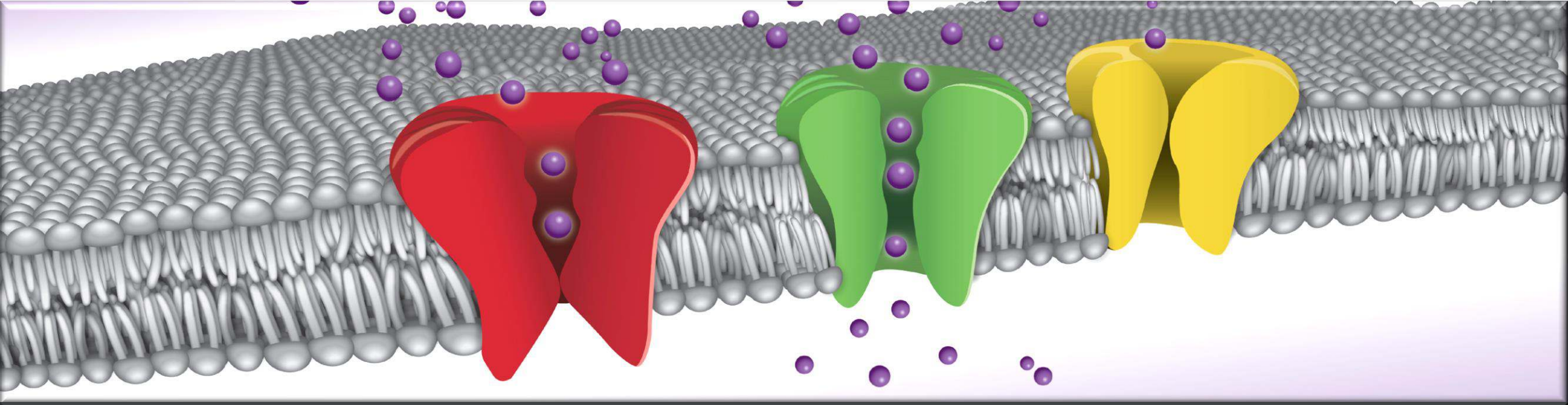

Metrion Biosciences: the ion channel specialists

Validation of an impedance-based phenotypic screening assay able to detect multiple mechanisms of chronic cardiotoxicity in human stem cell-derived cardiomyocytes

Marc Rogers, CSO

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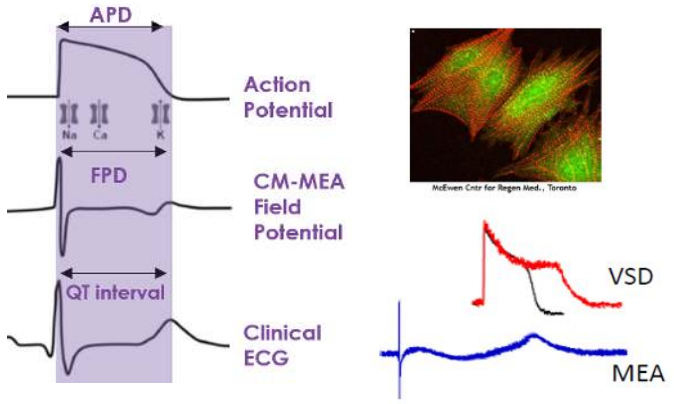
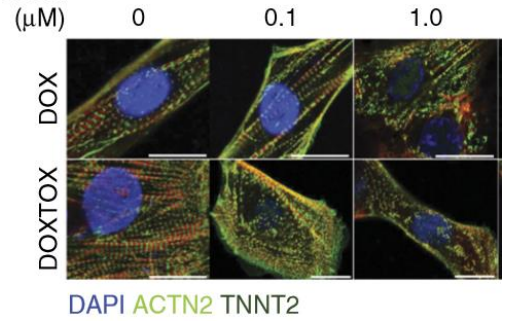
Drug-induced cardiomyocyte toxicity

- **CiPA** is designed to address <u>acute</u> drug-induced cardiac arrythmia (TdP)
  - Assay acute effects of drug discovery compounds (minutes – hours)
  - Focus on plasmalemmal ion channels that underlie the cardiac action potential
- **However, new and existing drugs can also cause chronic and structural cardiotoxicity**
  - Produced by a diverse set of chemical compounds and primary target mechanisms
  - Chronic effects can appear after days, weeks or months; not all are reversible
  - Classic examples include chemotherapy agents used for clinical oncology
    - Anthracyclines such as Doxorubicin (breast cancer)
    - Tyrosine kinase inhibitors (TKI) such as the 'nibs
    - Proteosome inhibitors such as Bortezomib
    - HDAC inhibitors such as the 'stats (some of which may also cause QTc prolongation)
- Safety pharmacology testing needs a reliable & predictive assay for chronic cardiotox

CiPA:



Cardiotoxicity:



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Anti-cancer drugs with clinical cardiotoxicity effects

Name	Type	Indication	Possible cardiovascular damage
Doxorubicin	SM	Anthracycline - solid tumor (breast cancer)	Congestive heart failure, Acute myocarditis, left ventricular dysfunction
Sunitinib	SM	Multi-targeted TKI (kidney cancer)	Arrhythmias
Dasatinib	SM	Multi-targeted TKI	QT prolongation
Ponatinib	SM	TKI inhibitor	Myocardial infarction, congestive heart failure (HF), cardiac arrhythmias
ibrutinib	SM	Bruton's tyrosine kinase	AF + other arrhythmias
Trametinib	SM	MEK inhibitor (MEK1, MEK2)	Cardiomyopathy
Bortezomib	SM	Proteasome inhibitor	Left ventricular dysfunction and atrioventricular block
Trastuzumab	Ab	antineoplastic ErbB2-targeted therapies	Decline left ventricular ejection fraction
Bevacizumab	Ab	VEGFA monoclonal antibody	Congestive heart failure
Rituximab	Ab	Non-Hodgkin's lymphoma	Arrhythmias (link to cytokine release by lymphocyte B?)
Vorinostat	SM	HDAC inhibitor	QT prolongation, thromboembolism
Amiodarone	SM	AF Class III antiarrhythmic	TdP, heart block, sinus bradycardia, CHF, VF
Paclitaxel	SM	Anti-microtubule agent	Arrhythmias (sinus bradycardia, ventricular tachycardia)
Capecitabine	SM	Antimetabolite	Myocardial ischemia + arrhythmias

