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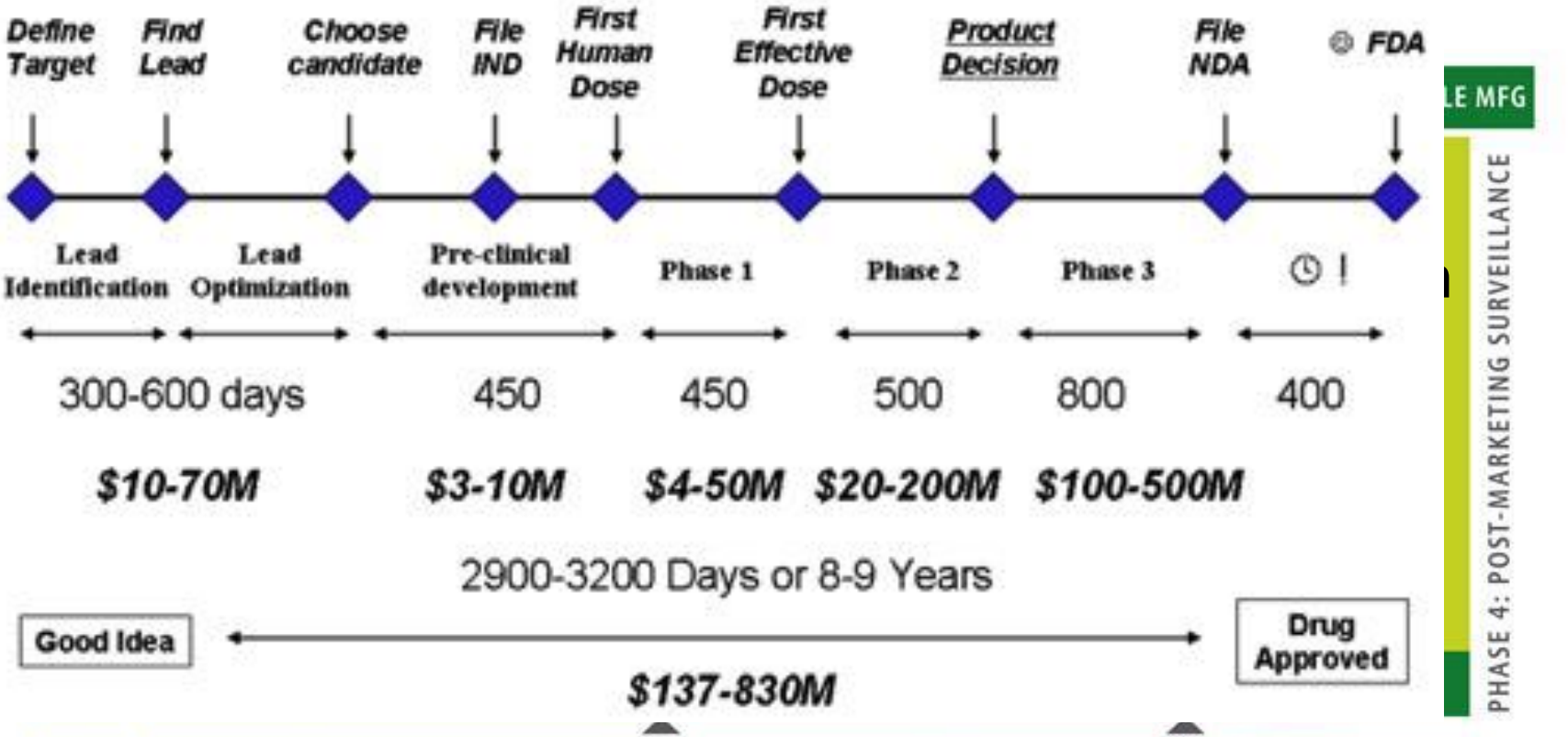
Rising to the Challenges of Human iPSC-derived Cells for Tox & Drug Screening

Paul Bello, PhD
Director of Operations
5th April 2016
SELECTBIO - Stem Cells in Drug Discovery
Cambridge, UK

Drug Discovery & Development
"A long, risky road"

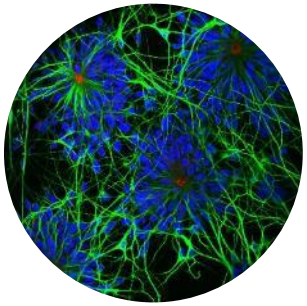
Need for early toxicity testing and improved prediction



Slide taken from the Pivotal Point Group, LLC

Source: Pharmaceutical Research and Manufacturers of America

Overview of Presentation

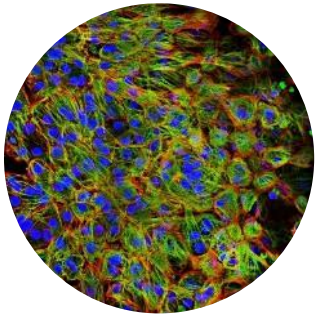


iPSC-Derived Neural Stem Cells

Neurotoxicity in drug safety testing

Functional Integrity

Gene Expression, Electrophysiology,
Multi-Electrode Array,
Effects of developmental neurotoxin



iPSC-Derived Cardiomyocytes

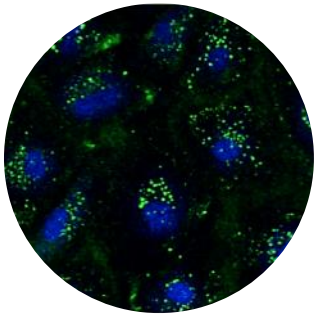
Cardiotoxicity in drug safety testing

Functional Integrity

Express major cardiac-selective markers
Beat spontaneously in culture, $Ca2^{+}$ imaging

Electrophysiology

Pharmacology



Hepatocytes

Hepatotoxicity in drug safety testing

Metabolism studies, Hepatotoxicity studies,
Genotoxicity micronucleus studies

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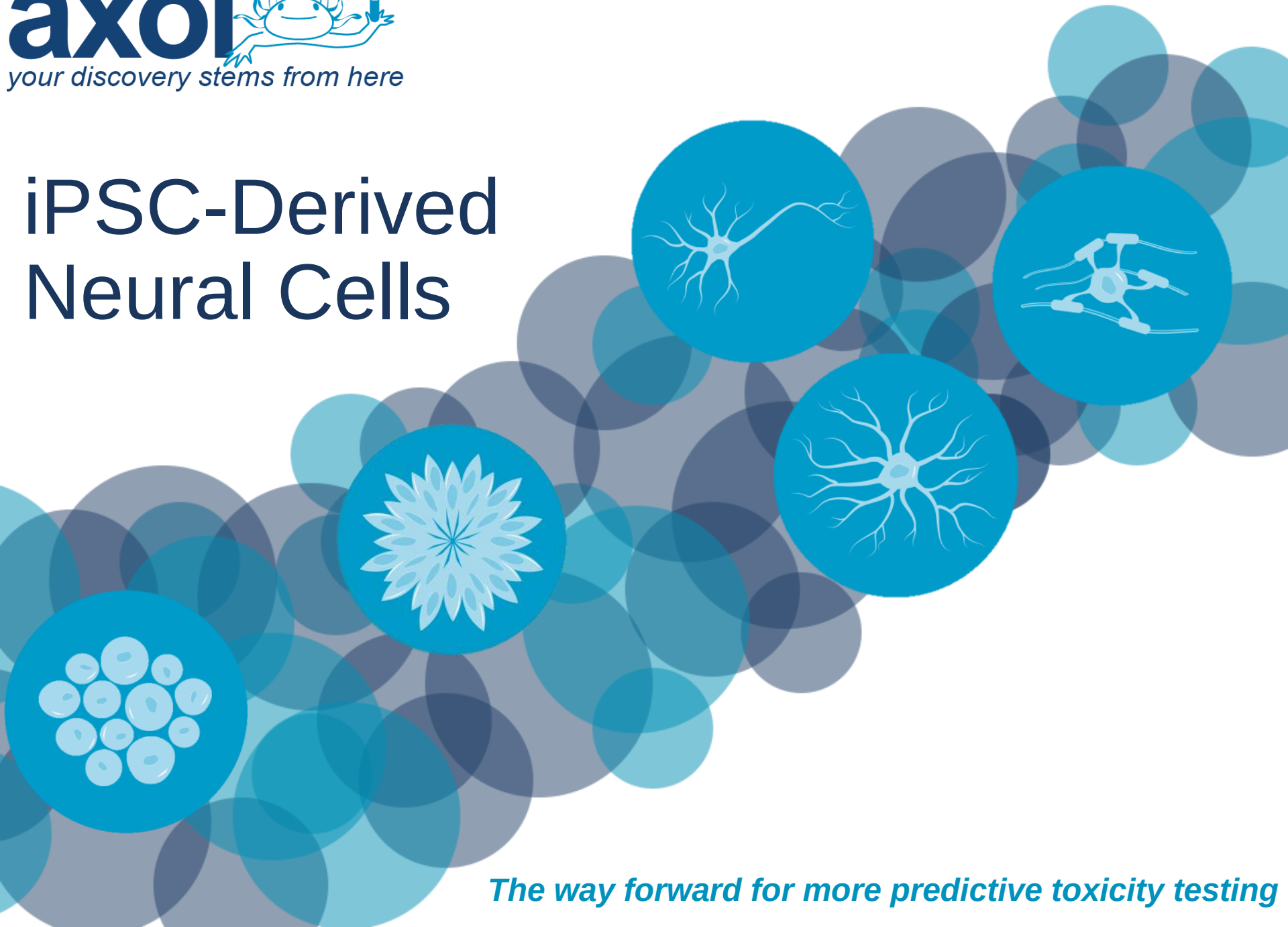
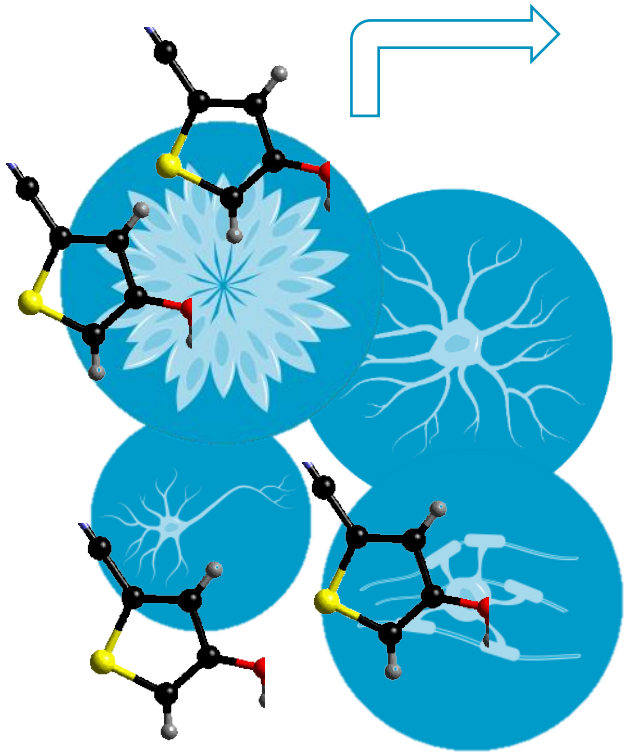
iPSC-Derived Neural Cells



