GREEN CHEMISTRY, RED FLAGS:

Multiparametric cardiotoxicity screening of phytochemicals using hiPSC-CMs-MEA Assay

- **Monday, 27th of November 2023**

- Laura-Sophie Frommelt

Importance of pre-clinical comprehensive cardiac safety studies

Cardiotoxicity:

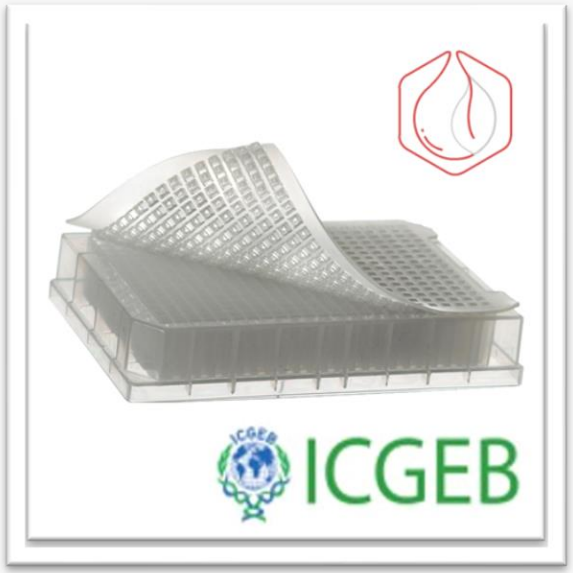
- is one of the most common forms of **drug-induced toxicity**
- Resulted in numerous costly **withdrawals of drugs**

Preclinical cardiac safety studies:

- Increase **drug safety**
- Reduce the **cost** of drug development

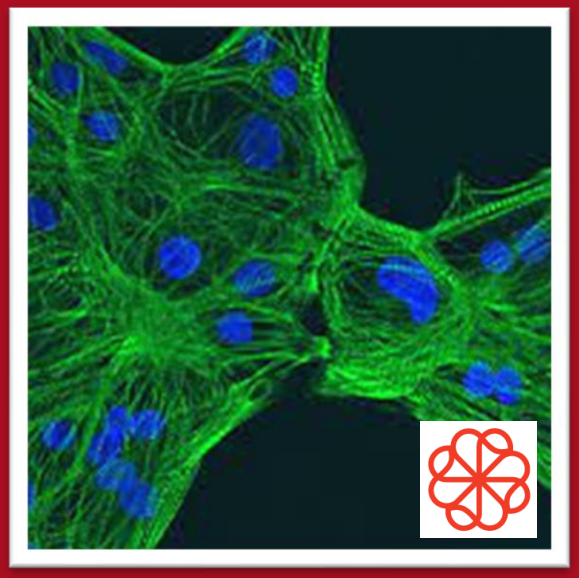
Aim: Assess the effectiveness of the **hiPSC-CMs-MEA assay** in detecting acute and chronic cardiotoxic effects of phytochemicals

Multiparametric drug assessment using hiPSC-CMs-MEA Assay



Prestwick Phytochemical Library:

- 320 purified well-known and novel compounds
- mostly from plants



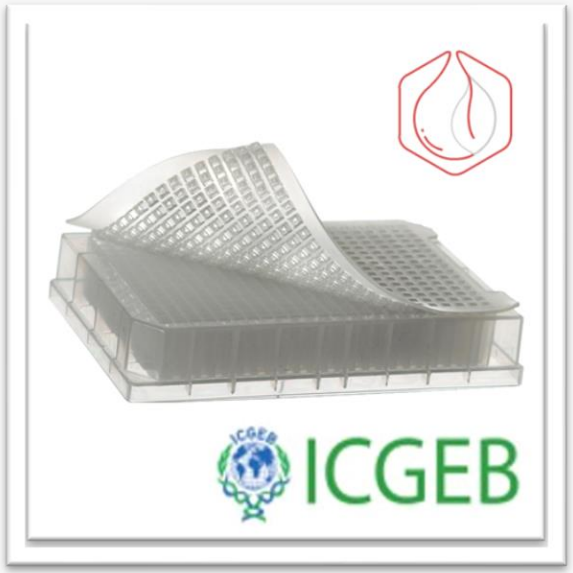
Ncyte Cardiomyocytes (Ncardia)

- Human iPSC-derived ventricular-like CMs
- Large-scale production
- Slow + uniform beating rate

Maestro Edge MEA system:

- CiPA-study (**C**omprehensive ***in vitro*** **P**roarrhythmia **A**ssay)
- Heartbeat in a dish
- Various cardiac endpoints: FP-signals, LEAP, contractility signals and cell viability

Multiparametric drug assessment using hiPSC-CMs-MEA Assay



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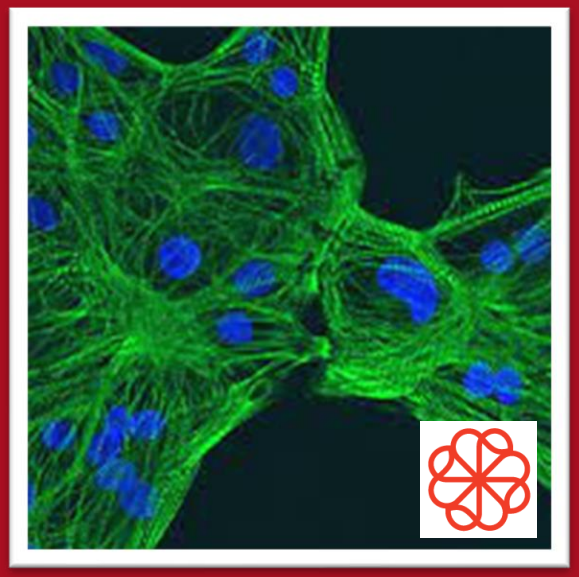

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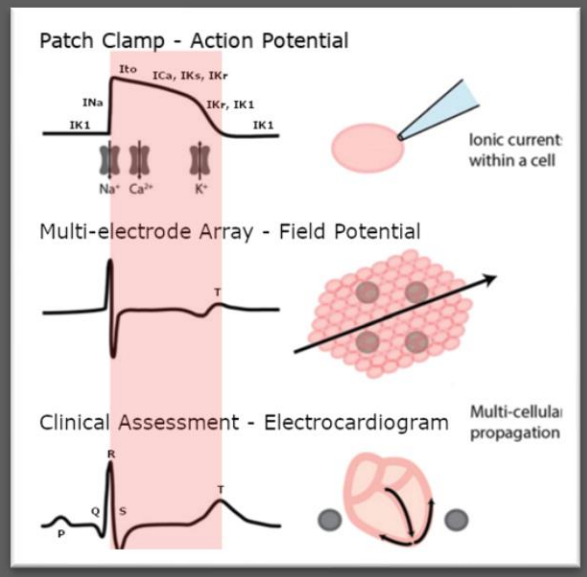