- A wide and growing list of chemotherapy agents produce a variety of serious cardiac side-effects in cancer patients, only some of which reverse after drug washout
  - Non-traditional, non-CiPA related cardiac liabilities are a challenge for current *in vitro* assays
  - As well as safety, iPSC cardiomyocyte assays could enable cardiac disease modelling

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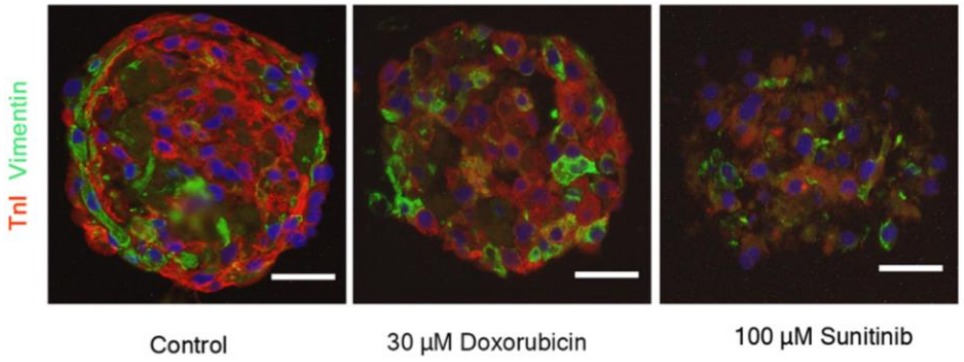
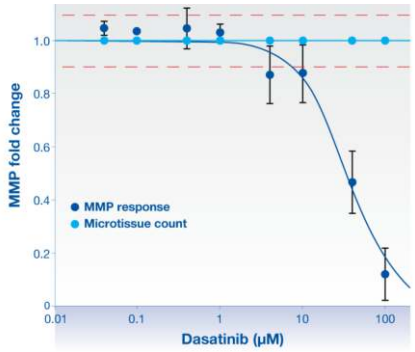
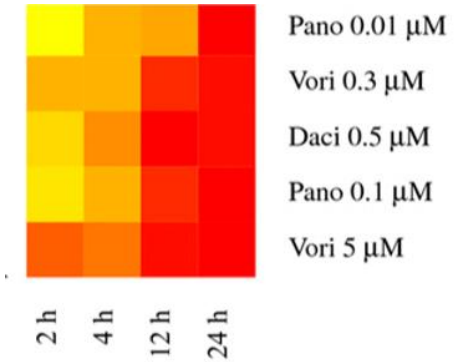
Chemotherapy-induced chronic cardiotoxicity

- Various cell-based screening assays and platforms can be used to monitor cardiomyocyte health and function as part of safety pharmacology testing
  - High content image analysis (sarcomeres, nucleus, cell morphology)
  - Functional imaging (Mitochondrial dyes, LDH, ATP, MTT, DNA damage)
  - Gene expression profiling
  - Secreted or expressed biomarkers are also gaining traction (translation to clinic)







- However, many of these assays require fixation of cells (end-stage assays)
- Other assays require labelling of cells, which adds expense and potential artefacts
- Impedance is useful as it is cheap, label-free, and physiologic over long time-course

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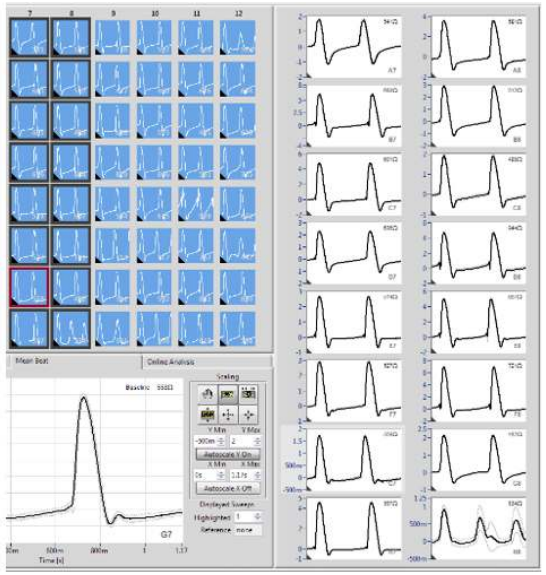
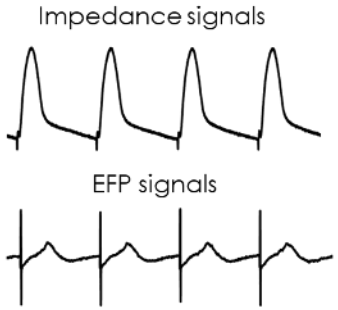
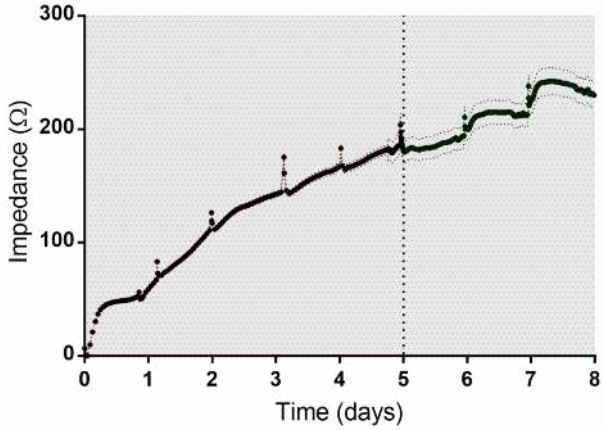
Nanion CardioExcyte 96: Chronic Impedance assay

