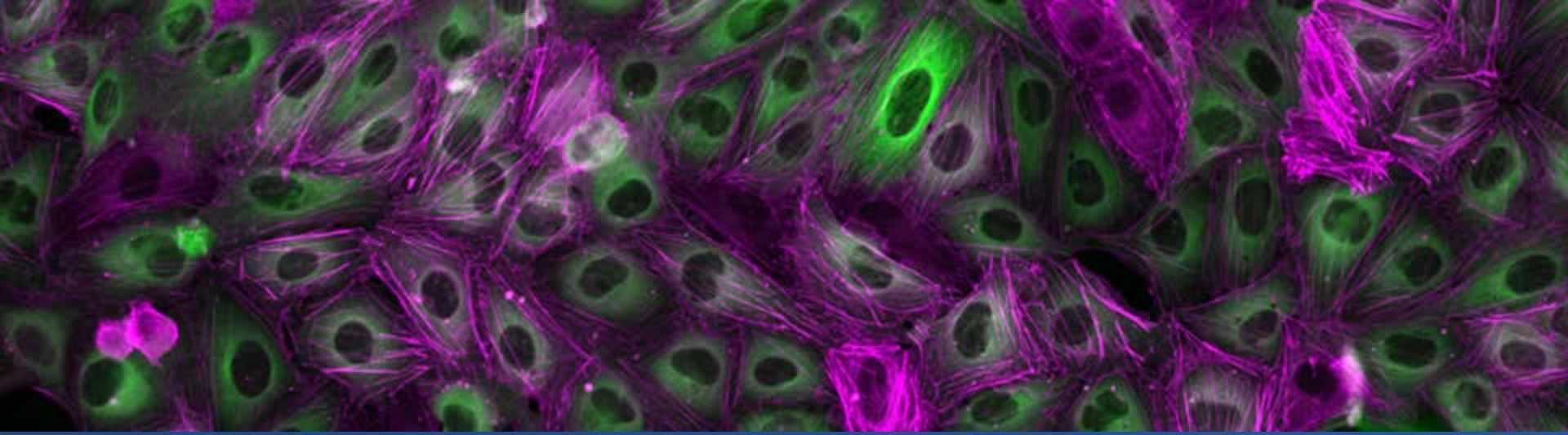


Drug-induced Delayed cardiac toxicities in HUMAN Stem Cell-DERIVED CARDIOMYOCYTES

Hua Rong Lu, MD, PhD, Global Safety Pharmacology

Art credit: *U2OS osteosarcoma cells genetically engineered to fluorescently label tubulin (green) and actin (magenta).*

March 2019

Nonclinical Safety | Janssen Research & Development, LLC

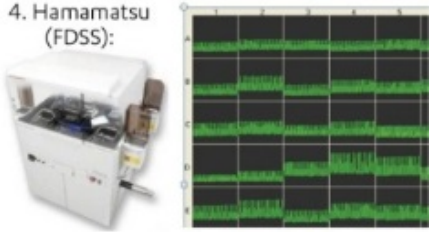
Janssen | PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Content of Presentation

1. Introduction & background of delayed cardiac toxicities in stem cell Research
2. Technologies for assessing delayed cardiac toxicities in h stem cell-CMs
3. Example compounds/mechanisms?
4. Conclusion

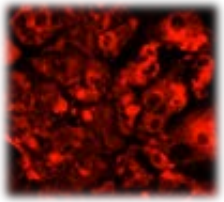
Introduction

Preclinical flow for de-risking compounds

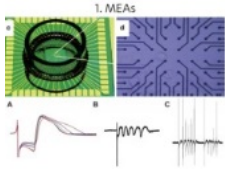


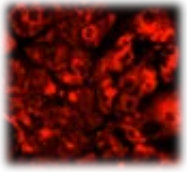
Calcium transient assay (CTCM)