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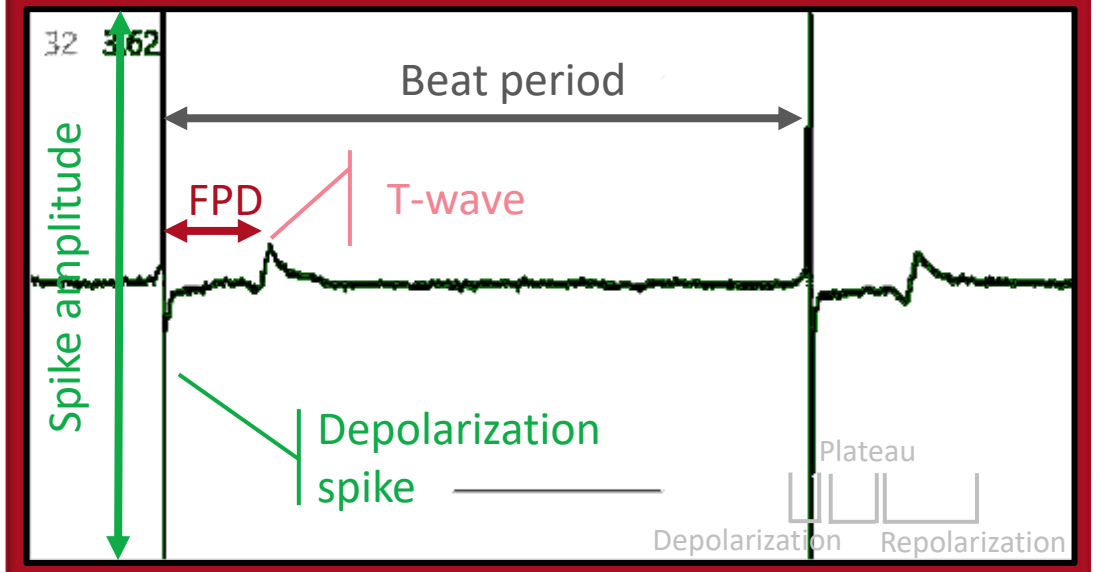
Cardiac endpoint parameters

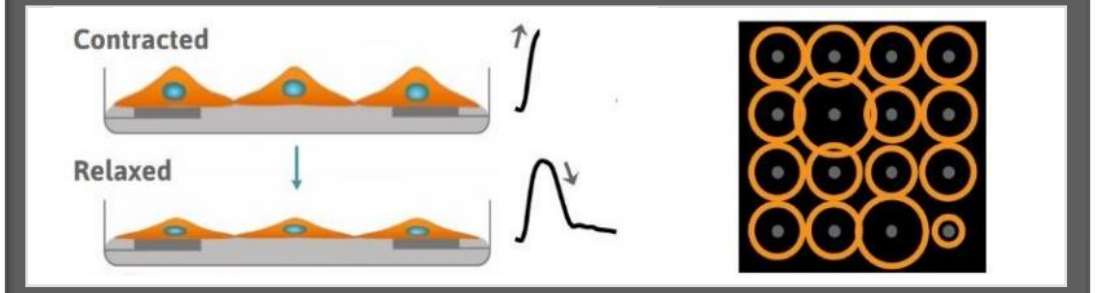
Field potential-based



Beat period mean
Beat period irregularities
Field potential duration (+corrected)
Spike amplitude mean
Arrhythmic events
Local extracellular action potential (LEAP)

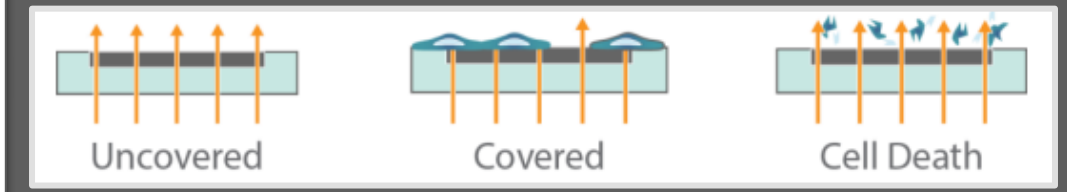
Impedance-based

Contractility (3.125kHz)



Beat amplitude
Excitation-contraction coupling

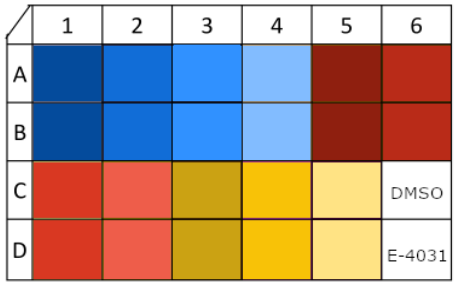
Viability (41.5kHz)



Weighted mean resistance
Average resistance

Experimental design of the library screening

Plate design



Timeline

Acute phase
(ion channel effects)

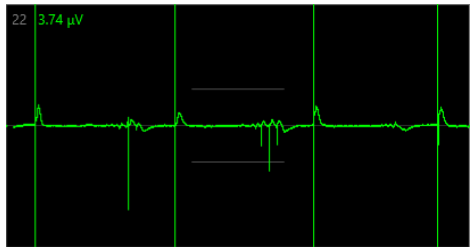
Chronic phase
(intracellular signal, ion channel trafficking)

Quality check → Baseline measurement → Addition of compounds → t = 0.5 h measurement → t = 2 h measurement → t = 24 h measurement → t = 48 h measurement → Protein pellets

Single Dosing (1µM)

Positive control

E-4031 (500nM)



Measurements:

- **Field Potential** (+paced)
- **Viability**
- **Contractility** (+paced)
- LEAP (2+24h)

Data analysis

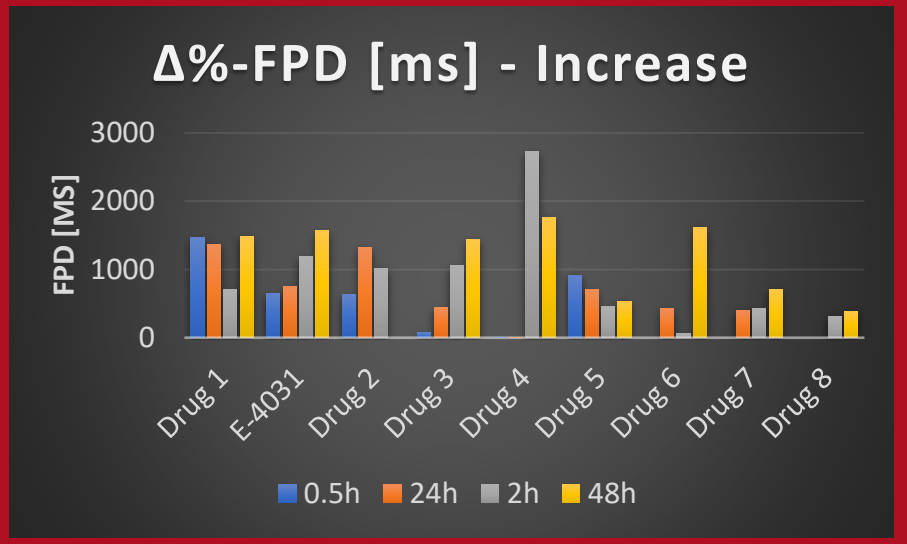
- **Normalized comparison:** %-change between baseline and dosed conditions
- **Subtraction** of the vehicle control (DMSO)
- **Min-Max Scalar normalization** → **ToxPi™ Ranking**