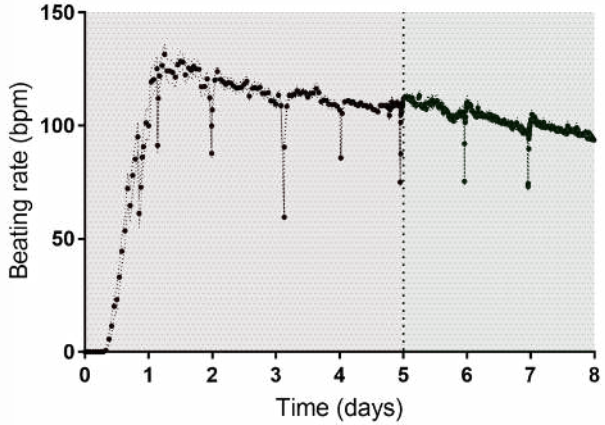


CiPA screening:

- Dual mode platform: impedance & microelectrode array (MEA)







Chronic cardiotox:

- Long-term monitoring of beat rate (arrythmia) and base impedance (growth and formation of a stable syncytium, cell viability)
- Beat rate and impedance stabilise after 1-5 days
- Chronic effects assayed over hours-days

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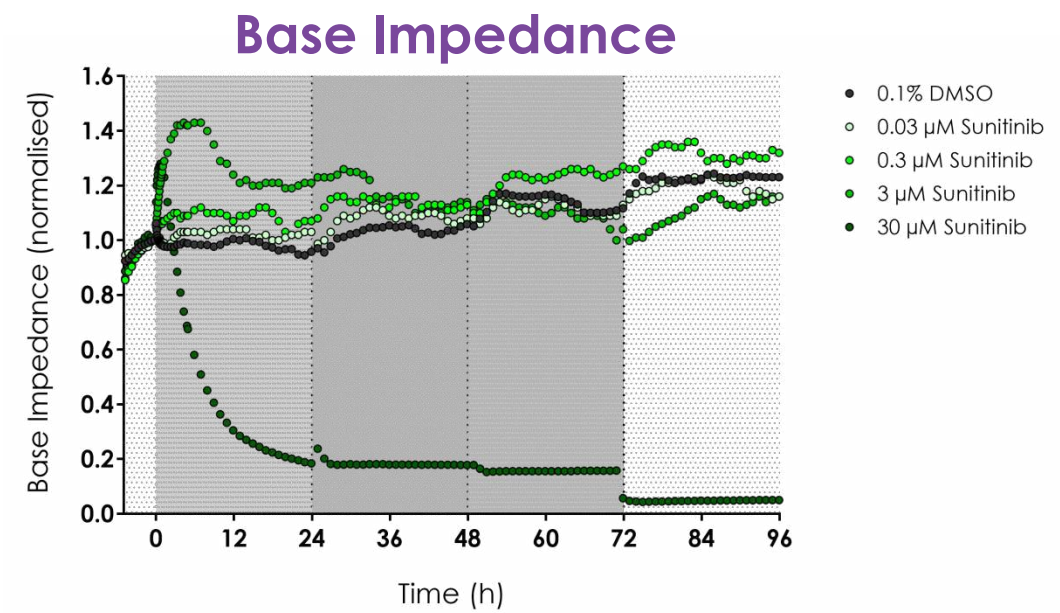
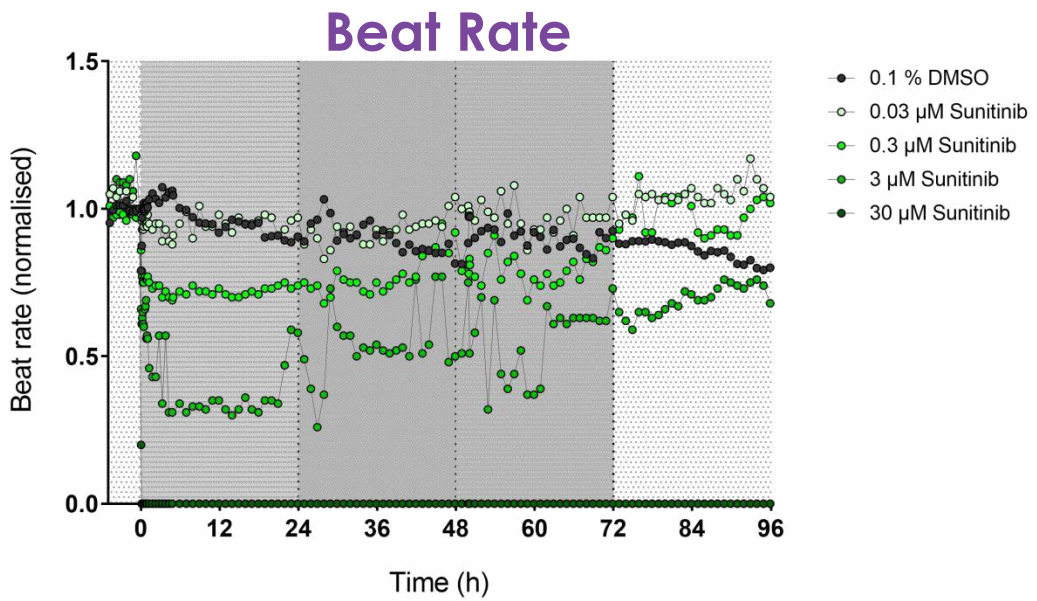
Cardiotoxicity assay: Sunitinib





- Broad spectrum tyrosine kinase inhibitor (TKI) used to treat kidney cancer
- Known cardiac side-effect is mostly ventricular arrhythmia
  - Other TKI's also produces congestive heart failure, hypertension, and myocardial ischemia