The way forward for more predictive toxicity testing

Neurotoxicity in Drug Safety Testing



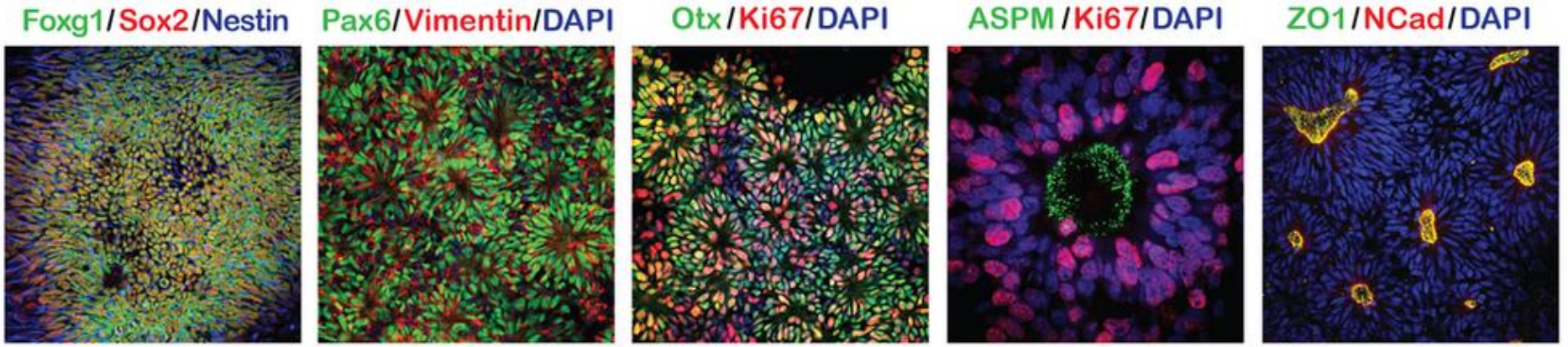
Functional Integrity
Gene Expression
Protein Expression
Electrophysiology
Multi-Electrode Array
Whole Cell Patch

Disease Modeling
Responsive to drug treatments
Expression disease-relevant phenotypes

- Patch clamp
- Gene expression
- Biochemical analysis
- Multi-Electrode Array
- Neurite outgrowth

General Characterization of NSCs

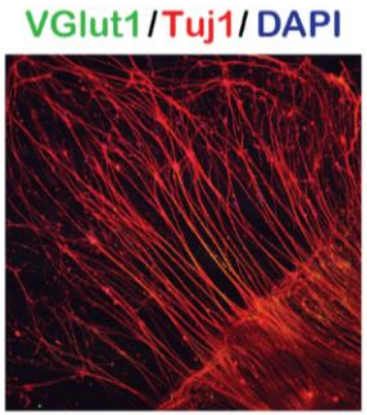
Axol confirms expression of neural stem cell markers like SOX2, PAX6, Ki67 and ZO1

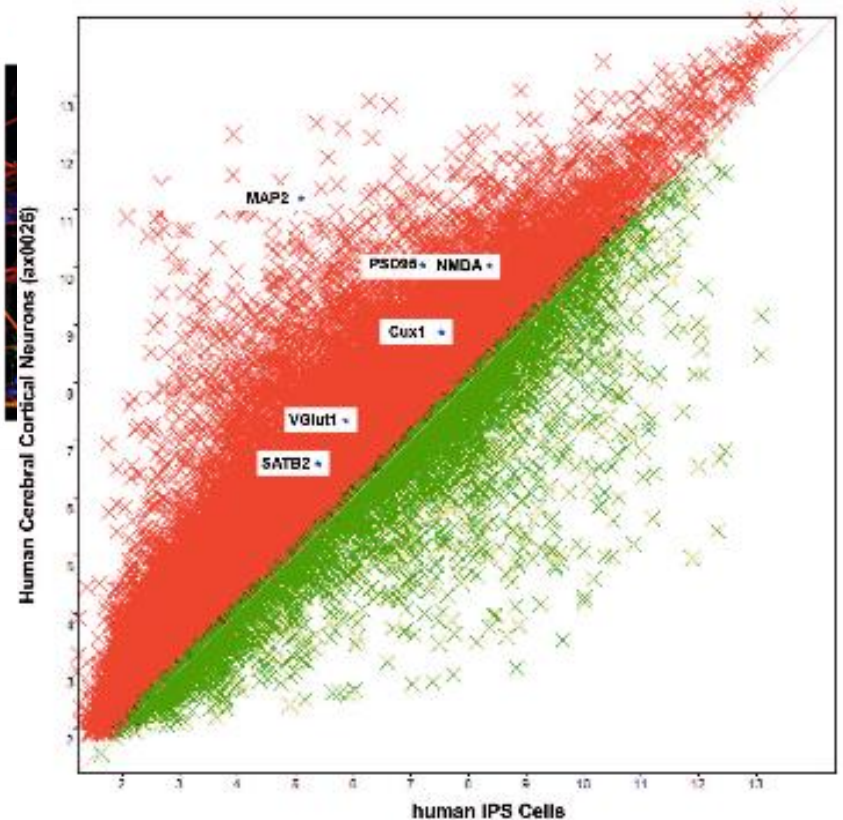


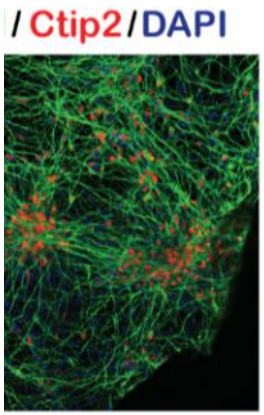
Characterization of Cortical Neurons

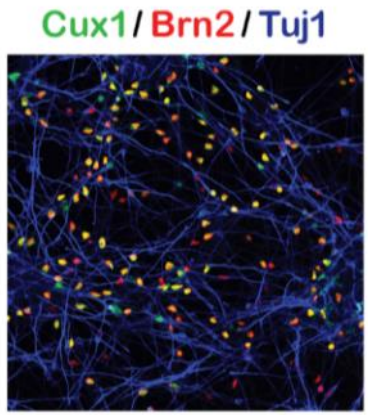
We looked at cerebral cortical neuronal markers like VGlut1, Tbr1, Cux1, Tuj1 by qPCR

Our transcriptomic data confirmed down regulation of iPSCs markers and up regulation of cortical neuronal markers like MAP2, NMDA, VGlut1, Cux1, etc.