Acute



Human stem cell-Cardiomyocytes





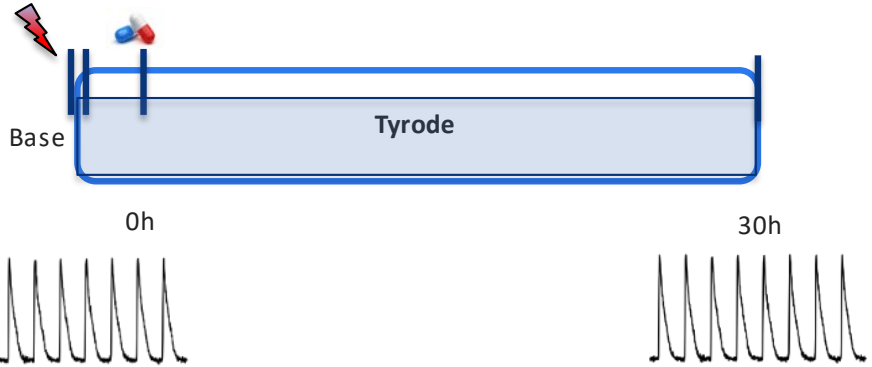
Chronic

MEA, Impedance, Sony, CTCM etc,

Delayed drug-induced cardiac toxicities

CTCM-human assay (Ca^{2+} transient assay)

- **The standard CTCM protocol (acute 30 min):**



2015

High Throughput Measurement of Ca^{++} Dynamics in Human Stem Cell-Derived Cardiomyocytes by Kinetic Image Cytometery: A Cardiac Risk Assessment Characterization Using a Large Panel of Cardioactive and Inactive Compounds

Hua Rong Lu,*,1 Ross Whittaker,† Jeffrey H. Price,† Raquel Vega,† Emily R. Pfeiffer,† Fabio Cerignoli,† Rob Towart,‡ and David J. Gallacher*

2018

J Pharmacol Toxicol Methods. 2018 May - Jun;91:80-86. doi: 10.1016/j.vascn.2018.02.004. Epub 2018 Feb 6.

Impact of calcium-sensitive dyes on the beating properties and pharmacological responses of human iPS-derived cardiomyocytes using the calcium transient assay.

Kopljar I[1], Hermans AN[2], Teisman A[2], Gallacher DJ[2], Lu HR[3].