Results:

- Complex dose-dependent impedance increase (*cell hypertrophy?*), followed by profound and rapid decrease in at highest dose (cardiotoxicity) over 24 hours
- Effects on beat rate (QT) occurred quickly and at lower concentrations than impedance

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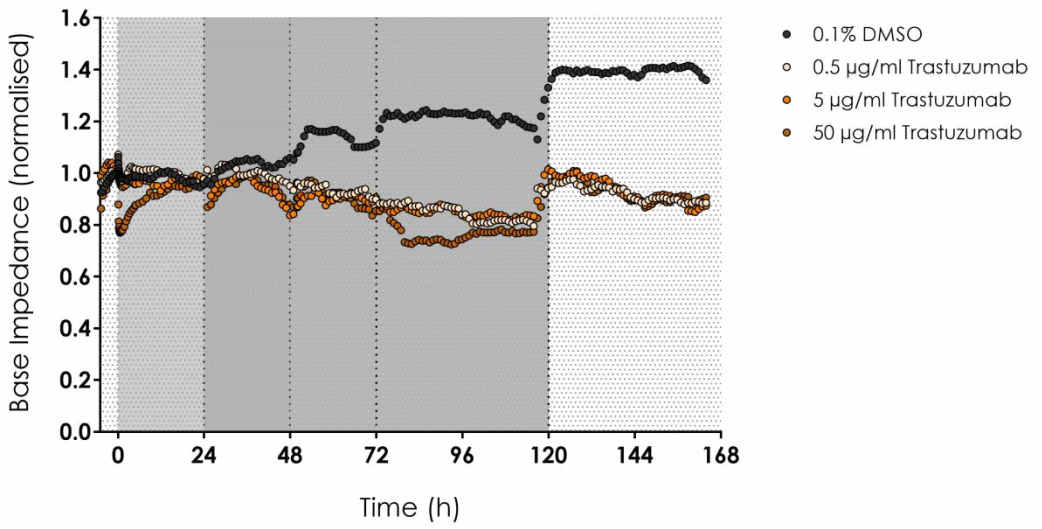
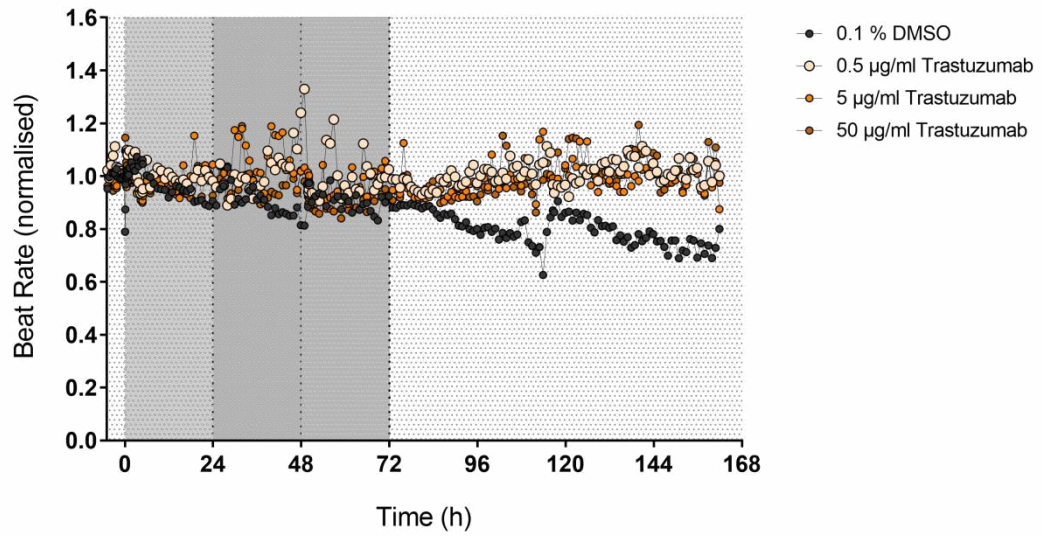
Cardiotoxicity assay: Trastuzumab (Herceptin)

Base Impedance



Beat Rate



- Monoclonal antibody against Her2/ErbB2 receptor, used to treat breast cancer
- Known cardiac side-effects are CHF and changes in left ventricular ejection fraction

Results:

- Very slow, dose-independent decrease in base impedance (cardiotoxicity) > 72 hours
- No effects on beat rate suggest little occurrence of chronic arrythmias or QT changes

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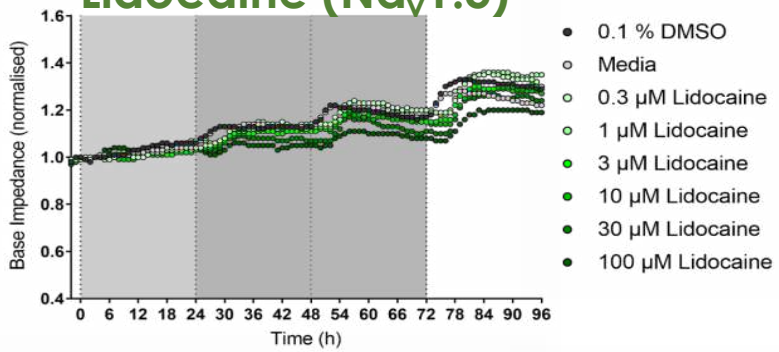
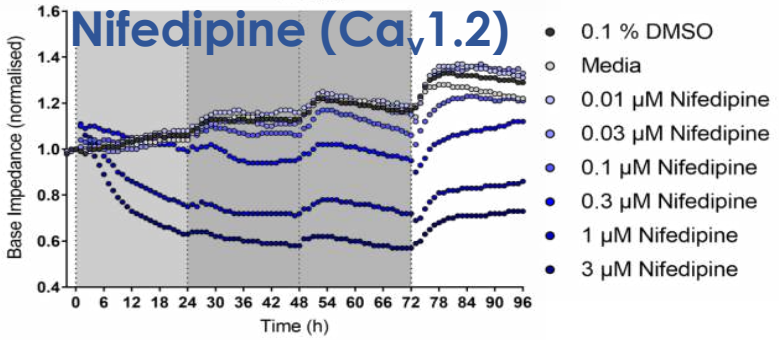
Cardiotoxicity assay: Ion channel modulators

