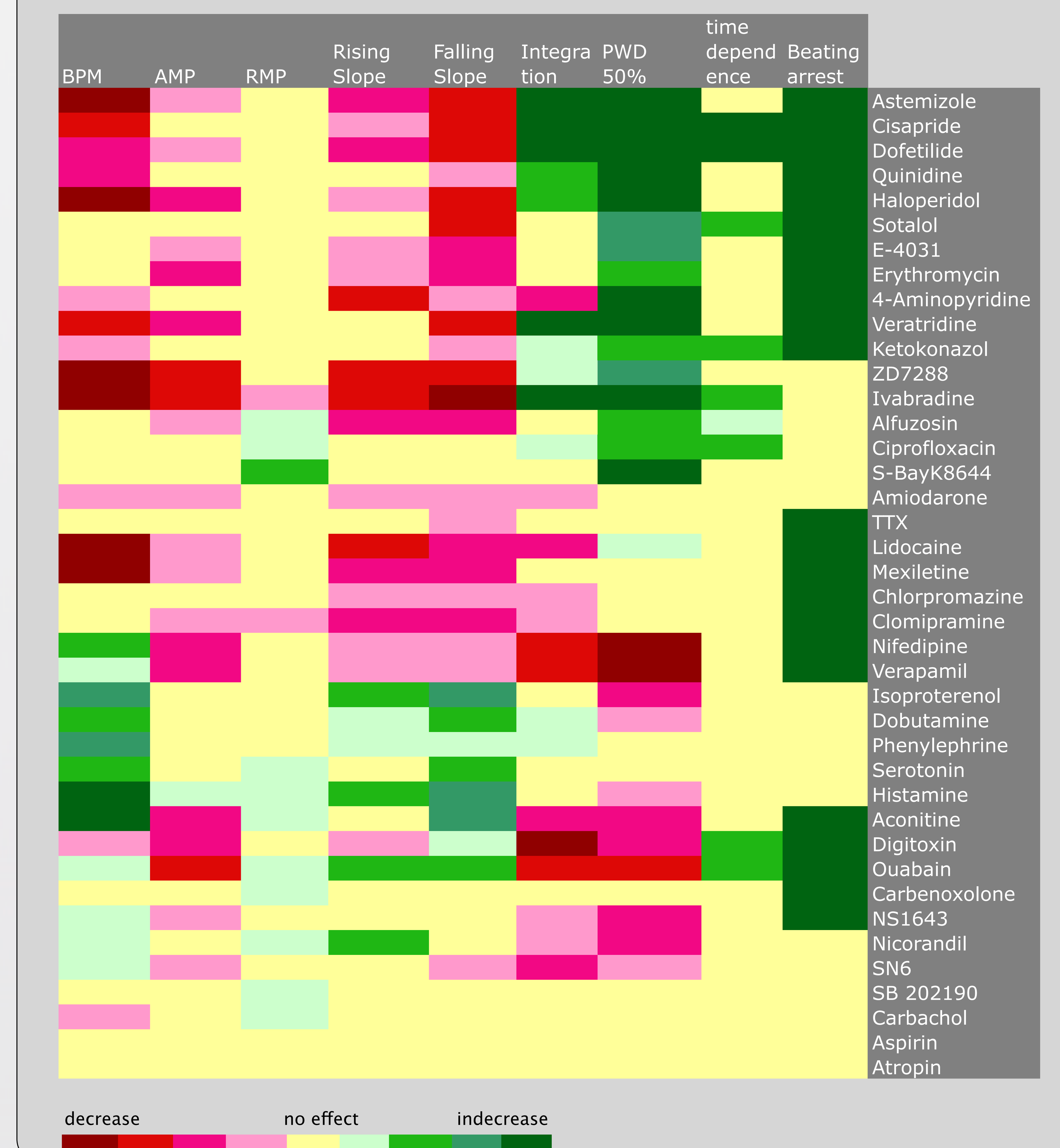
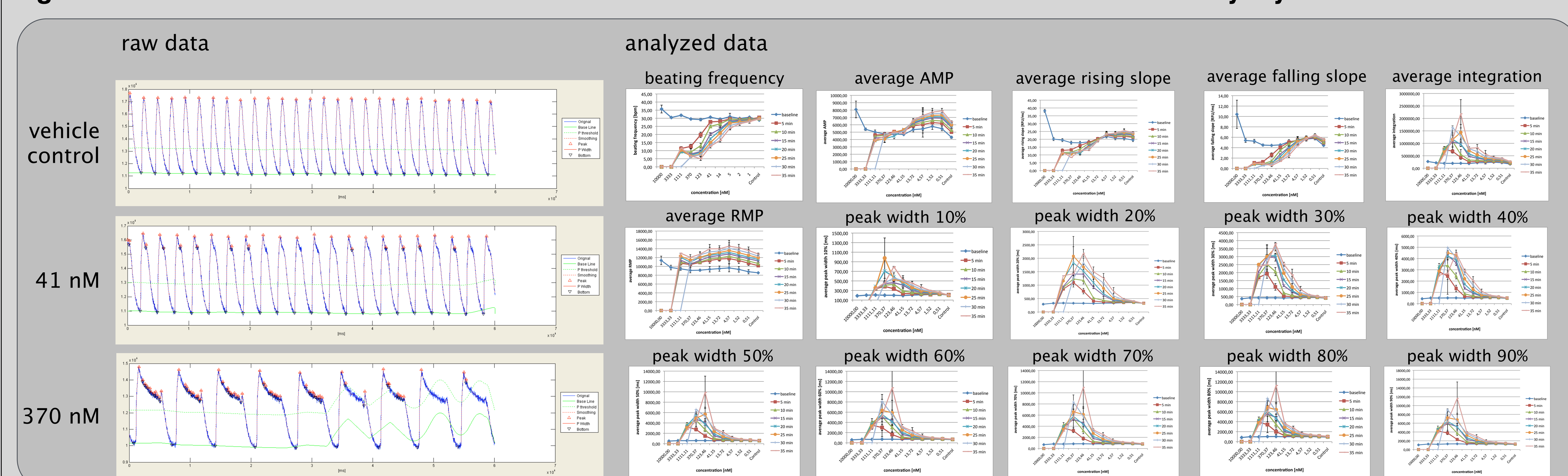


Fig. 4 Detection of Astemizole effects on Ca^{2+} transients in iPSc-derived human Cor.4U® cardiomyocytes



Results

The hiPSC-derived Cor.U cardiomyocytes have been successfully introduced into an HTS assay to monitor intracellular free calcium with a calcium sensitive fluorescent dye under serum-free condition with the Hamamatsu FDSS/μCell. Within a period of 10 days 39 compounds were tested in 10 concentration dose responses and at least triplicates for each concentration and vehicle controls. Using the CalDio software it was possible to analyze the action of the compounds on the calcium transients and it was possible to cluster the compounds due to their action on the analyze parameter.

Conclusion

- Human iPS-derived Cor.4U cardiomyocytes can be easily applied to fast HTS screening assay to monitor drug-induced intracellular free calcium transients.
- The fast camera of the FDSS/μCell allows for a reliable and stable recording of Ca^{2+} transients in a 384 well format.
- Thus this assay is an ideal and cost effective, fast medium to high throughput screening assay to assess cardiotoxicity and drug efficacy at an early time point of the drug development process.

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