2018

Stem Cell Reports

ISSCR

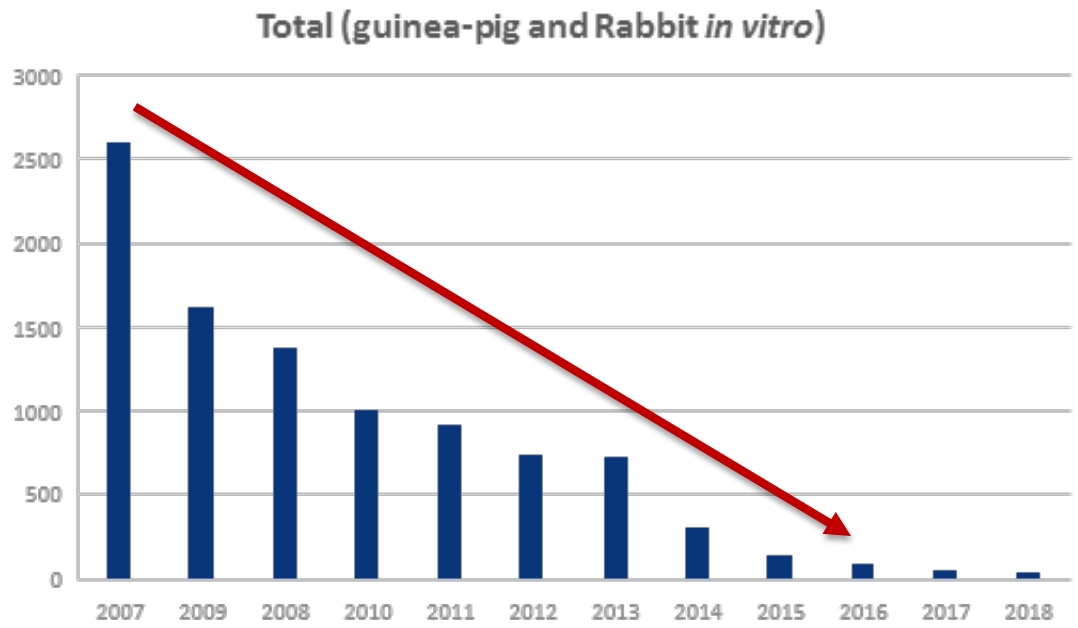
Article

OPEN ACCES

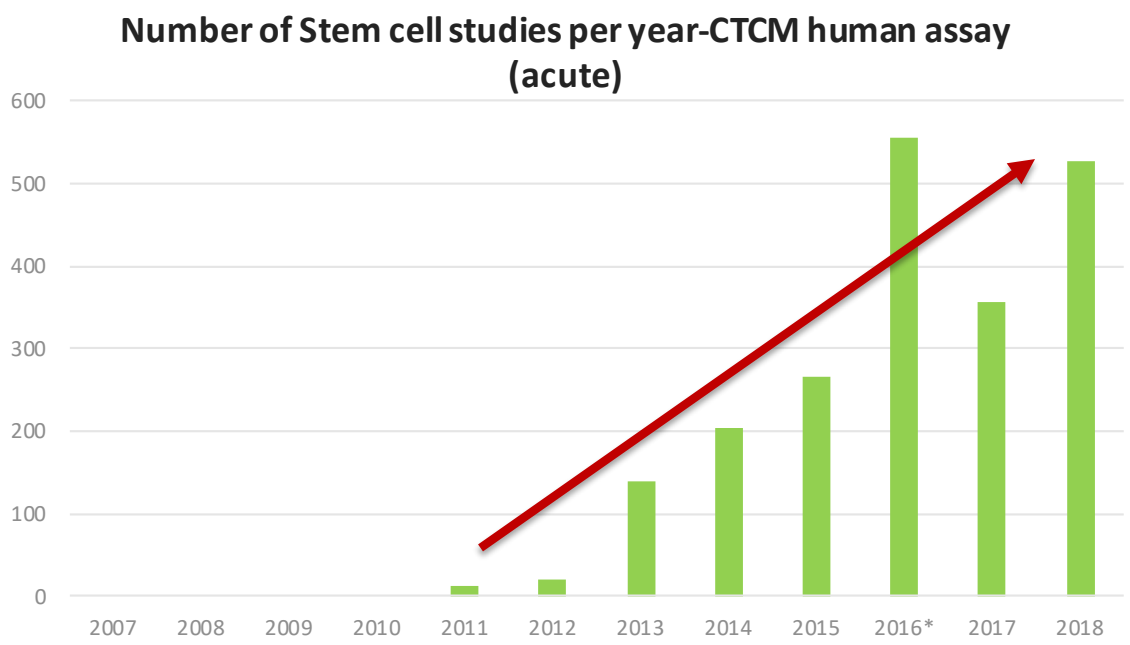
Development of a Human iPSC Cardiomyocyte-Based Scoring System for Cardiac Hazard Identification in Early Drug Safety De-risking

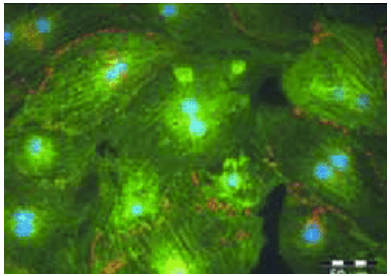
Ivan Kopljar,[1,3,*] Hua Rong Lu,[1,3,*] Karel Van Ammel,[1] Martin Otava,[2] Fetene Tekle,[2] Ard Teisman,[1] and David J. Gallacher[1]

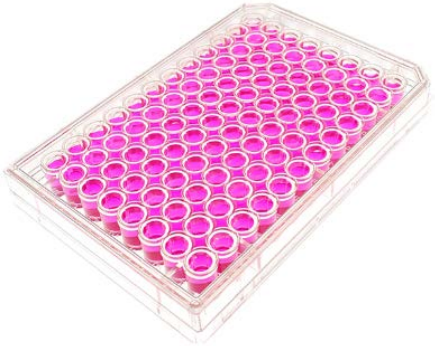
Steady Decrease in Animal Use 2007 to 2018