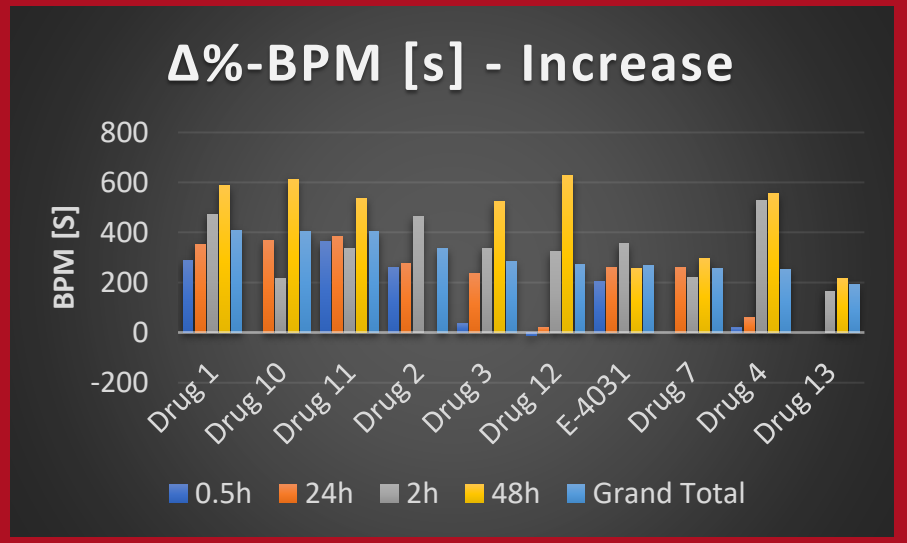
Validation – K^+- channel blockers (K_v11.1 - hERG)

E-4031 dihydrochloride (positive control)

Expectations

- Prolongation of Field Potential Duration (FPD)
- Increase in Beat Period Mean (BPM)
- Arrhythmic events



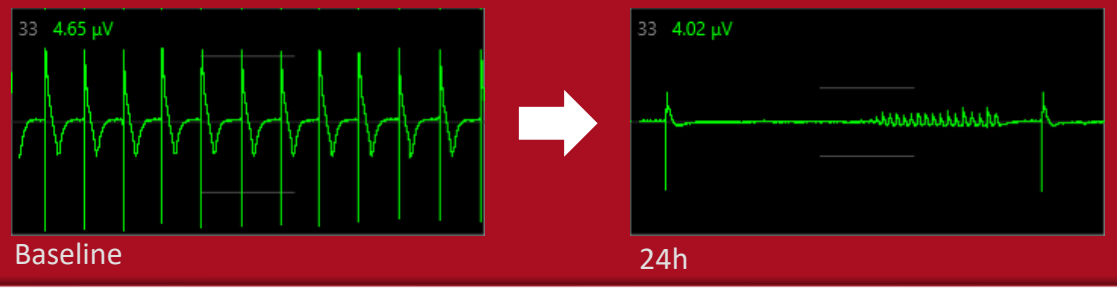


Validation – K^+- channel blockers (K_v11.1 - hERG)

E-4031 dihydrochloride (positive control)

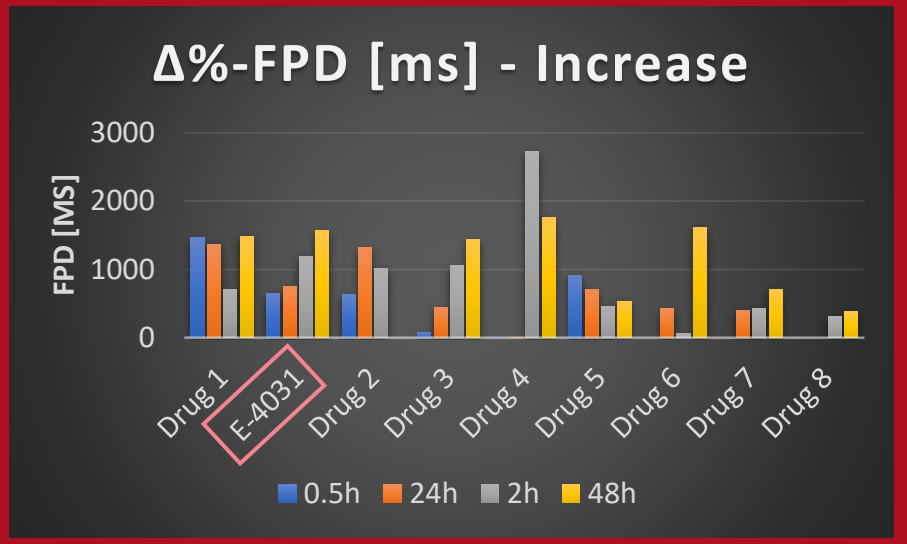
Results:

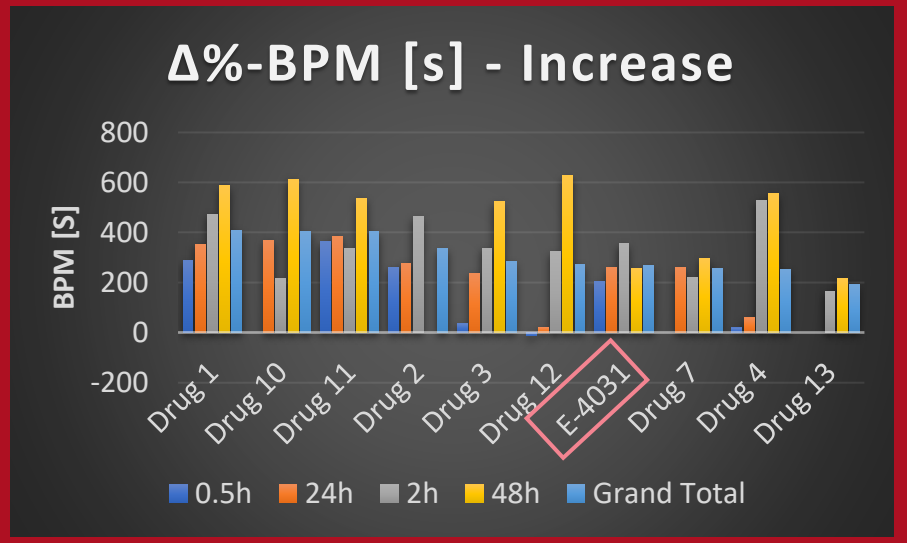
- Prolongation of Field Potential Duration (FPD): **2nd place**
- Increase in Beat Period Mean (BPM): **6th place**
- Arrhythmic events: **1st place (20 events)**



Baseline

24h

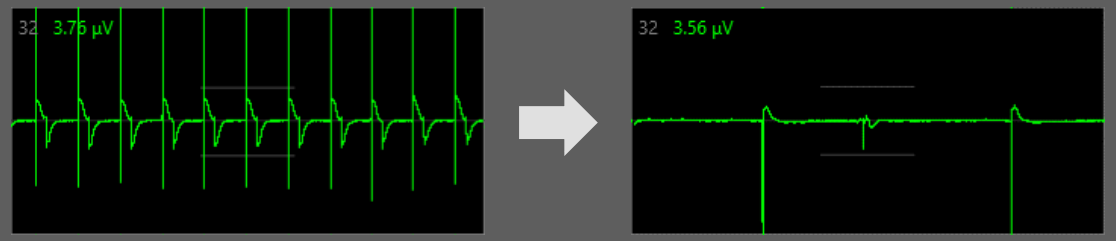




Validation – K^+- channel blockers

Drug 1: Indole - Cardiovascular active compound, Vasodilator

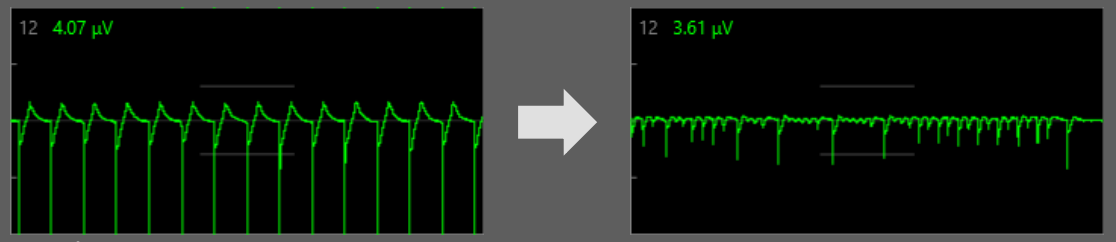
- Arrhythmic events: **3rd place (17 events)**