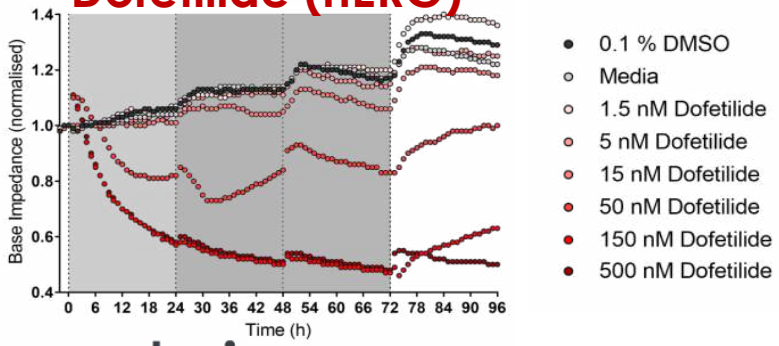
A. **Lidocaine ($Na_V1.5$)**



B. **Nifedipine ($Ca_V1.2$)**



C. **Dofetilide (hERG)**



- **Compound effects on ion channels are usually only studied over short periods for acute toxicity**
- **We decided to see if CiPA drugs could also produce long-term effects in a chronic toxicity assay**

- Along with beat rate and contraction amplitude, we could monitor base impedance over several days on the CE96 platform to assess effects of ion channel blockers on cell viability and sustained cardiotoxicity
  - Lidocaine ($Na_V1.5$) had no effect on cell viability
  - Nifedipine ($Ca_V1.2$) produced time- and dose-dependent decreases in iPSC-CM viability
  - High concentrations of Dofetilide (hERG) also decreased iPSC-CM base impedance

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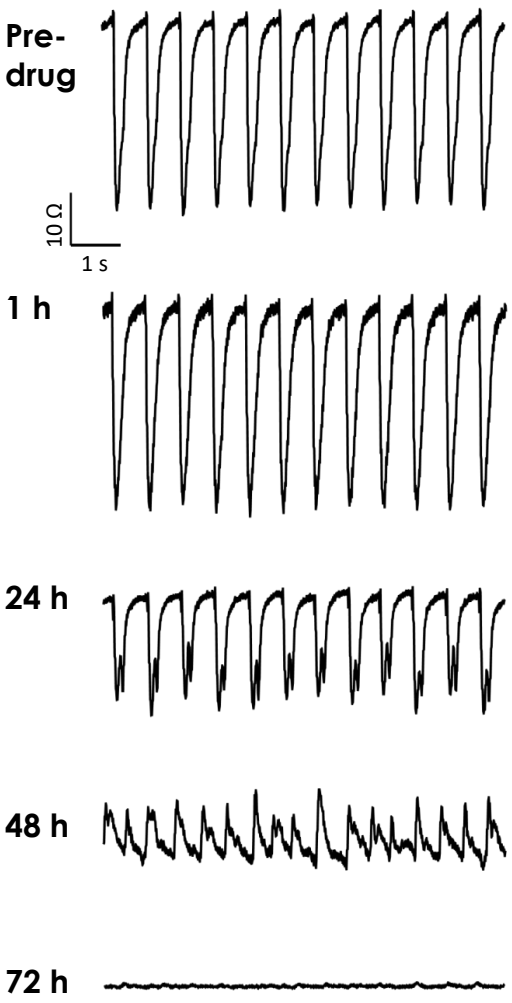
Cardiotoxicity assay: Doxorubicin

A. **1 µM Doxorubicin**



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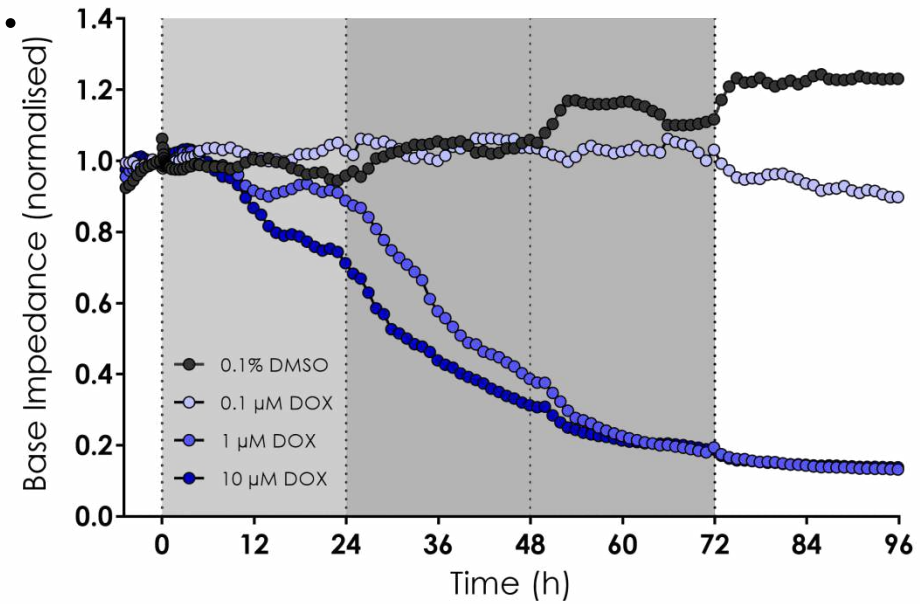