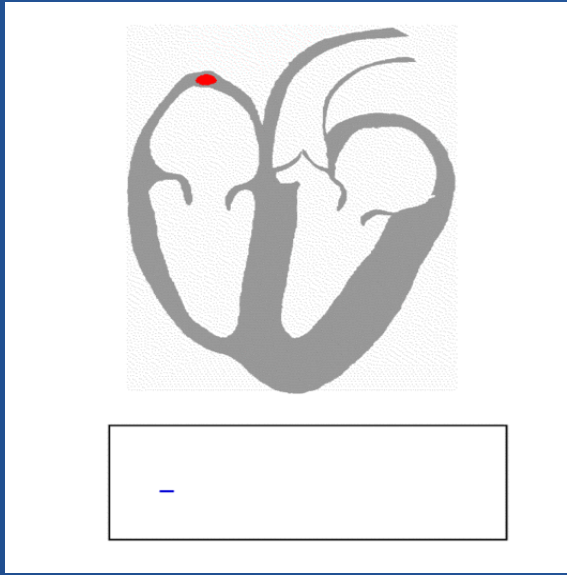
Increase in Use of Human-Based Cell Assays (hiPSC-Cardiomyocytes)







Assays for drug-induced Delayed cardiac toxities



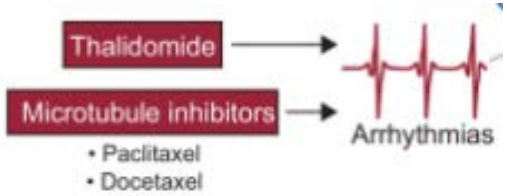
Many Drugs known to induce delayed cardiotoxicity- *different classes of drugs*

Cardiotoxicity of Anticancer Therapeutics

*Jerry Dong[1,2,3] and Hong Chen[1,3]**

2018

frontiers in Cardiovascular Medicine



Cardiac Dysfunction Associated With a Nucleotide Polymerase Inhibitor for Treatment of Hepatitis C

Tariq Ahmad,[1] Philip Yin,[2] Jeffrey Saffitz,[3] Paul J. Pockros,[4] Jacob Lalezari,[5] Mitchell Shiffman,[6] Bradley Freilich,[7] Joann Zamparo,[2] Kyle Brown,[2] Dessislava Dimitrova,[8] Monica Kumar,[8] Doug Manion,[8] Margo Heath-Chiozzi,[2] Robert Wolf,[9] Eric Hughes,[9] Andrew J. Muir,[1] and Adrian F. Hernandez[1]

HEPATOLOGY

2014

Development of BMS-986094 in Stopped in Phase 3

Cardiac Toxicity Associated with HCV Direct Antiviral Agents

Claudio Ucciferri[1-2], Alessandro Occhionero[1], Jacopo Vecchiet[1] and Katia Falasca[1].

Mediterranean Journal of Hematology and Infectious Diseases

2018

Cardiotoxicity of Histone Deacetylase Inhibitor Depsipeptide in Patients with Metastatic Neuroendocrine Tumors

Manisha H. Shah,[1] Philip Binkley,[2] Kenneth Chan,[3] Jim Xiao,[3] Daria Arbogast,[1] Minden Collamore,[1] Yasser Farra,[2] Donn Young,[4] and Michael Grever[1]

Cancer drug-induced cardiac side effects:

- doxorubicin and cardiac toxicity (4771)
  PubMed
- cancer drugs and cardiac toxicity (1593)
  PubMed
- cancer drugs and cardiac arrhythmias (314)
  PubMed
- kinase inhibitors and atrial fibrillation (96)
  PubMed

