



Cardiotoxicity testing in human iPSC-derived cardiomyocytes for the CiPA pilot study using all-optical electrophysiology

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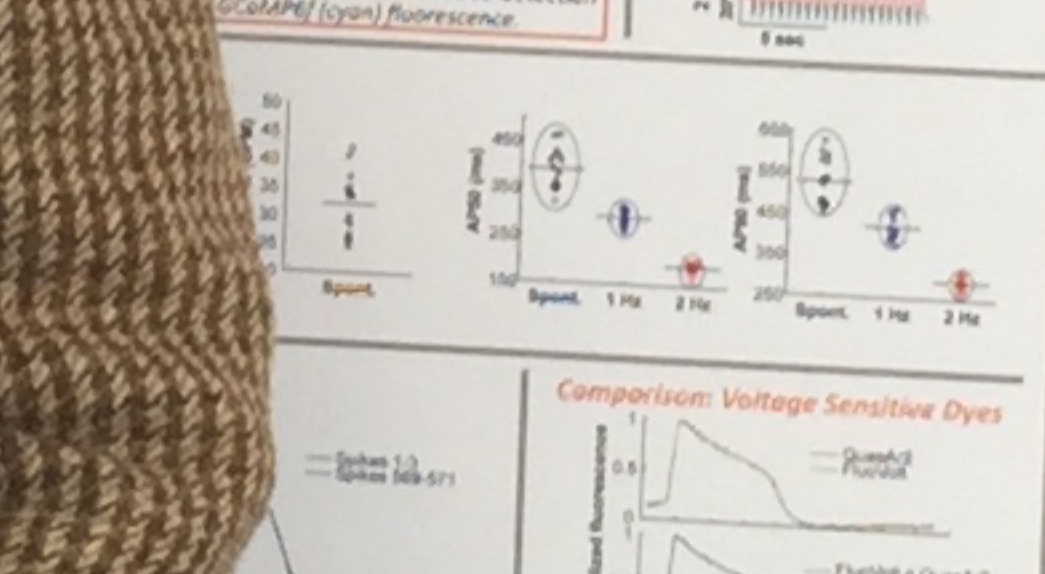
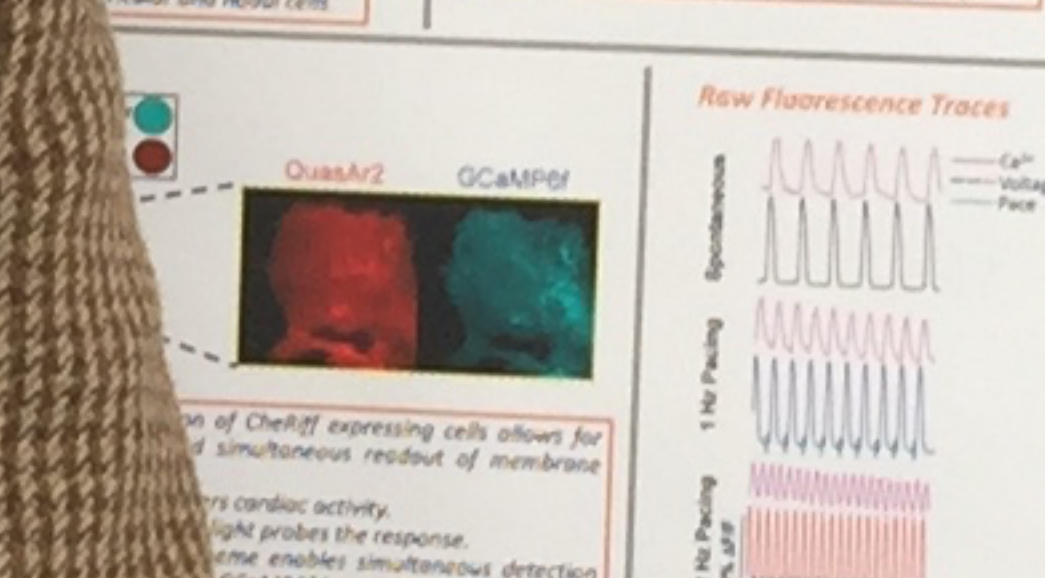
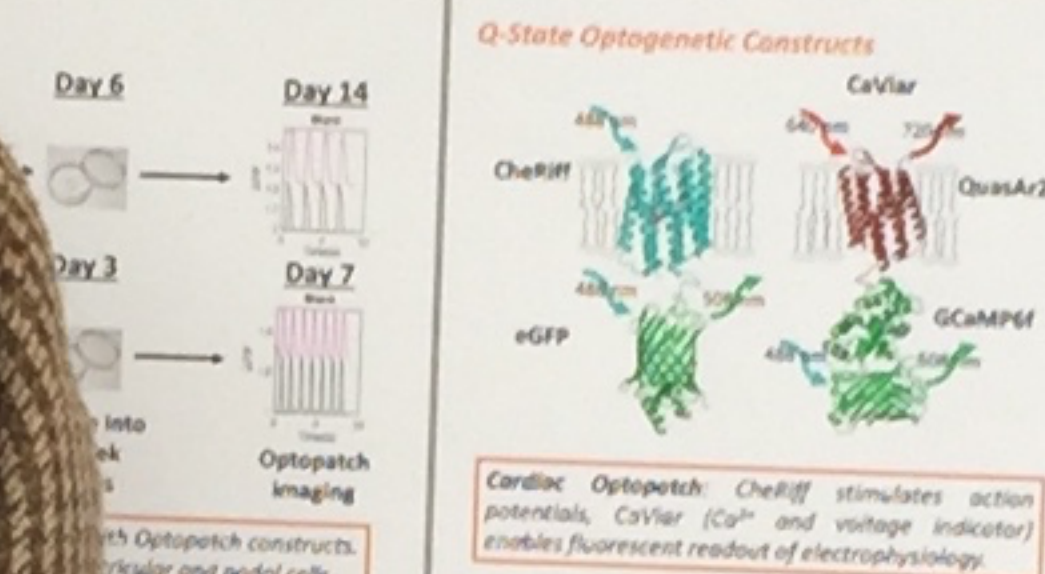
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Introduction