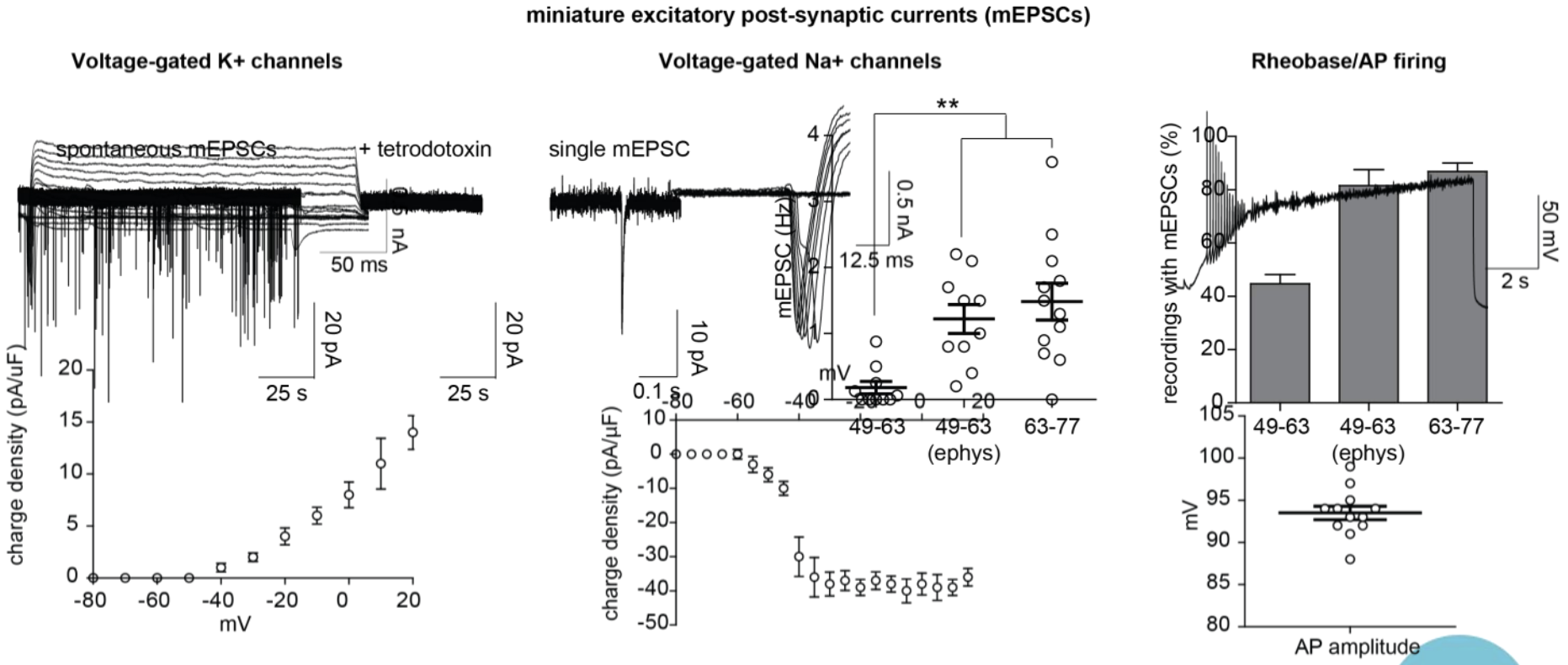








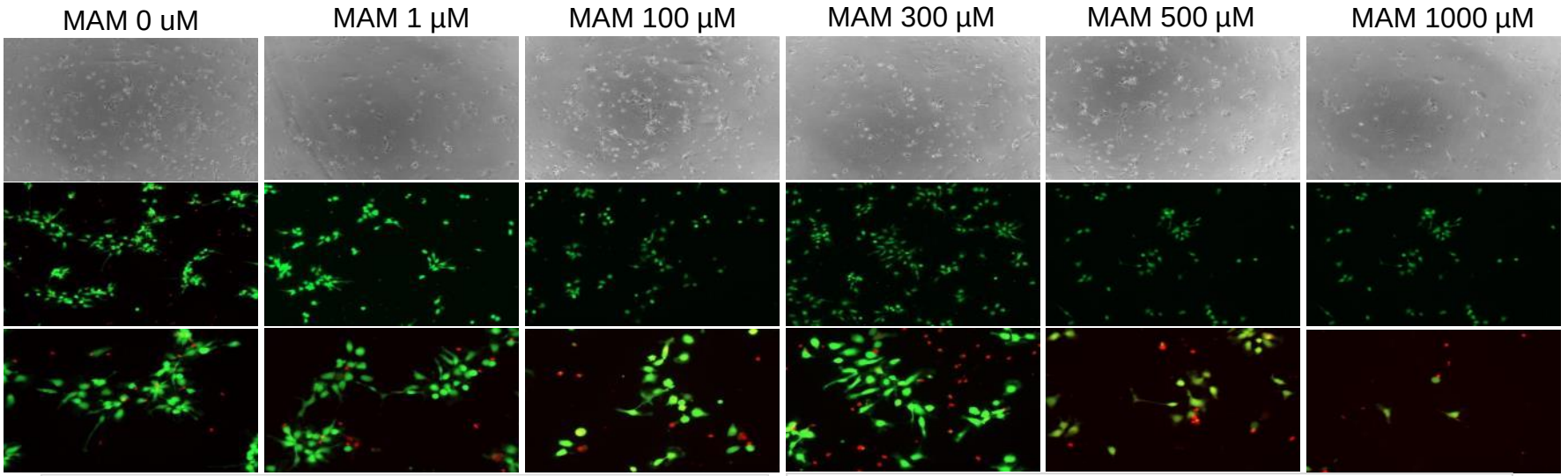
Electrophysiological Characterization

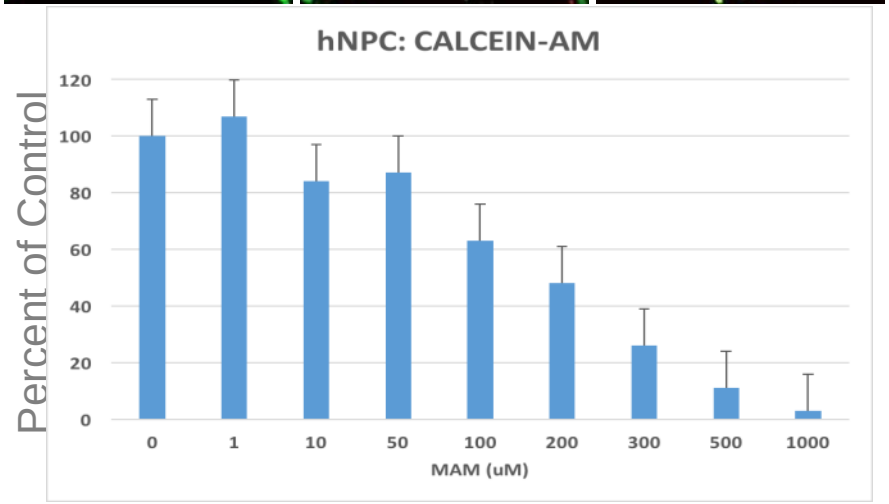


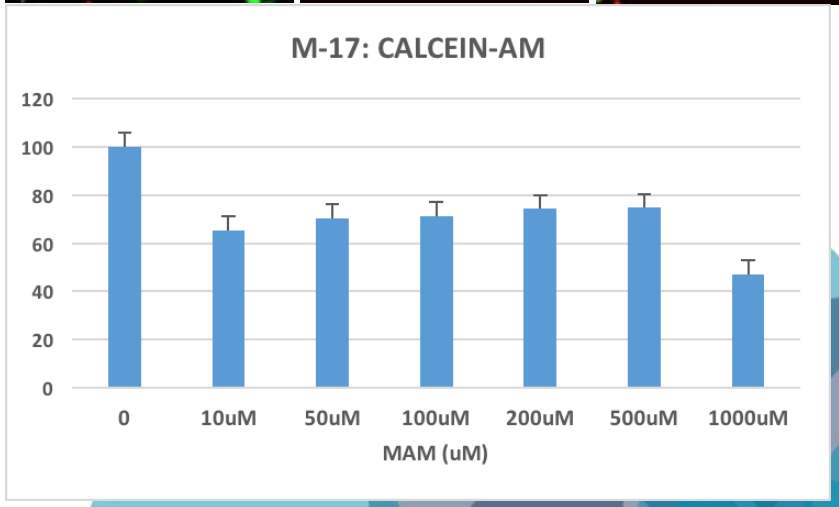
Spontaneous activity

Data from our collaborators

Neurotoxin Effects on iPSC-Derived Neural Stem Cells







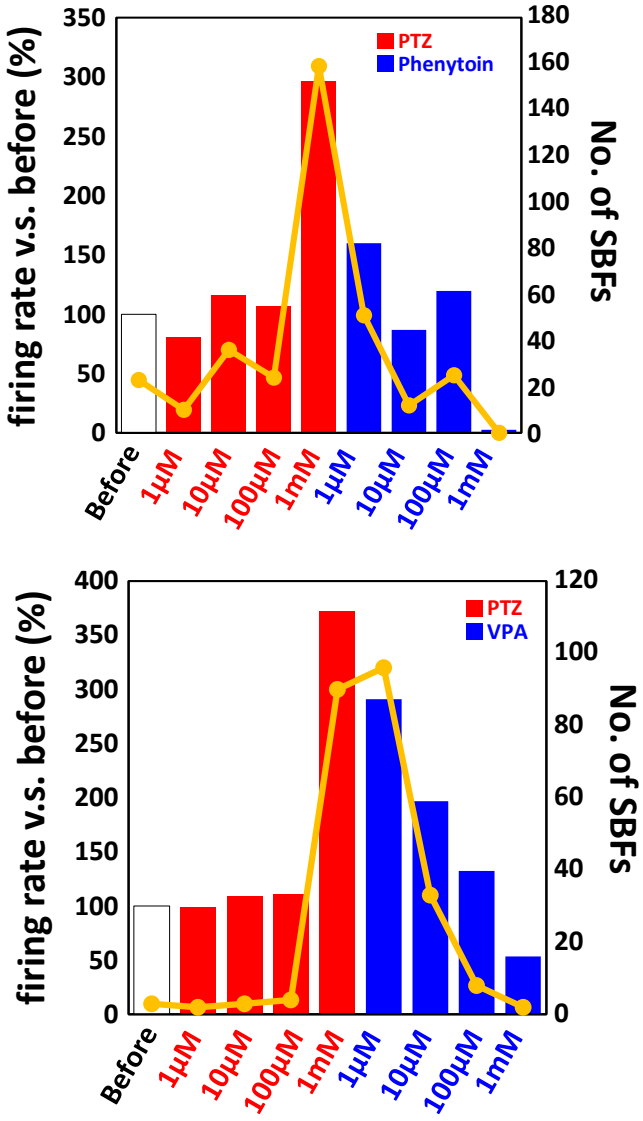
Data provided from Dr Kisby's lab by Michael Czulinski and Morgan Florek: MAM, Methylazoxymethanol

Induction of Epileptiform Activity & Effects of Anti-Epilepsy Drugs

- Induced epilepsy by adding PTZ (pentylentetrazole) (>1mM)

- Anti-epilepsy drugs, **phenytoin** & **sodium valproate (VPA)** were able to reverse the high frequency synchronized bursts evoked with PTZ

These results suggested that long-term electrophysiological measurements in iPSC-derived neurons using a MEA system may be beneficial for ***drug screening applications (ePoster 107)***



Neurotoxicity Summary

- iPSC-derived NSC
    - *Express neural markers at gene & protein level*