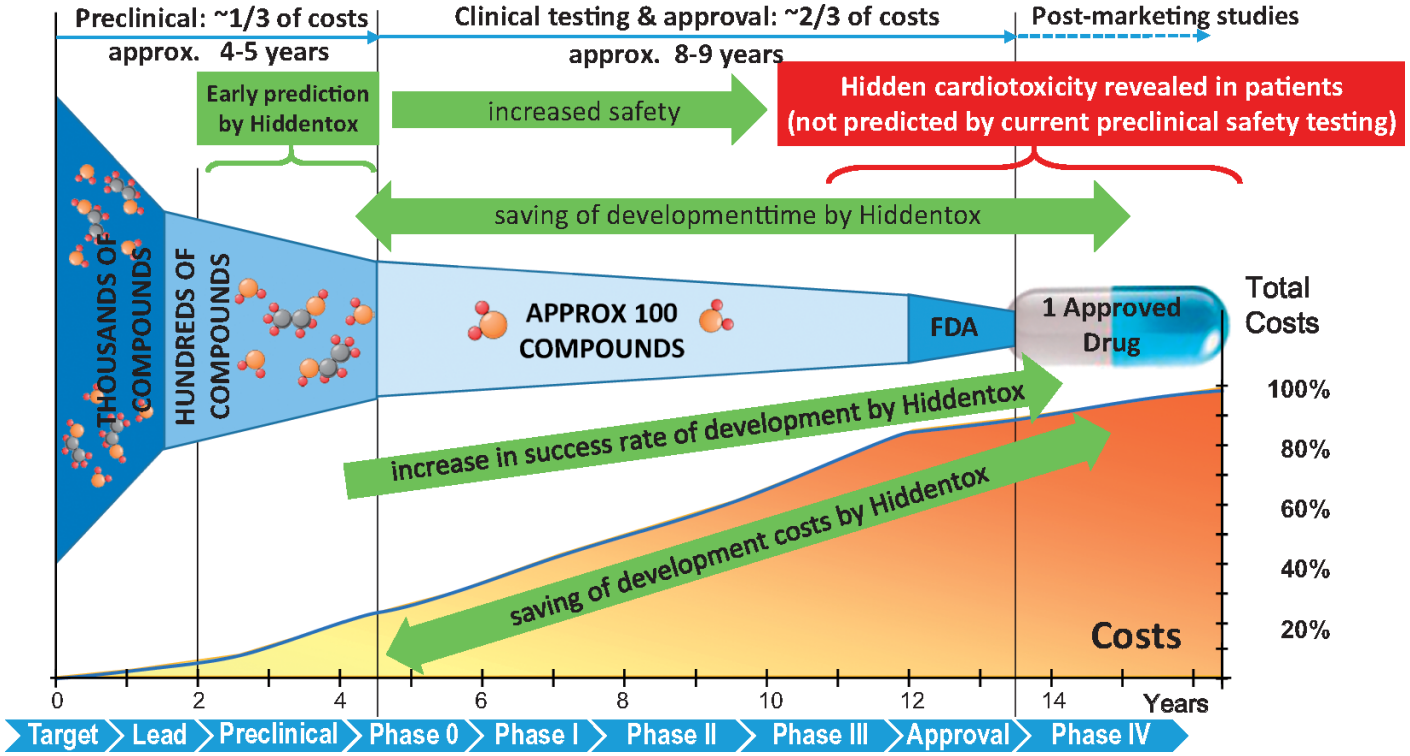
Some Internal Projects / Research Presented with Delayed Cardiac Toxicities