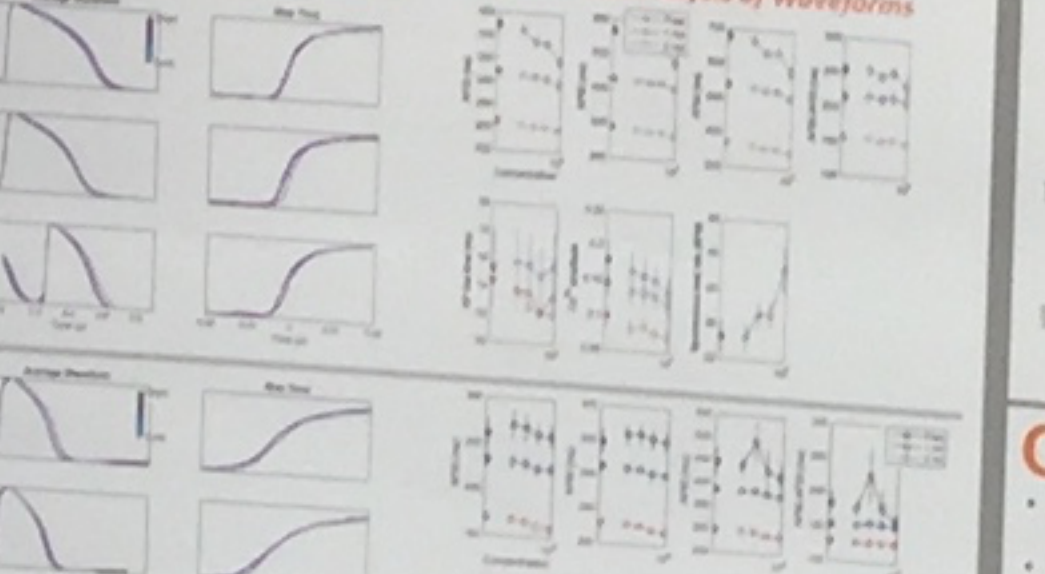
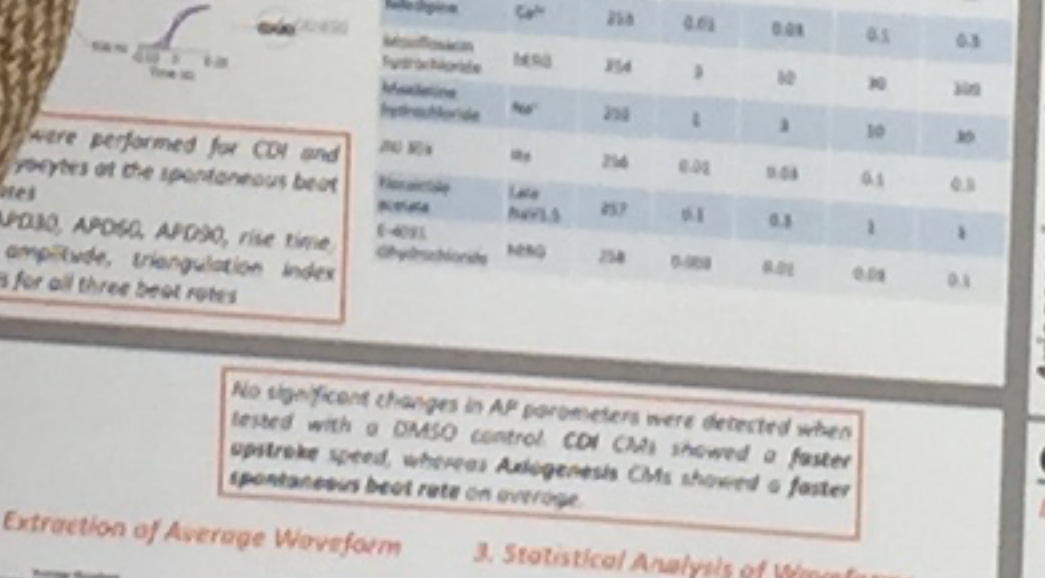
The goal of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative is to create a new model for evaluating potential cardiotoxic effects of compounds through improved cellular assays coupled with *in silico* modeling. A primary endpoint of this effort is to evaluate human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs) as a cellular substrate for cardiotoxicity testing. Studies have shown that hiPSC-CMs recapitulate a number of phenotypic properties of native cardiac tissue and are sensitive to different classes of ion channel modulators.