    - *Excellent neurite outgrowth & branching*
    - *Electrophysiologically functional*
    - *Capable of synaptic plasticity*
- iPSC-derived NSCs are more sensitive to the developmental neurotoxin MAM & can replace cell lines for neurotoxin screening
- Responsive to drug treatment
- Can be cultured long-term
- Physiologically relevant tool for toxicity & drug discovery studies

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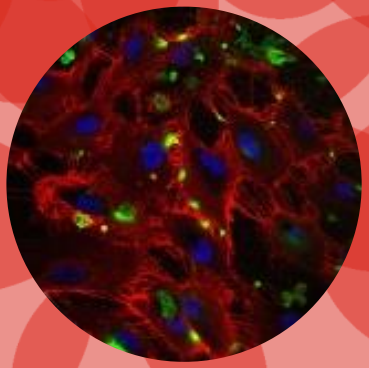
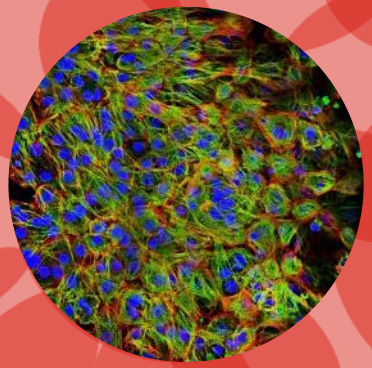
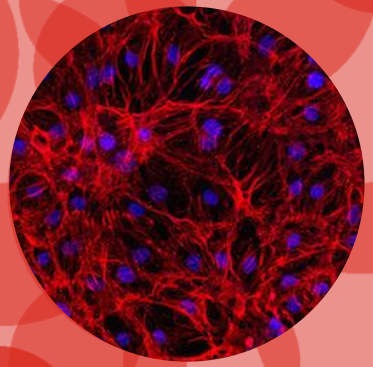
iPSC-Derived Cardiomyocytes

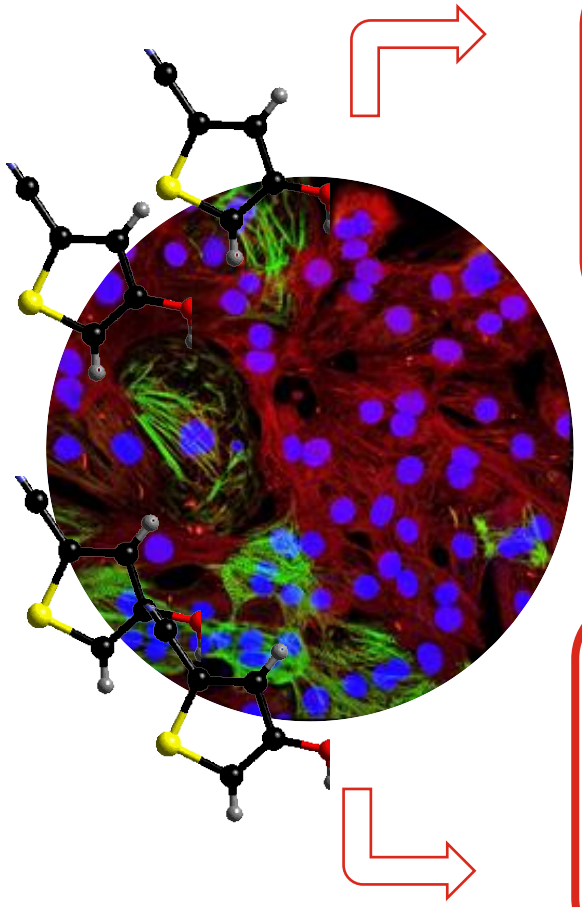






A way forward for more predictive toxicity testing

Cardiotoxicity in Drug Safety Testing



Electrophysiology
Contractility
QT prolongation
Na^+ & Ca^{2+} channels
Pharmacology

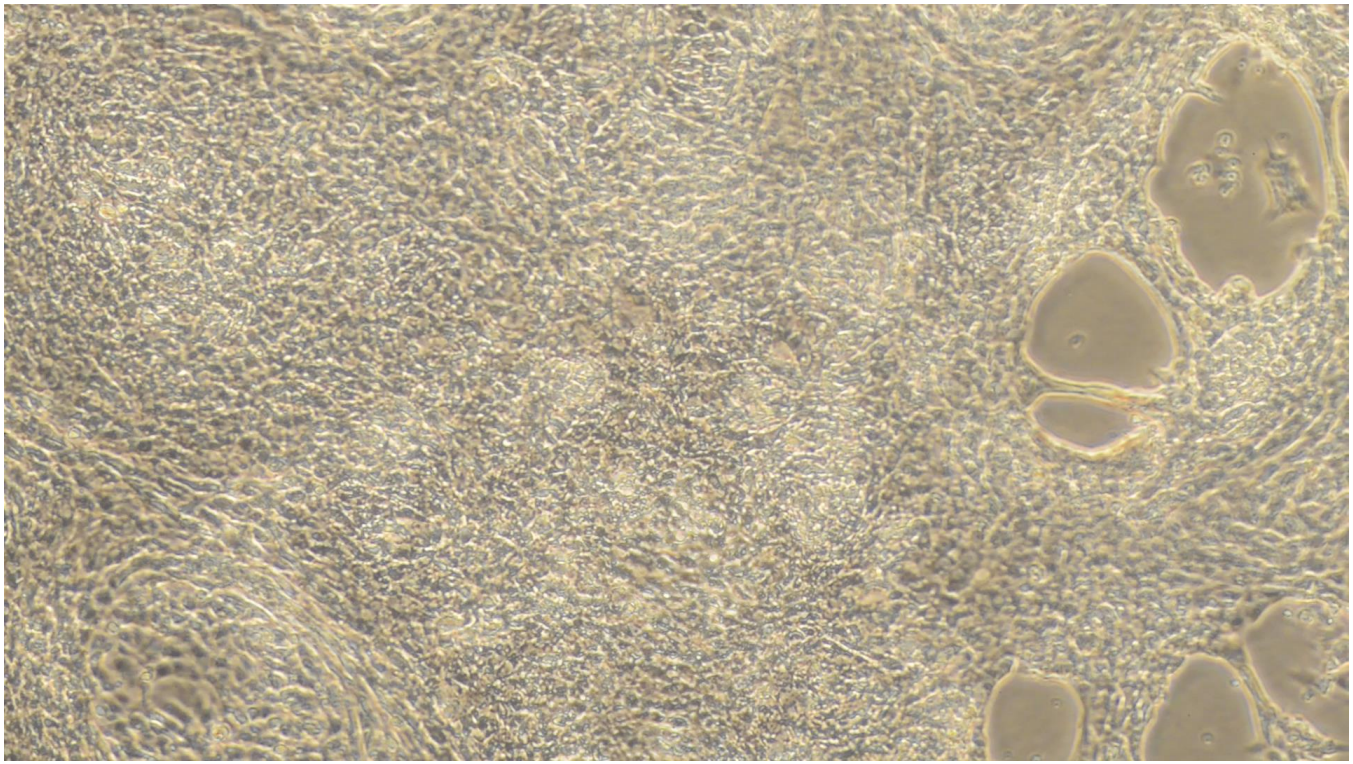
Functional Integrity
Ca^{2+} signaling
Morphology
Stress & toxic response markers

- Patch clamp
- Impedance
- Biochemical analysis
- Immuno-cytochemistry
- Multi-electrode Array

Why iPSC-Derived Cardiomyocytes?