Project X (JNJ-X): delayed QT ↑↑↑ /SCD in GLP study dogs

Anti-infectious drugs: delayed QT, Hypertrophy, cardiac arrhythmias

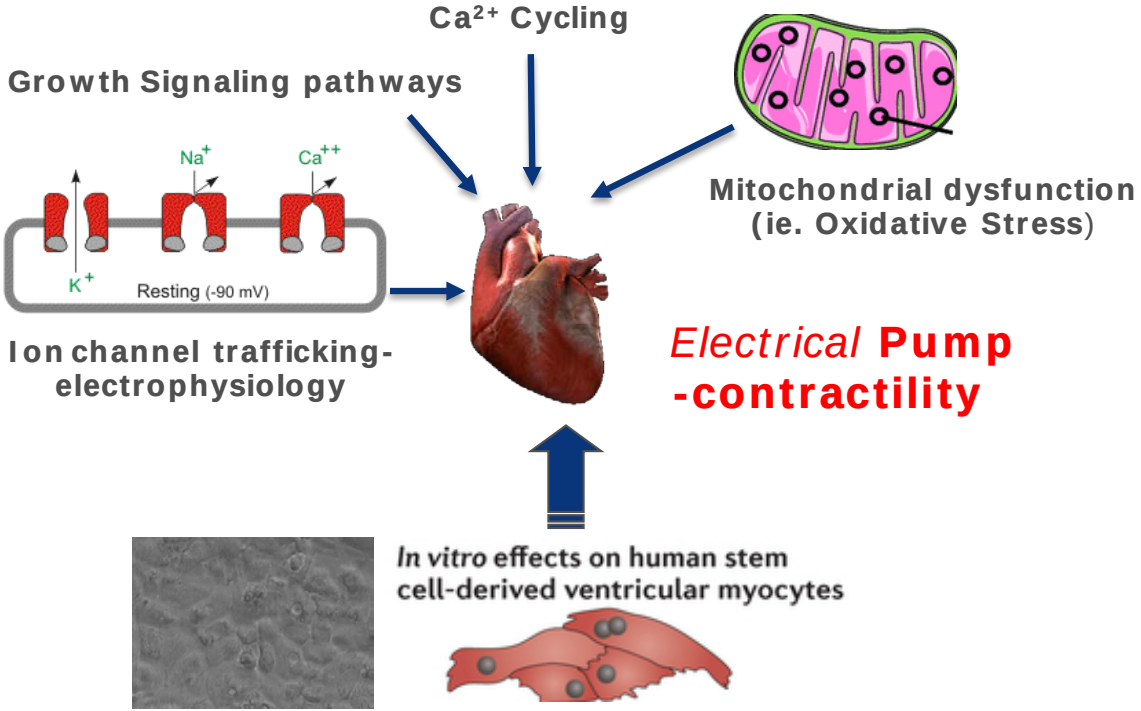
Oncological drugs: could cause delayed atrial fibrillation, ventricular arrhythmias and heart failure (HF)

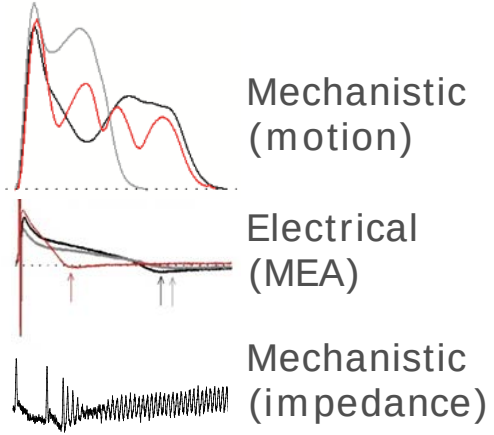
Delayed Cardiac Toxicities are not Detected by the current standard Safety Pharmacology Assays: expensive



Ferdinandy et al., 2018. Eu Heart J

Chronic Cardiac Liabilities Result From Multiple Mechanisms





Research on Delayed Cardiac Liabilities in human stem cell-CM

Janssen Global Safety Pharmacology

Project I: Differentiating cardiotoxicity within HDAC inhibitors

Impedance-assay (xCelligence)

STEM CELLS TRANSLATIONAL MEDICINE

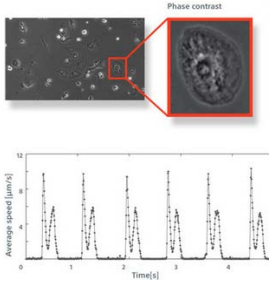
CELL-BASED DRUG DEVELOPMENT, SCREENING, AND TOXICOLOGY

Functional and Transcriptional Characterization of Histone Deacetylase Inhibitor-Mediated Cardiac Adverse Effects in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

IVAN KOPLJAR,[a] DAVID J. GALLACHER,[a] AN DE BONDT,[a] LAURE COUGNAUD,[b] EDDY VLAMINCKX,[a] ILSE VAN DEN WYNGAERT,[a] HUA RONG LU[a]

Project II: Detection of various mechanisms of cardiac liabilities

Video Microscopy (Sony)