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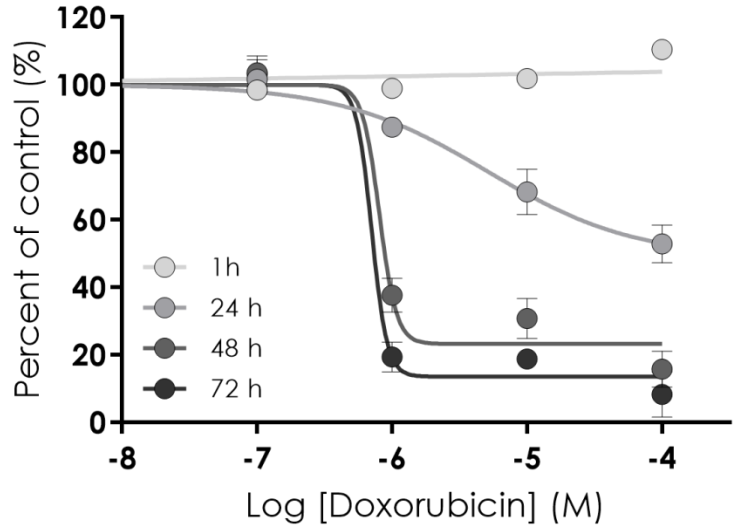

- Broad spectrum anti-cancer medication belonging to Anthracycline family
- DNA intercalating agent
- Known cardiac side effects:
  - Acute atrial and ventricular arrhythmia
  - Chronic cardiomyopathy and congestive heart failure

Results:

- Concentration-dependent decrease of base impedance after 24 hours
- Concomitant decrease in beat amplitude and onset of arrythmia (24-48 hours)

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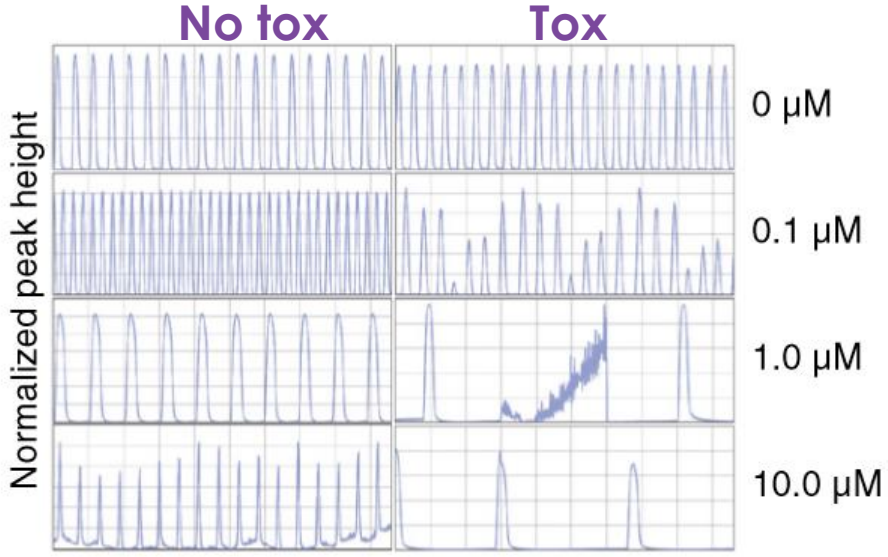
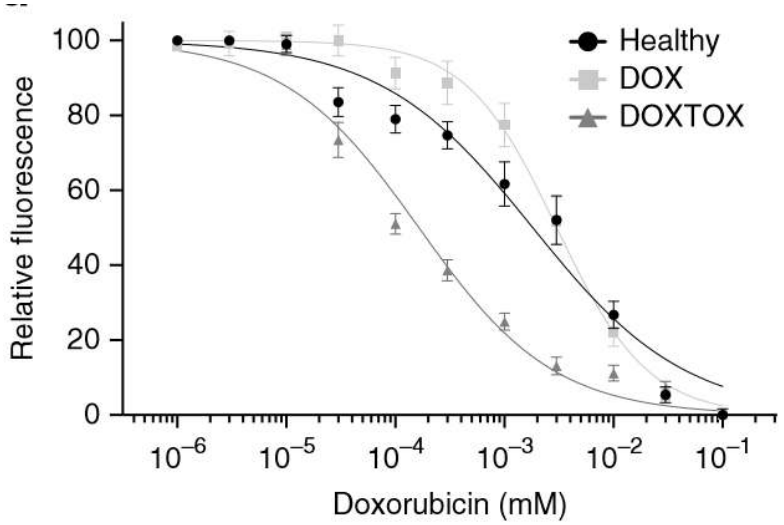
Cardiotoxicity disease modelling: Doxorubicin

- Not all breast cancer patients show chronic cardiotoxicity in response to Doxorubicin
- Stanford group explored this phenomena using patient-derived iPSC cardiomyocytes
- Clear differences in cardiotoxicity measures in sensitive *vs* insensitive patients
  - Cell damage markers, functional contractility and arrythmia, gene expression profiles, etc





- Impedance-based cardiotoxicity personalised medicine assays could be used to:
  - Model other types of patient-specific drug cardiotoxicity
  - Select patients for safest type of chemotherapy and other drug treatments

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HL-1 mouse atrial cardiomyocytes: Chronic atrial cardiotox

- HL-1 cells are an immortalised mouse 'atrial' CM cell line
  - Exhibit immature physiology and de-differentiated morphology
  - A subset of cells demonstrate spontaneous contractility for a few passages
  - Express atrial-like ion channel expression profile (incl IK_{ACh})
- We wanted to test them as a model for chronic atrial cardiotoxicity
  - Assess acute and long-term changes in cell impedance as a measure of cell toxicity
  - Assess any changes in spontaneous contractility (beat rate)

Results:

- HL-1 cells have similar sensitivity to a wide range of chronic cardiotoxicity agents as human ventricular iPS cardiomyocytes
  - Ion channel antagonists (esp mixed blockers like Amiodarone)
  - Doxorubicin
  - 'nib kinase inhibitors
- HL-1 cells have greater sensitivity to drugs causing atrial-specific damage
  - Bortezomib

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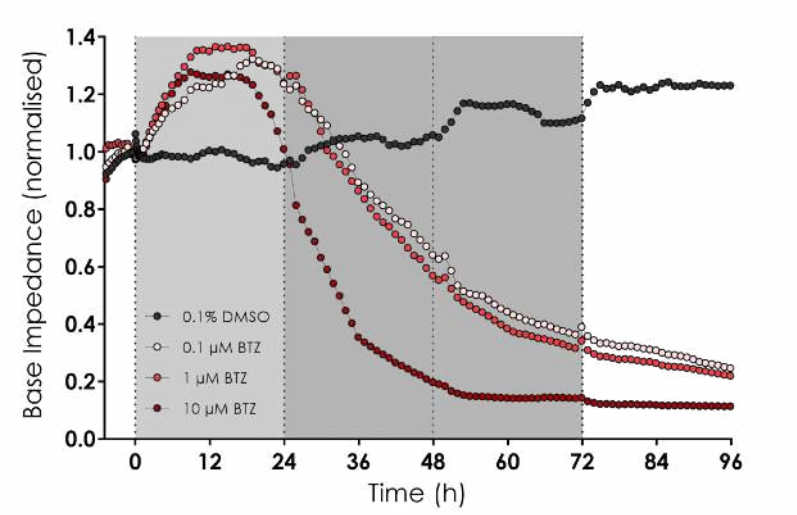
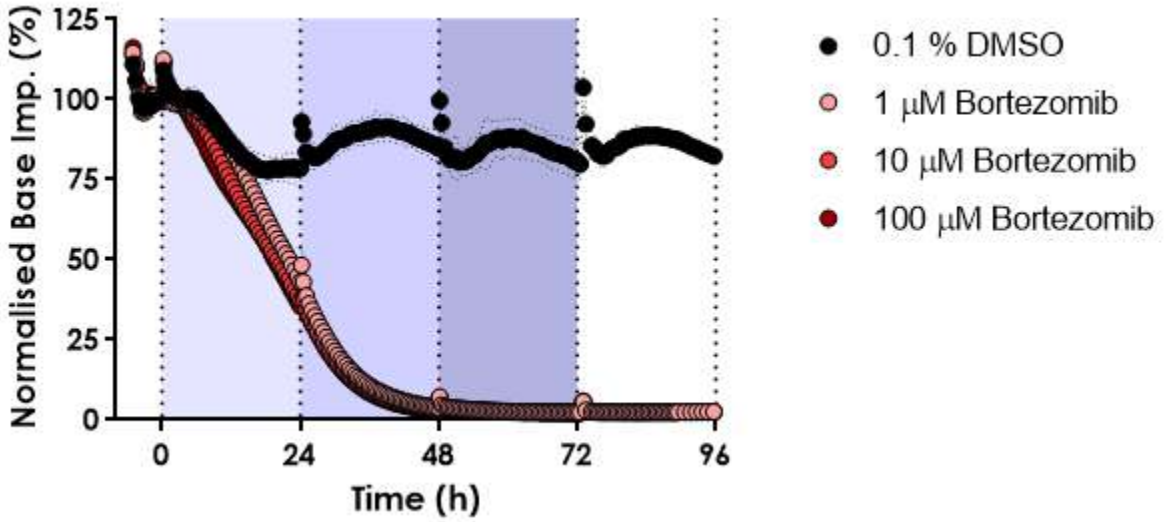
Bortezomib: Oncology agent with atrial cardiotoxicity

Human iPSC-CM (ventricular)



HL-1 cells (atrial CM)



- Bortezomib is a proteasome inhibitor with known cardiotoxicity (*incl AV block*)
- Large time-dependent decrease in impedance, indicating significant cardiotoxicity
- We saw slower effects with lower sensitivity in ventricular human iPSC-CMs, suggesting that HL-1 atrial cells may be a useful model for chronic toxicity studies of agents such as Bortezomib with atrial-specific side-effects

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Acknowledgments

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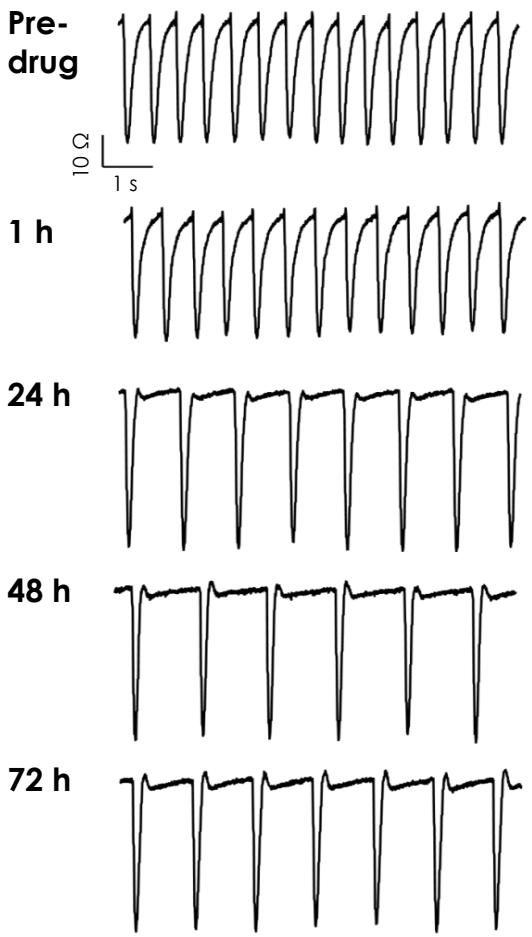
Thanks