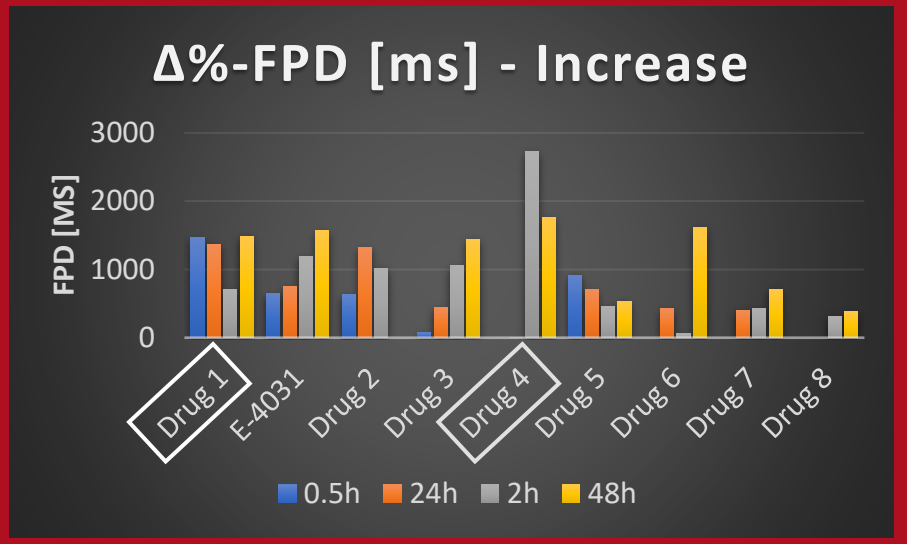
Baseline 24h

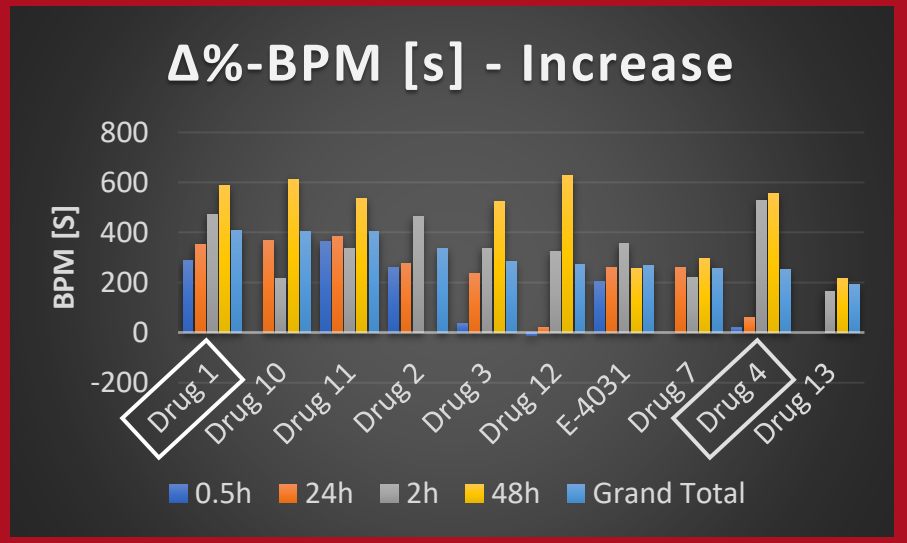
Drug 4: Alkaloid - Cardiovascular active compound, Antihypertensive

- Arrhythmic events: 11th place (12 events)



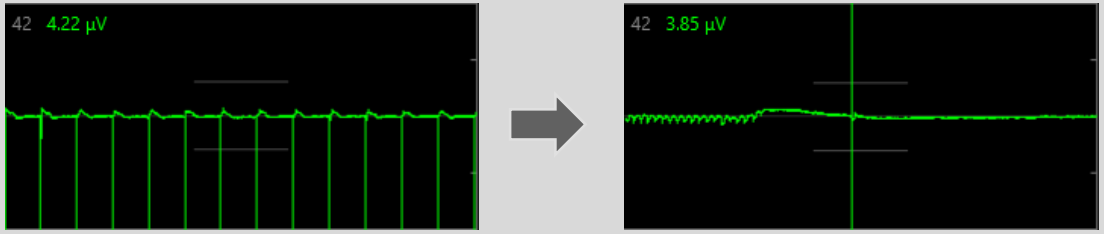
Baseline 24h





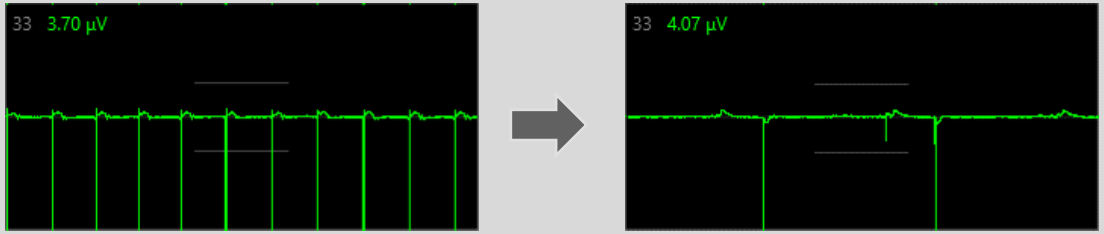
New Hits – possible K^+- channel blockers

Drug 2: semisynthetic alkaloid –
CNS active compound, Neuroprotectant
Arrhythmic events: **2nd place (19 events)**



Baseline 48h

Drug 3: Isoquinoline, Infectiology,
antibacterial effect
Arrhythmic events: **5th place (15 events)**



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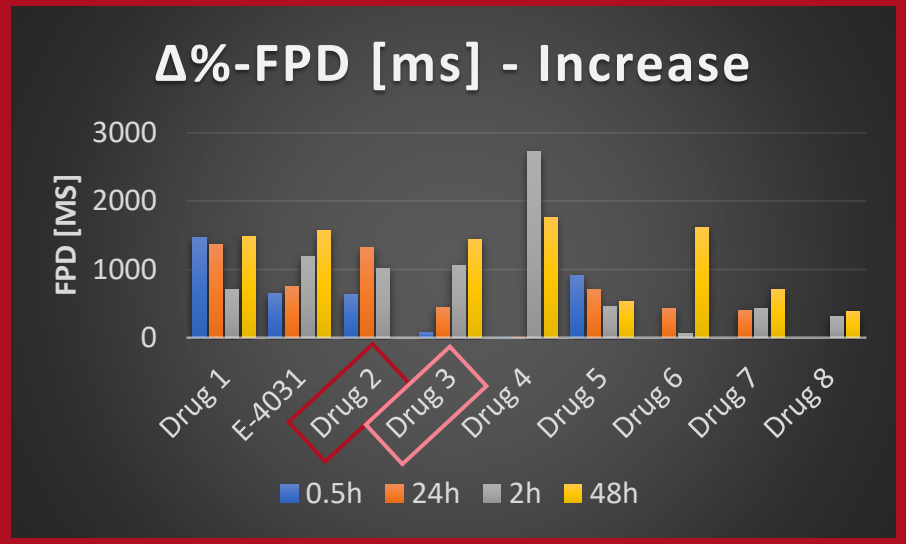