- Benefits of a synchronously beating monolayer
  - *React as a unit syncytium of cells, electrically coupled*
- Robust & Reproducible
- Large quantities available
- High purity
- Functional on xCelligence, for calcium imaging & for electrophysiology

iPSC-Derived Cardiomyocytes Showing Synchronized Beating

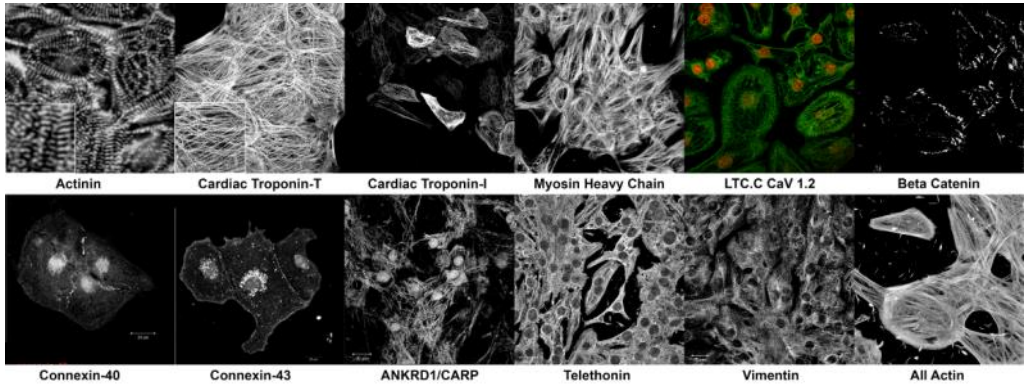


Benefits of a synchronously beating monolayer

- Electrically coupled,
- Physiologically relevant to human heart

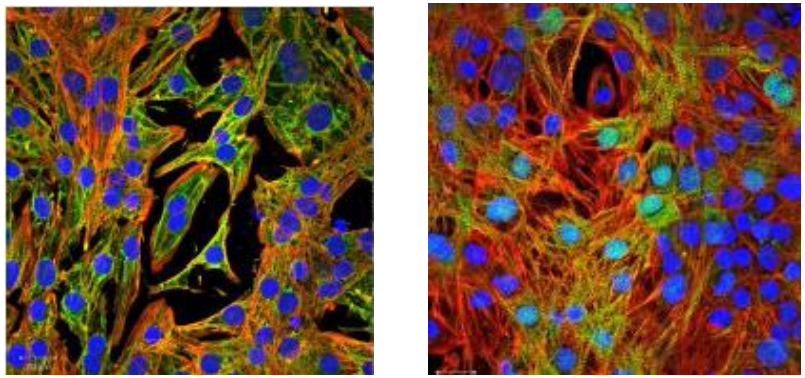
Functional iPSC-Derived Cardiomyocytes

Protein Expression



Data from Dr Christian Zuppinger

Signaling & Stress-Response



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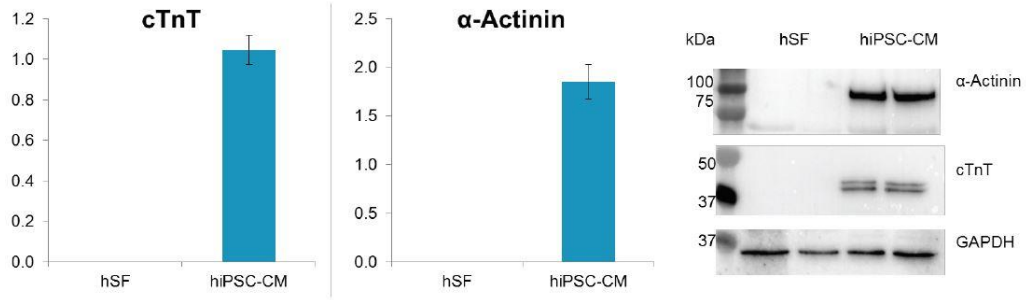


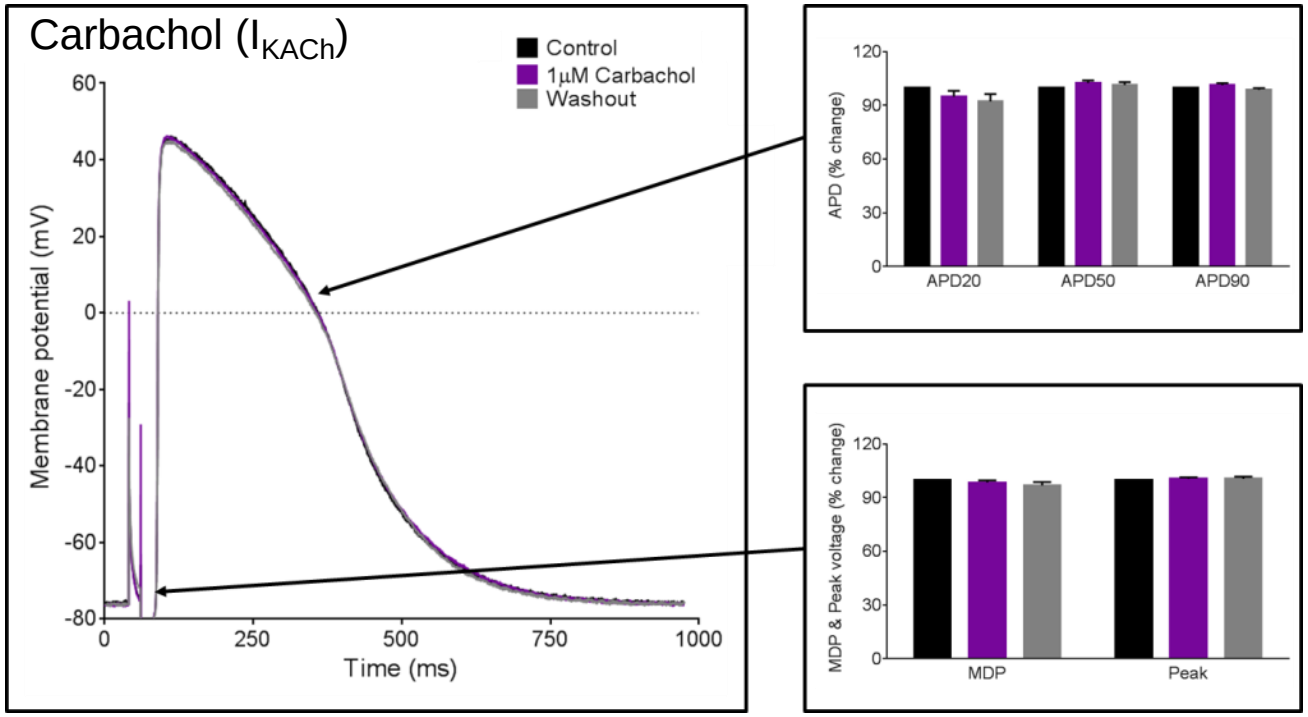
Human iPSC-CMs (hiPSC-CMs) express more cardiac troponin-T (cTnT) & α-Actinin than human skin fibroblasts (hSFs)

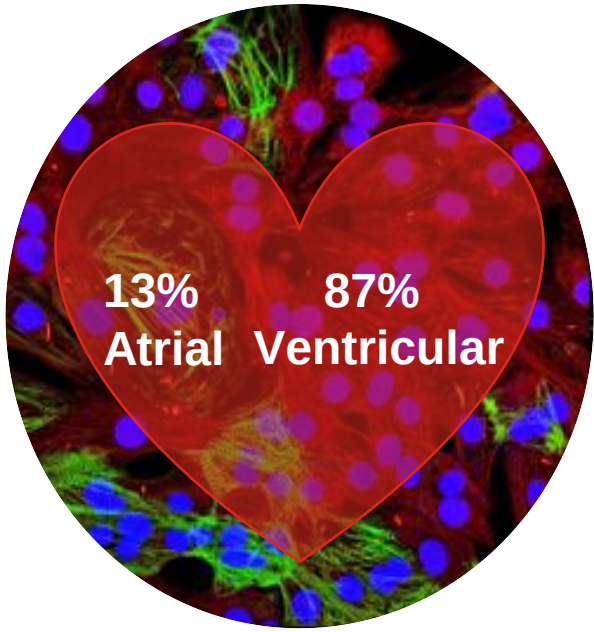
Data from Abigail Robertson from University of Manchester