At Q-State Biosciences, we have developed an all-optogenetic platform called Cardiac Optopatch that enables simultaneous stimulation and detection of electrical activity in hiPSC-derived CMs. Stimulation is achieved with a channelrhodopsin variant, ChR2, which provides a stable electrophysiological background. Simultaneous fluorescent detection of action potential (AP) waveforms and calcium transients (CTs) is achieved using a fast, sensitive dual-reporter fusion protein called Cavilar (calcium and voltage indicator).

As part of the CiPA pilot study, we used this platform to test the effects of eight ion channel modulatory compounds (binned) on cell lines from two providers, CDI and Asigenesis. Measurement of electrophysiological parameters included changes in AP waveforms (APD30, APD50, APD90), triangulation, AP rise time, spontaneous beat rate and CTs (amplitude) at the spontaneous beat rate and at two paced frequencies (1 Hz, 2 Hz). These results demonstrate the utility of Cardiac Optopatch to be used for assessment of drug responses in hiPSC-derived cardiomyocytes.

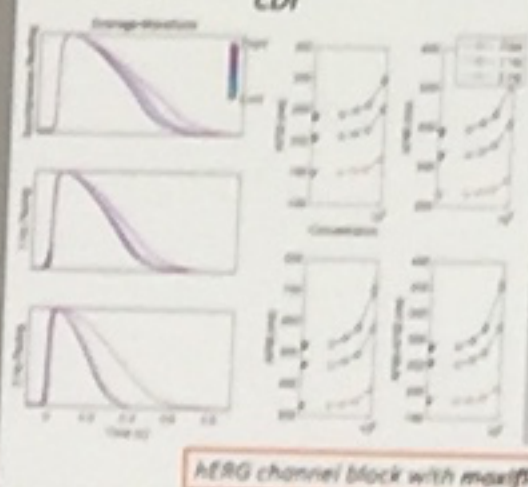


Drug	APD30	APD50	APD90	AP Rise Time	CT Amplitude
Control	20.0	30.0	40.0	1.0	1.0
Drug 1	21.0	31.0	41.0	1.1	1.1
Drug 2	22.0	32.0	42.0	1.2	1.2
Drug 3	23.0	33.0	43.0	1.3	1.3
Drug 4	24.0	34.0	44.0	1.4	1.4
Drug 5	25.0	35.0	45.0	1.5	1.5
Drug 6	26.0	36.0	46.0	1.6	1.6
Drug 7	27.0	37.0	47.0	1.7	1.7
Drug 8	28.0	38.0	48.0	1.8	1.8



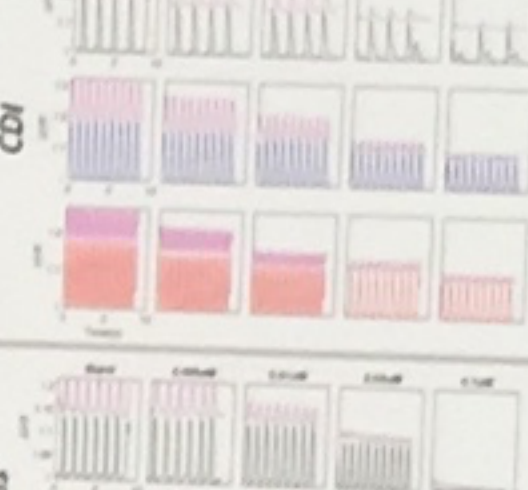
K⁺ channel modulators

Drug 254: Moxifloxacin - HERG channel block



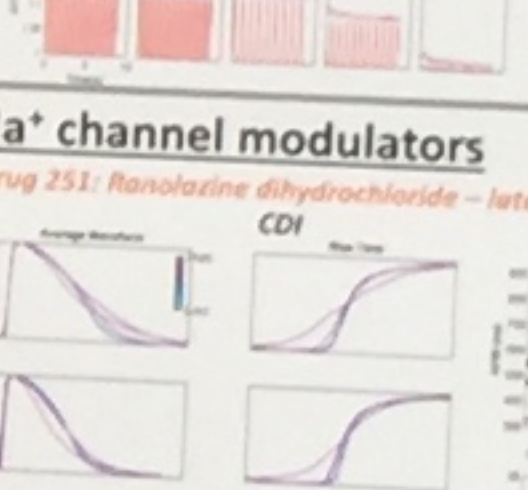
HERG channel block with moxifloxacin leads to prolonged APs.

Drug 258: E-4031 dihydrochloride - HERG channel block



E-4031 increases APD, rise time and decreases CT amplitude. EADs are observed at 30 nM and above with no longer steps at 2 nM and below steps at 200 nM.

Drug 256: JNJ 303 - IKs block



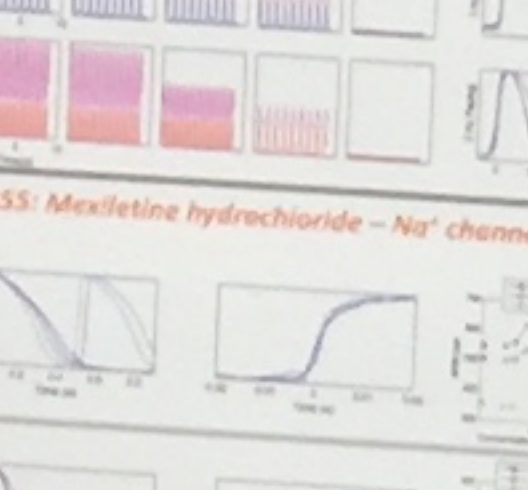
IKs channel block with JNJ 303 shows modest effects on AP parameters.