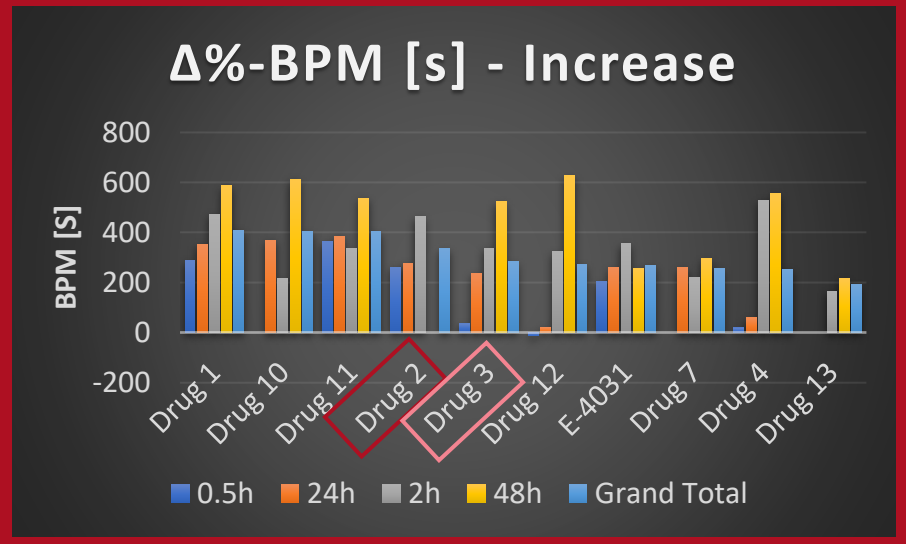

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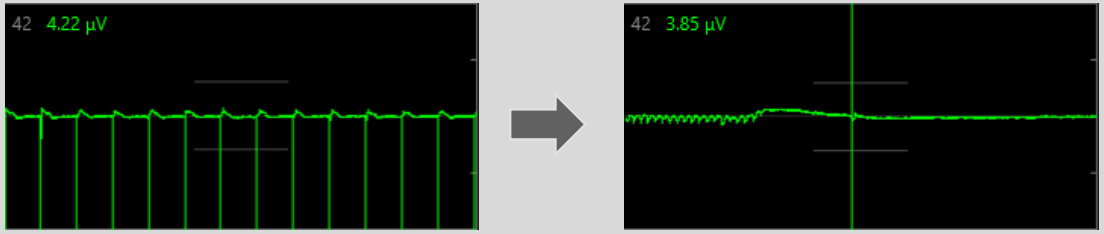

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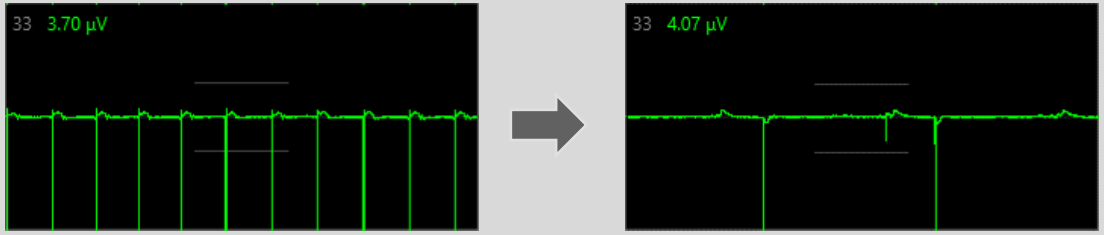


Baseline 48h

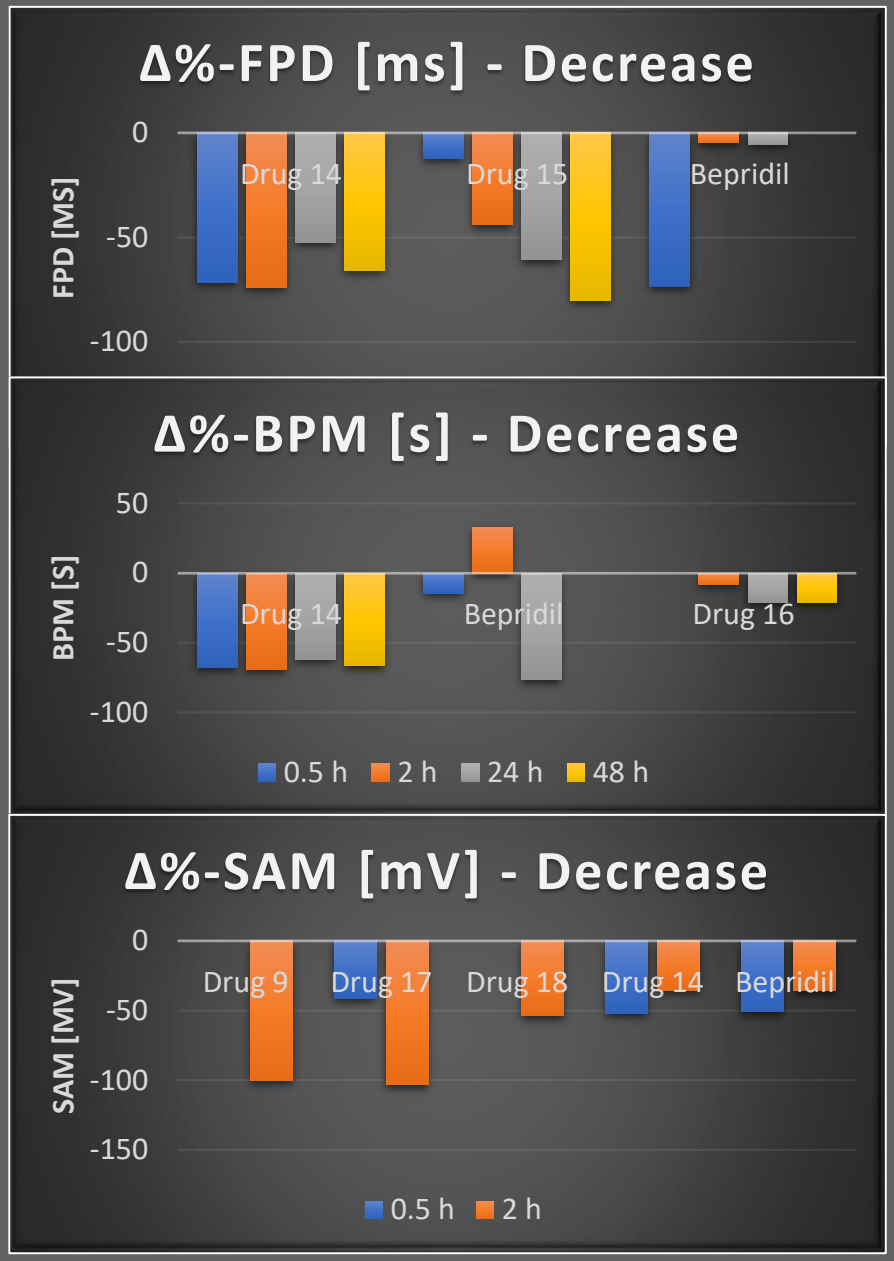
NO reported link with arrhythmia so far

Validation – Na^+/Ca^{2+}-channel blockers

Bepridil (positive control) - Na^+ and Ca^{2+}-channel blocker, antiarrhythmic and antihypertensive

Expectations:

- Shortening of Field Potential Duration (FPD)
- Decrease in Beat Period Mean (BPM):
- Decrease in Spike Amplitude Mean (SAM)