Telethonin (green) suggested signalling & stress-response functions is present iPSC-CMs with a pattern of sarcomeric striation observed in patches inside some cells. (All actin, red)

Ankyrin repeat domain 1 (ANKRD1) (green) could be used a marker of toxic stress, showed similar expression to telethonin. (All Actin, red)

Near Pure Population Ventricular Cardiomyocytes

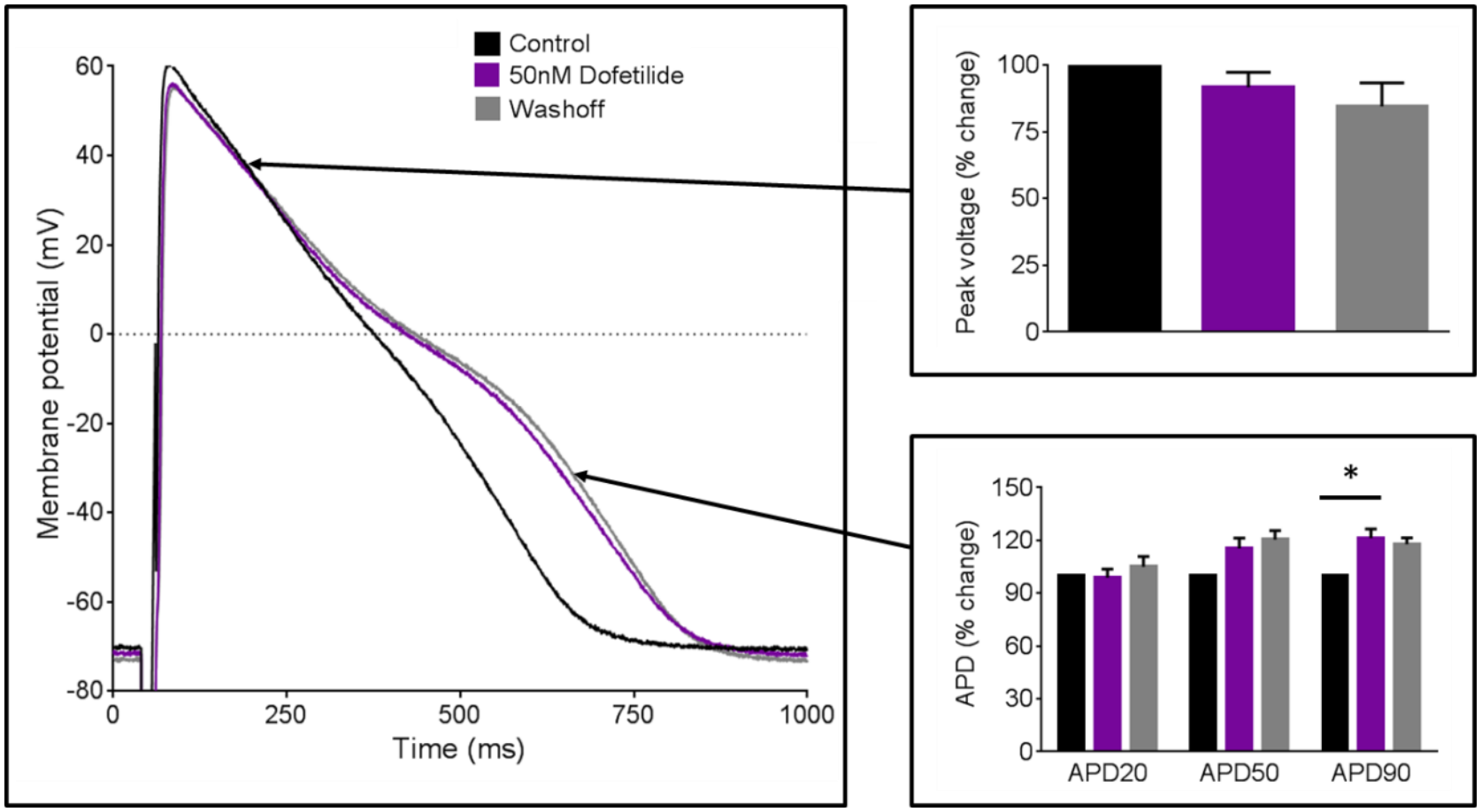




- Negligible effect on AP parameters (n=8)
  - Positive effect of carbachol observed with atrial-derived HL-1 cells
  - Suggests majority of cells do not display an atrial phenotype

Ventricular myosin light chain (87%) and atrial myosin light chain (13%)
(Does not include nodal population)

Dofetilide (I_{Kr})