Drug 251: Ranolazine dihydrochloride - late NaV1.5 block



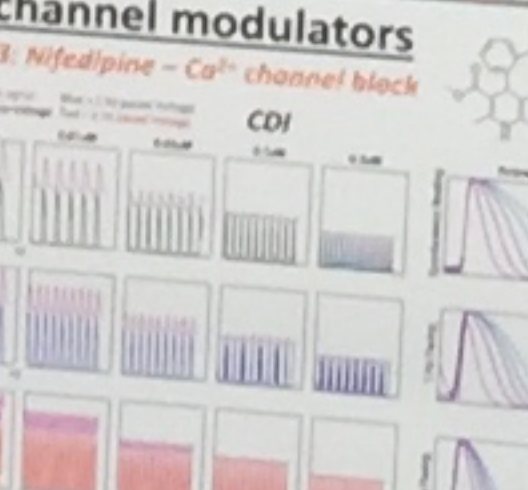
Ranolazine increases APD, rise time and decreases CT amplitude. EADs are observed at 30 nM and above with increasing concentrations of drug.

Drug 252: Quinidine - Na⁺ channel block



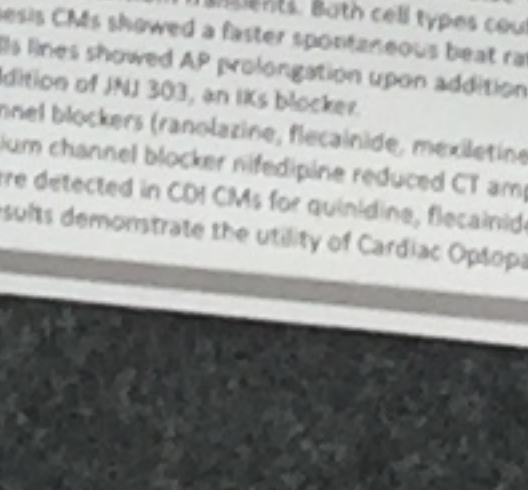
Quinidine increases APD, rise time and decreases CT amplitude. EADs are observed at 3 μM.

Drug 255: Mexiletine hydrochloride - Na⁺ channel block



Mexiletine increases APD, rise time and decreases CT amplitude. EADs are observed at 3 μM.

Drug 257: Flecainide acetate - Late NaV1.5 block



Flecainide increases APD, rise time and decreases CT amplitude. EADs are observed at 3 μM.

Drug 253: Nifedipine - Ca²⁺ channel block



Nifedipine decreases APD and increases CT amplitude, consistent with Ca²⁺ channel block. Asigenesis cells showed no effect at 30 nM.

Conclusions

- Both CDI and Asigenesis CMs expressed Cardiac Optopatch vectors efficiently, enabling high SNR, simultaneous recordings of AP waveforms and calcium transients. Both cell types could be paced at different stimulus frequencies using this platform.
- Both cell lines showed a faster spontaneous beat rate compared to CDI CMs. CDI CMs showed a faster upstroke velocity upon addition of JNJ 303, an IKs blocker.
- Na⁺ channel blockers (ranolazine, flecainide, mexiletine, quinidine) increased rise time and AP prolongation, larger in effect for CDI cells.
- The calcium channel blocker nifedipine reduced CT amplitude and AP width.
- EADs were detected in CDI CMs for quinidine, flecainide and E-4031.
- These results demonstrate the utility of Cardiac Optopatch to be used for assessment of drug responses in hiPSC-derived cardiomyocytes.