Cardiotoxicity assay: Amiodarone

A. **1 µM Amiodarone**



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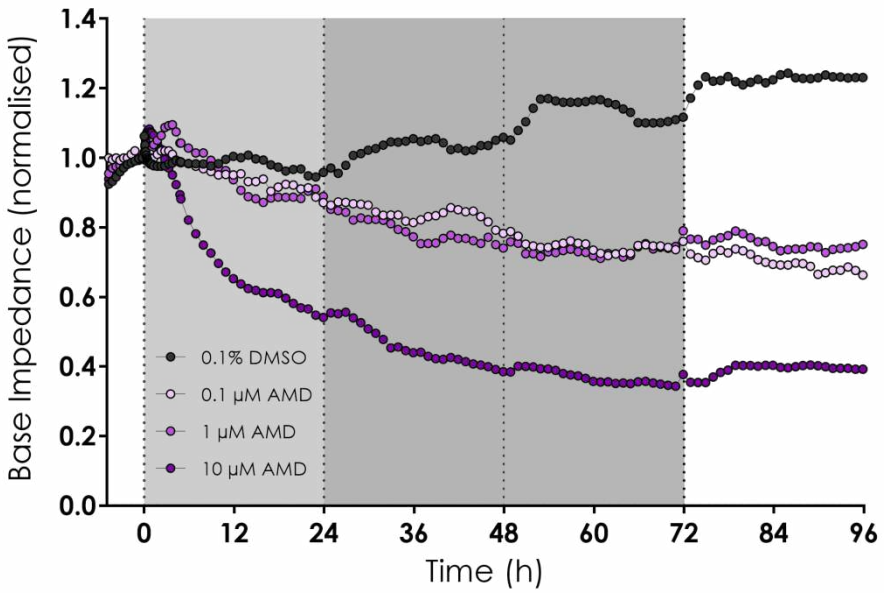

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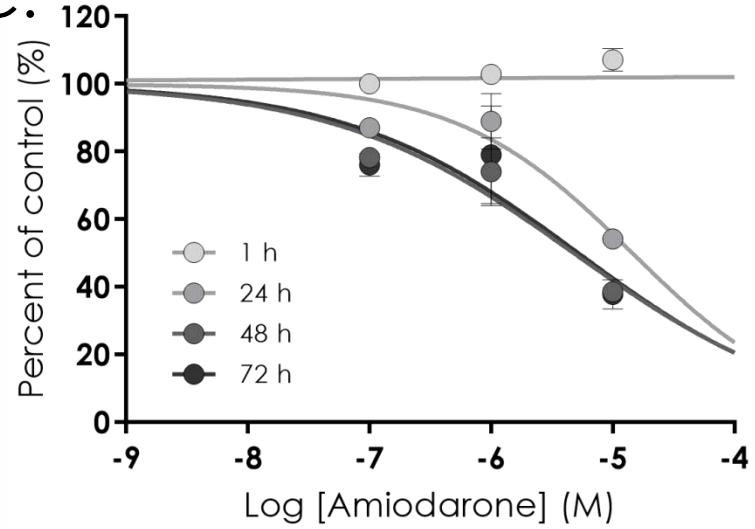

- Class III antiarrhythmic widely used for treatment of atrial fibrillation
- Known cardiac side effects:
  - Arrhythmia: Torsade de Pointe, VF and sinus bradycardia
  - Congestive Heart Failure

Results:

- Base impedance decreased rapidly in dose-dependent manner (12 hr)
- Corresponding slowing of beat rate, increased contractility

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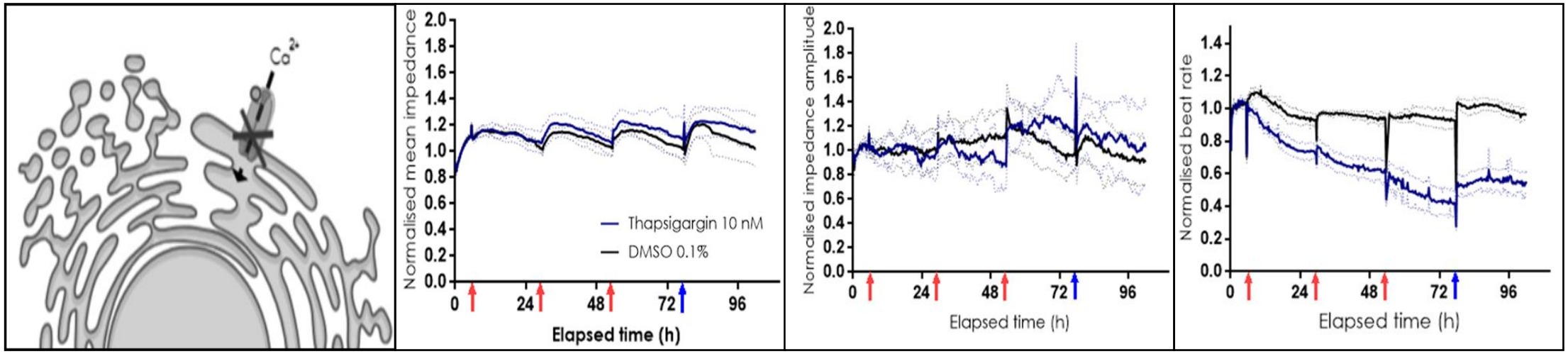
Cardiotoxicity assay: Thapsigargin (Ca^{2+} pump)

Molecular mechanism | **Base impedance** | **Impedance amplitude** | **Beat rate**



- Tendency of increased basal impedance, suggestive of hypertrophy effect?
  - We have seen other compounds also produce chronic increases in impedance
- Decreased beat rate as expected with SR Ca^{2+} store depletion after SERCA block
- These data may support the contention that spontaneous beating in iPSC-CMs is due to Ca^{2+} leak from SR, and is a sign of Ca^{2+} signalling immaturity

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