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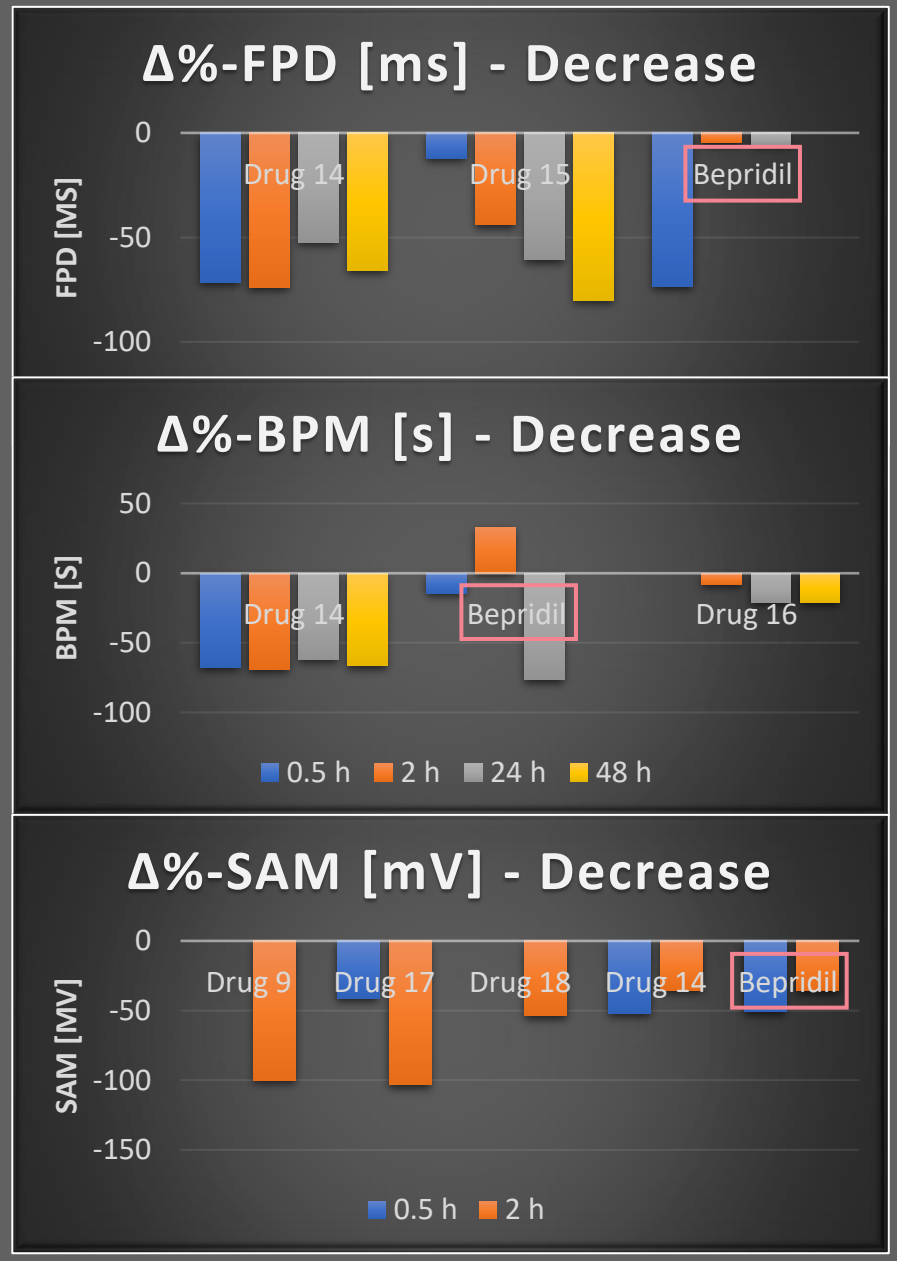
Results:

- Shortening of Field Potential Duration (FPD): **3rd place**
- Decrease in Beat Period Mean (BPM): **2nd place**
- Decrease in Spike Amplitude Mean (SAM): **5th place**

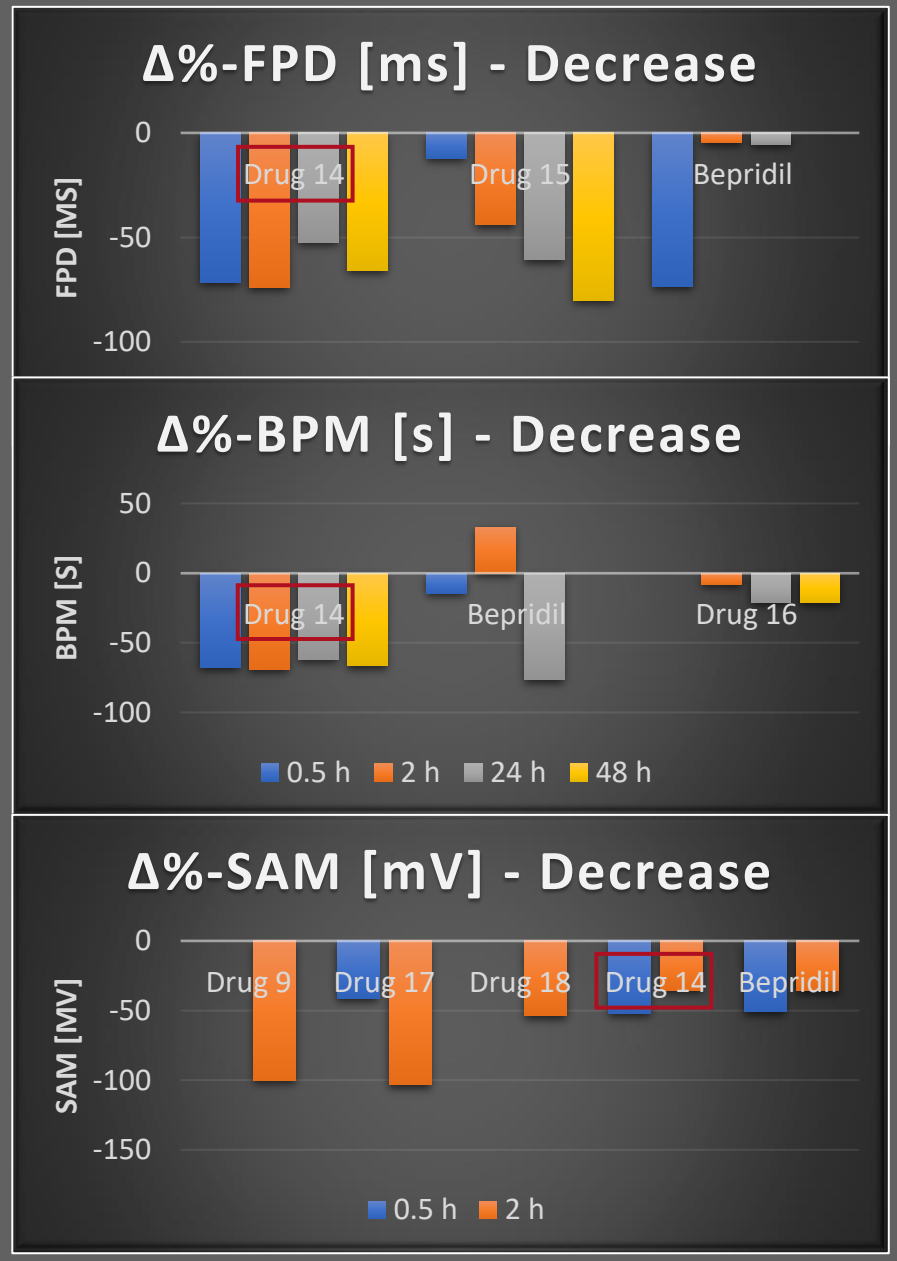


Validation – Na^+/Ca^{2+}-channel blockers

Drug 14: Alkaloid, Cardiovascular active compound, antihypertensive effect

- Shortening of Field Potential Duration (FPD): **1st place**
- Decrease in Beat Period Mean (BPM): **1st place**
- Decrease in Spike Amplitude Mean (SAM) : **4th place**