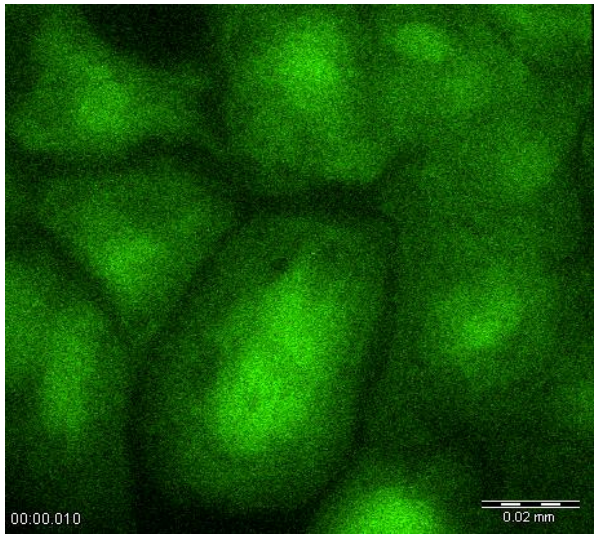


- Significant prolongation to APD90
- Negligible effect on other AP parameters

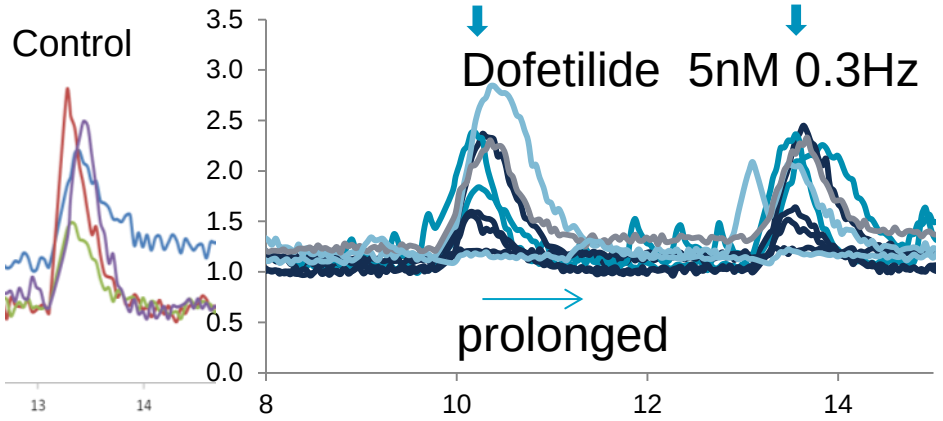
Effect of Dofetilide on Calcium Imaging

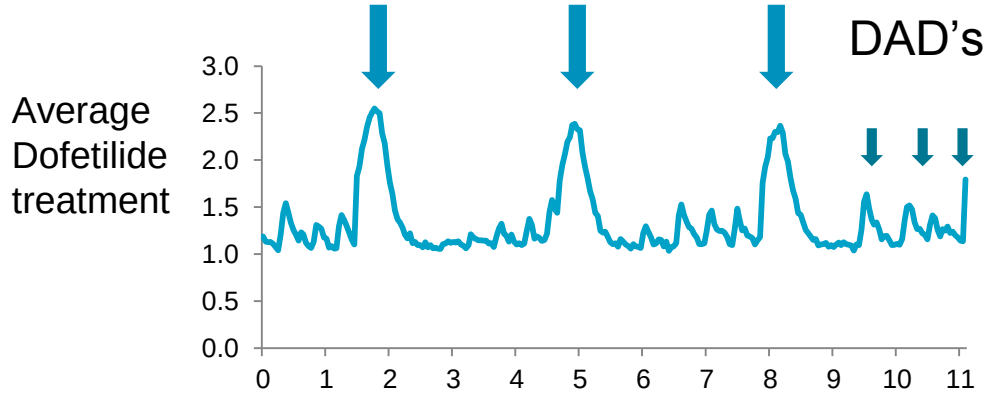
Without treatment

Using Fluo-4 calcium dye to measure calcium transients



Dofetilide treatment prolongs the calcium transient

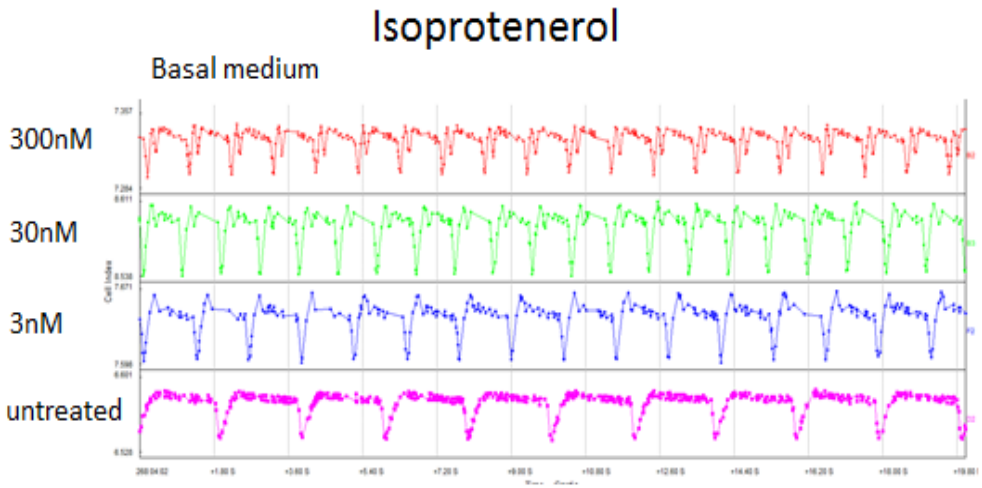




Delayed after depolarization (DAD) apparent in some cells

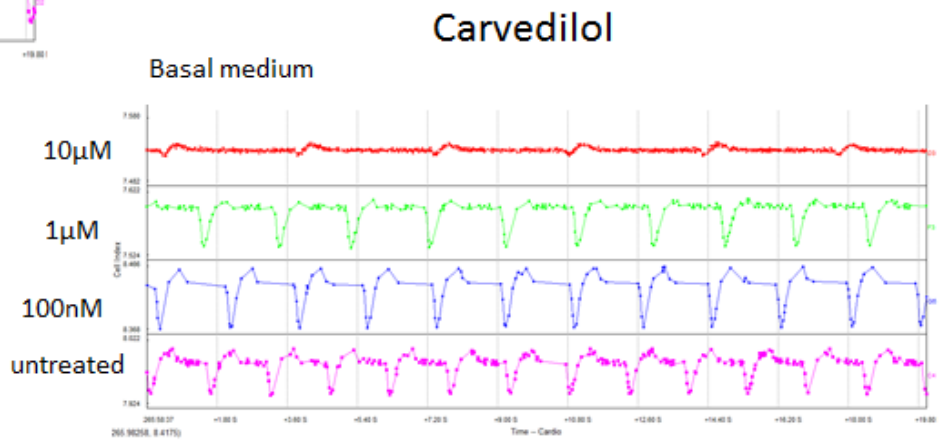
Data provided by Dr Frances Brook at Oxford University

In-Vitro Models for Cardiotoxicity Studies



Isoproterenol (β-adrenoceptor antagonist) & **Carvedilol** (β-adrenoceptor agonist) were added at 265h

Data shared by Dr Jason Gill, Durham University



iPSC-derived cardiomyocytes responded to both compounds in a dose-dependent fashion & strongly indicate the clinical relevance of these cells & their utility for drug screening applications

Cardiotoxicity Summary

iPSC-derived cardiomyocytes (CMs) could be used in cardiotoxicity & pharmacology studies

- iPSC-derived CMs express definitive cardiac markers & form organized sarcomeres
- iPSC-derived CMs show synchronized beating as a monolayer culture at high confluency
- Functional on xCelligence & for calcium imaging
- Pharmacology consistent electrophysiological measurement of Aps
- iPSC-derived CMs can form ‘cardiospheroids’ (ePoster 107)

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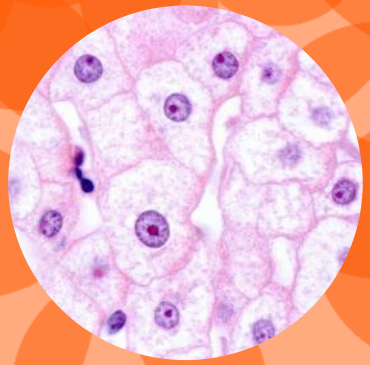
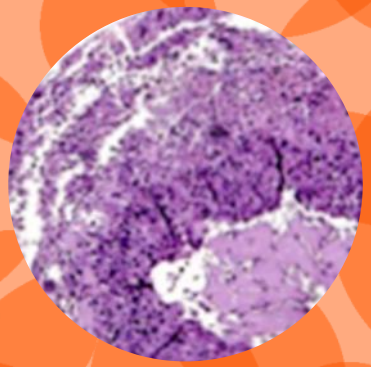
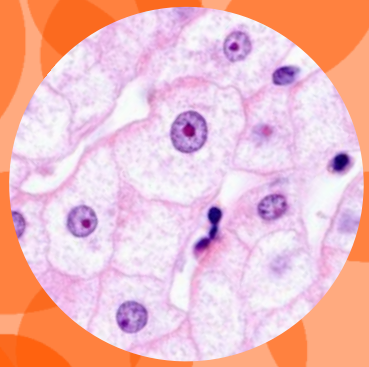
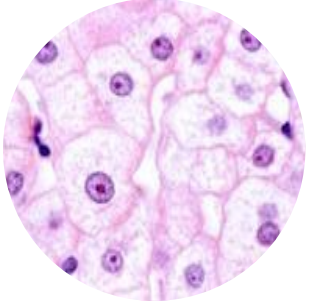
Hepatocytes







A way forward for more predictive toxicity testing

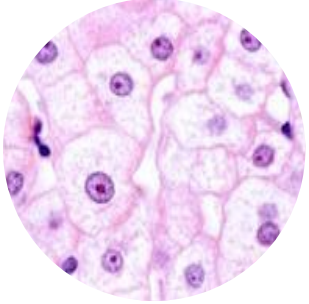


Hepatotoxicity in Drug Safety Testing

We need:

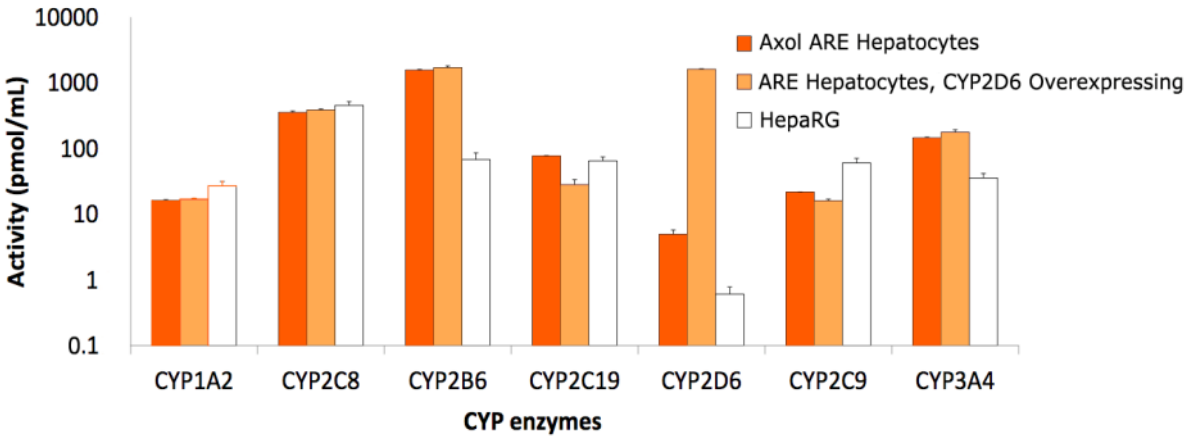
- Reliable genotoxicity testing, predictive hepatotoxicity screens
- Cells expressing adult hepatocyte markers & no fetal phenotype
- Large batch sizes from the same donor for consistency for toxicity and high-throughput screening

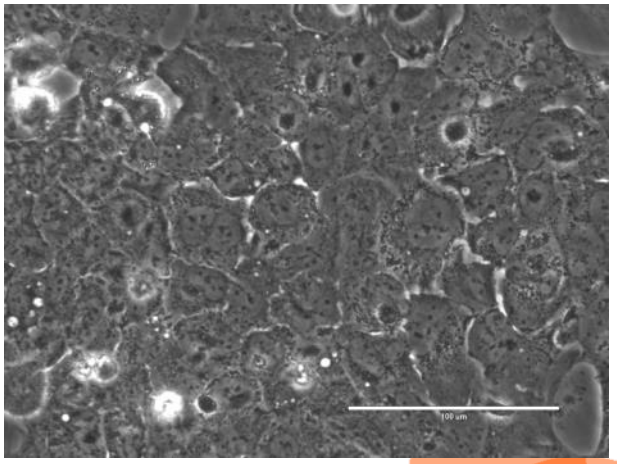
TO DATE, human primary hepatocytes have much greater functionality than iPSC-derived hepatocytes



Assay-Ready Expanded (ARE) Hepatocytes

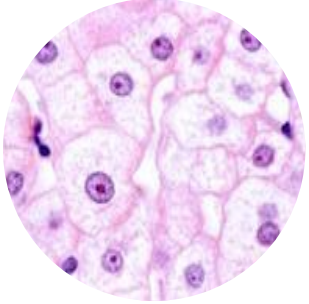
- Expanded hepatocytes that retain many characteristics of primary human hepatocytes
- Metabolically functional & express cytochrome P450 (CYP) enzymes