→ has been used in the treatment of hypertension but has largely been replaced by drugs with fewer adverse effects



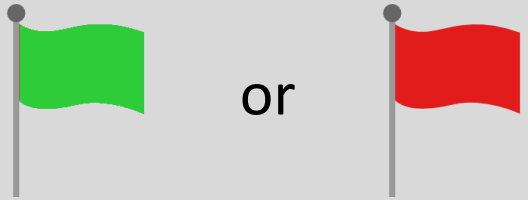
Moving from single parameter to multiparameter analysis

Individual parameters:

Beat Amplitude: 69 compounds
Field Potential Duration: 13 compounds
Beat Period Mean: 17 compounds
Beat Period Irregularities: 23 compounds
Spike Amplitude Mean: 39 compounds
Severe arrhythmic effects: 27 compounds
Viability: 14 compounds

Multiparametric analysis aims to:

- quantify the actual arrhythmogenic risk rather than sole focus on a single parameter
- comprehensive characterization of cardiotoxicity
- early and informed decision:



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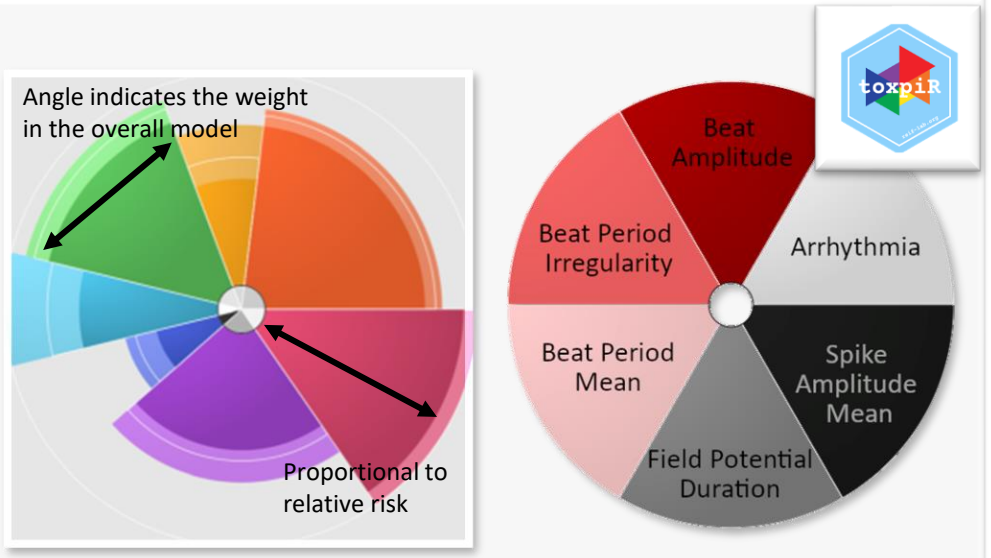

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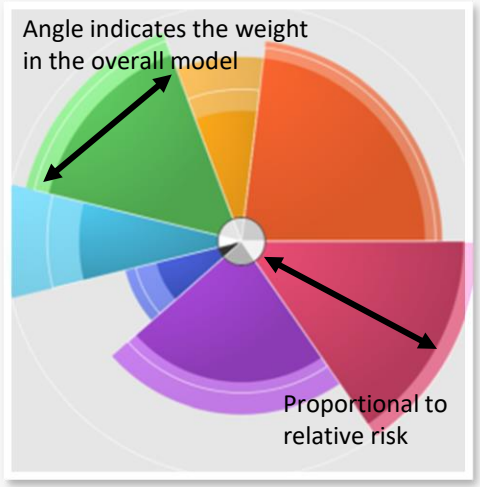
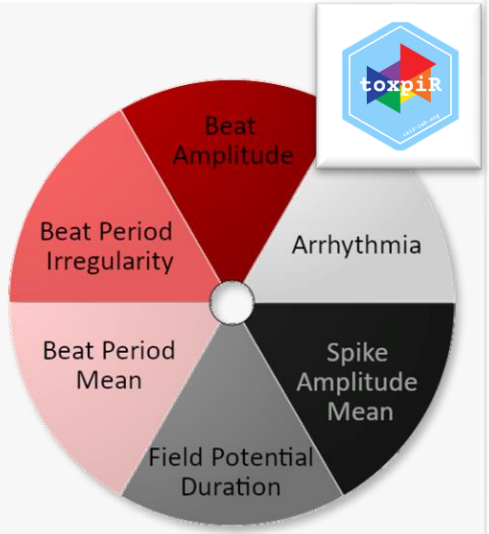
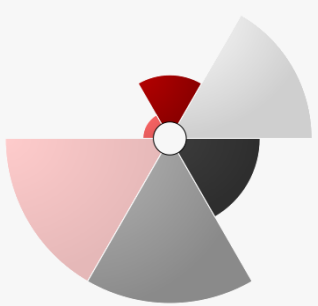
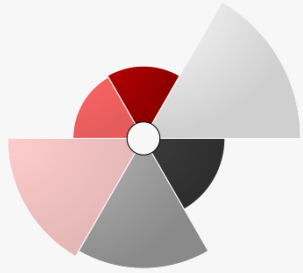




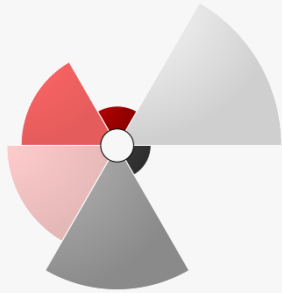
Top six compounds:



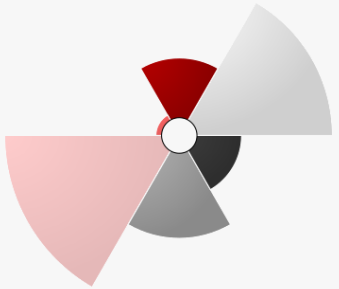
Drug 1



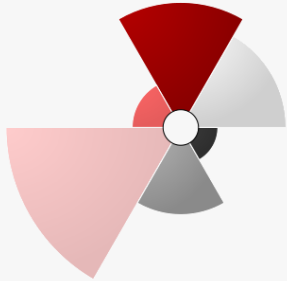
Drug 2



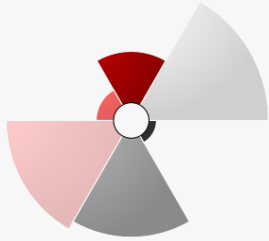
E-4031



Drug 5

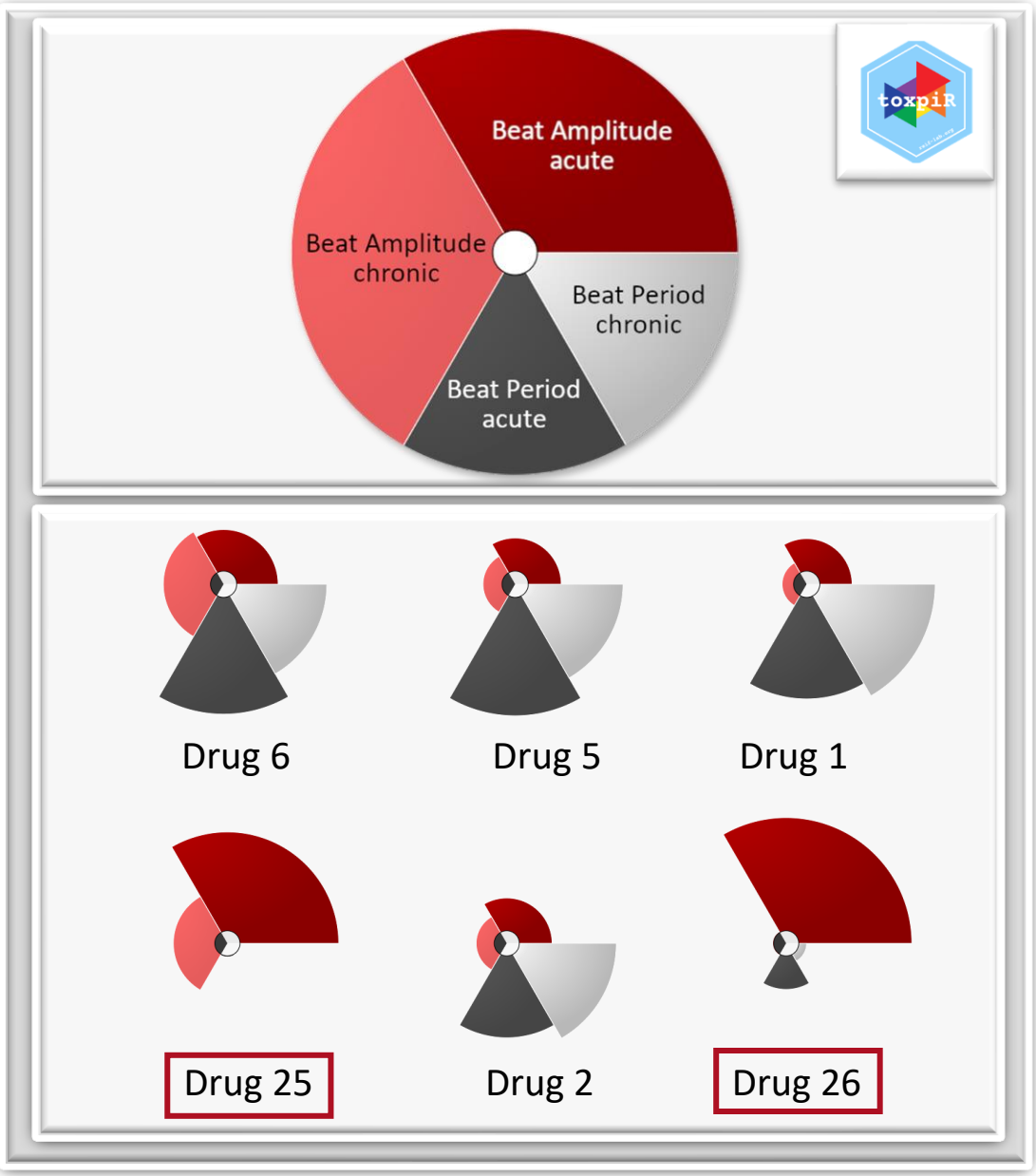


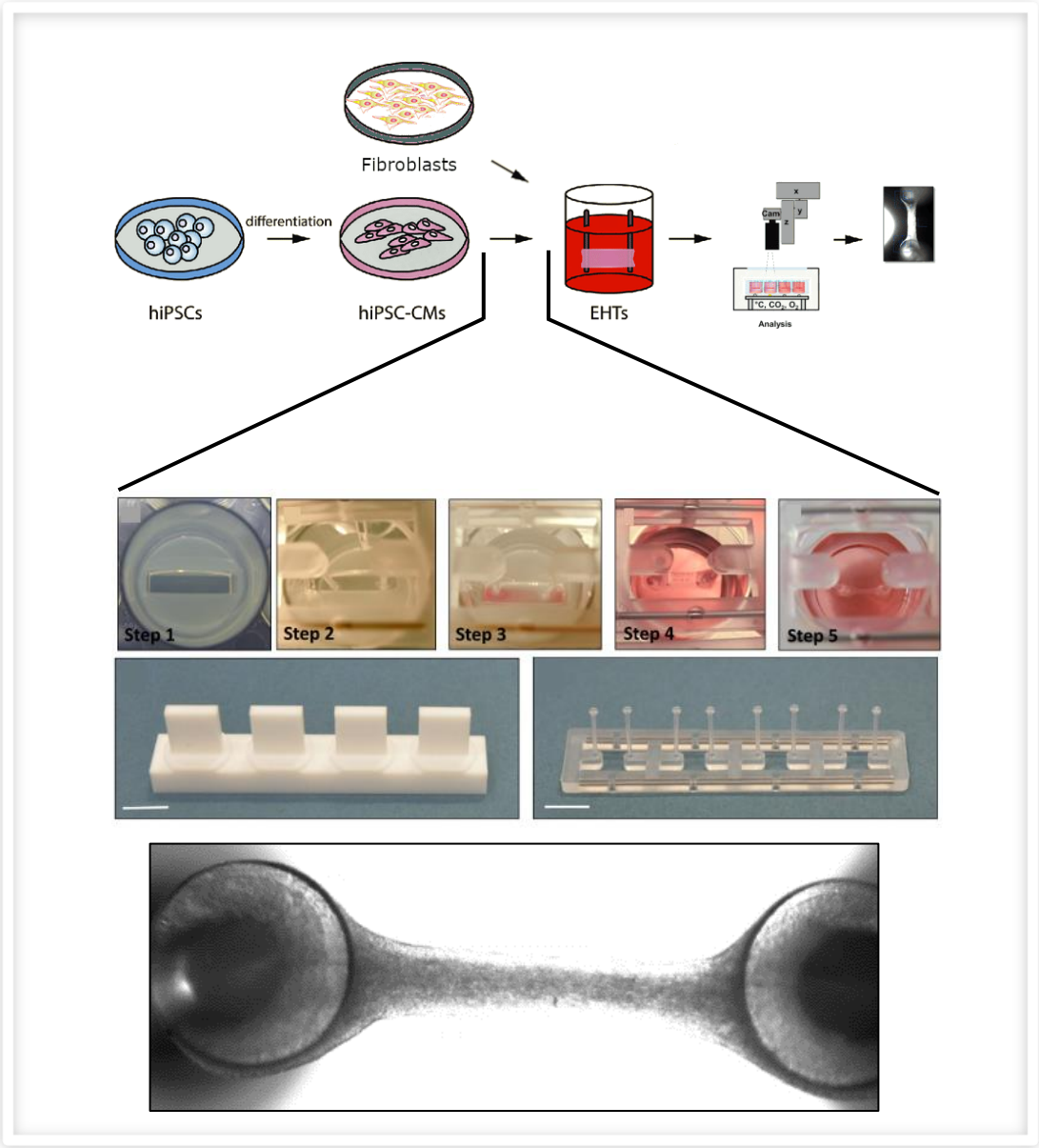
Drug 6



Drug 3

Inotropic compounds





Summary

hiPSC-CMs-MEA Assay is a useful tool for preclinical assessment of cardiotoxic effects

- Positive controls (E-4031, Bepridile, etc.) are among the highest ranked compounds
- 81 compounds have a significant effect on 1 or more of the analyzed cardiac parameters
- Multiparameter analysis

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