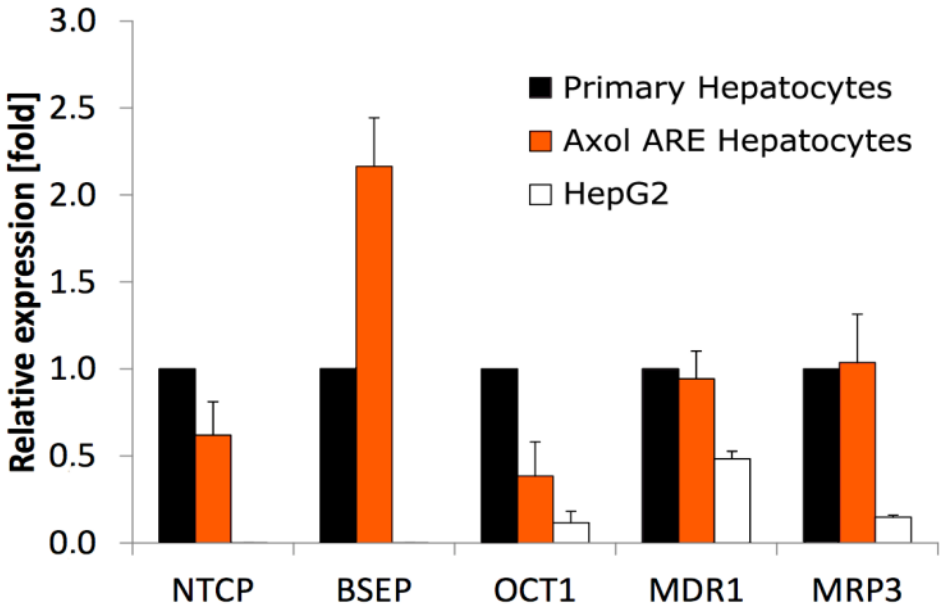
Cobblestone morphology

Comparison of the Phase I CYP enzyme activity between ARE Hepatocytes, ARE Hepatocytes (CYP2D6 Overexpressing) & HepaRG cells



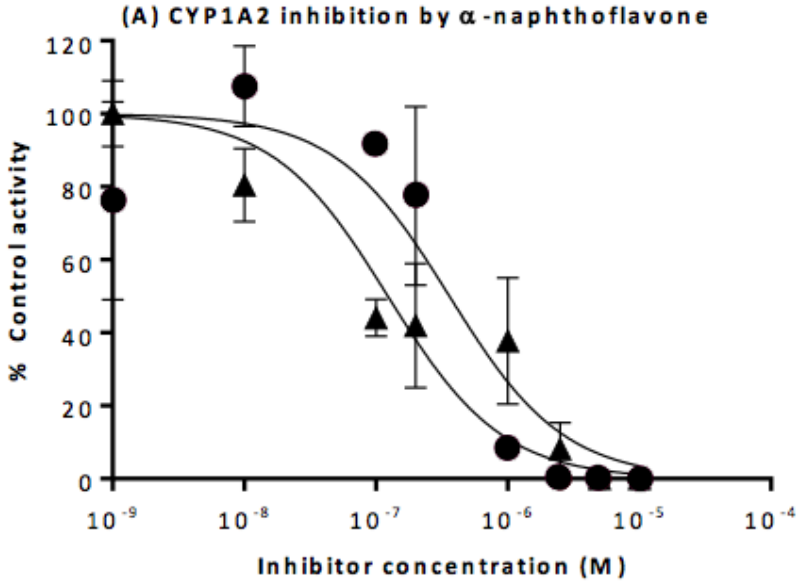
Assay-Ready Expanded (ARE) Hepatocytes

Compound uptake studies

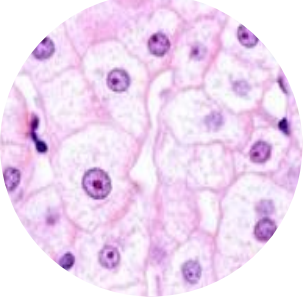


Expression of hepatic transporter genes in primary hepatocytes, ARE hepatocytes & HepG2 cells

Inhibition Studies

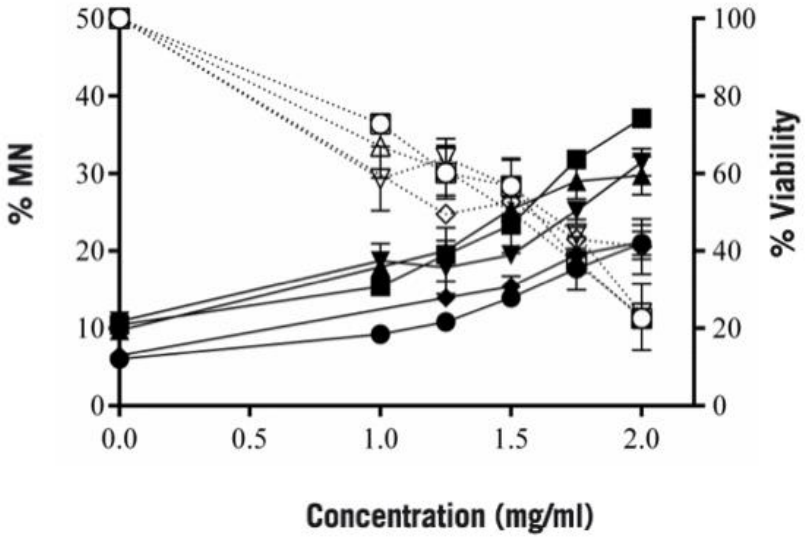


Reproducible CYP induction & inhibition in a donor-specific manner by prototypical inducers and inhibitors, for example, Naphthoflavone inhibition(N=2)



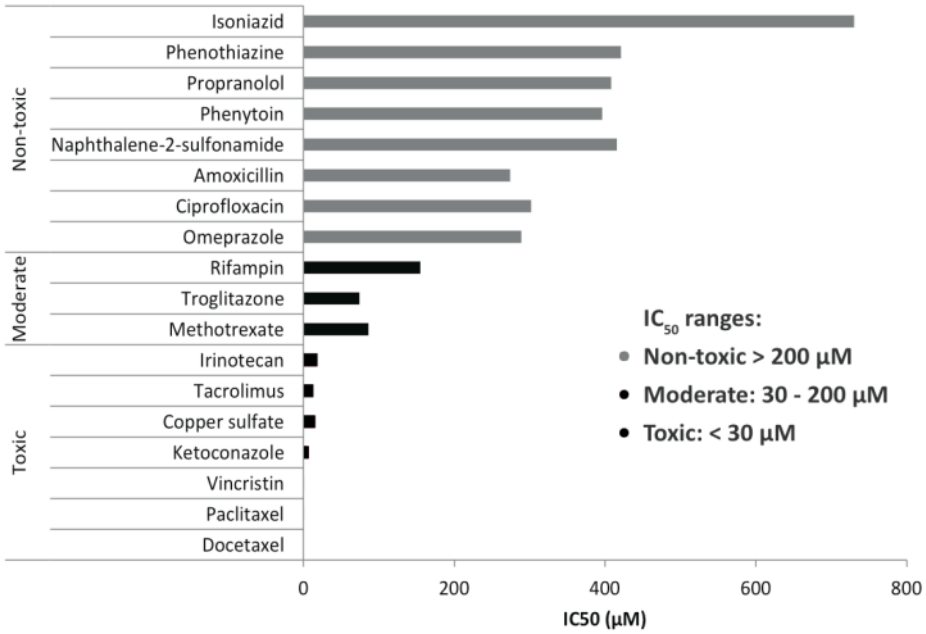
Assay-Ready Expanded (ARE) Hepatocytes

Genotoxicity studies



Increasing cyclophosphamide concentration affects the percentage of cells with MicroNuclei (% MN) & cell viability

Hepatotoxicity studies



Sensitivity to hepatotoxic compounds

Hepatotoxicity Summary

- ARE Hepatocytes display a primary liver cell phenotype
- ARE Hepatocytes are metabolic-competent cells expressing liver specific transporters and metabolizing enzymes
- Large batch sizes from the same donor for consistency in toxicity & high-throughput screening
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing
- *ARE Hepatocytes can be co-cultured with liver sinusoidal endothelial cells (data not shown)*

Conclusions

Our aim is to provide physiologically relevant *in-vitro* disease models for toxicity & drug discovery campaigns

Axol iPSC-derived NSC

- Express neural markers at gene and protein level
- Excellent neurite outgrowth
- Electrophysiologically functional
- Capable of synaptic plasticity

Axol iPSC-derived Cardiomyocytes

- Expressing definitive cardiac markers and form organized sarcomeres
- Synchronous beating monolayers, electrophysiologically functional,
- Functional on xCelligence & for calcium imaging

ARE Hepatocytes

- Display a primary liver cell phenotype
- Metabolic competent cells expressing liver specific transporters and metabolizing enzymes
- Sensitivity to hepatotoxic compounds & reliable genotoxicity testing

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Thank you!

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