BJP British Journal of Pharmacology

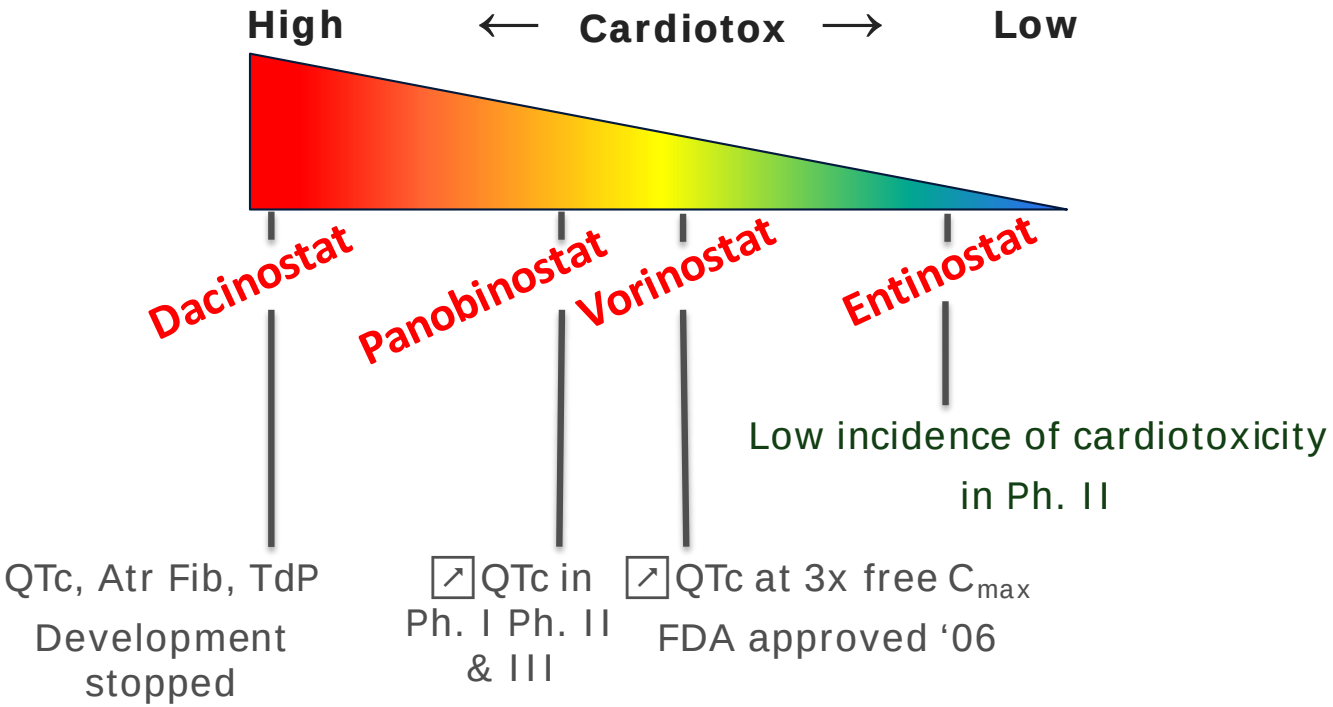
RESEARCH PAPER | Free Access

Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes

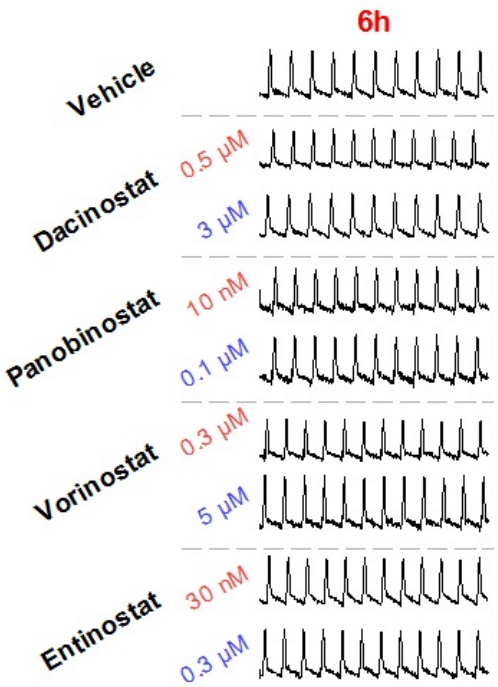
Ivan Kopljar, An De Bondt, Petra Vinken, Ard Teisman, Bruce Damiano, Nick Goeminne, Ilse Van den Wyngaert, David J Gallacher, Hua Rong Lu

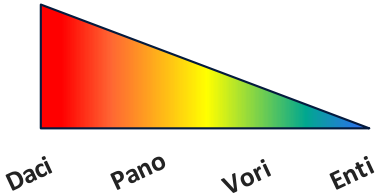
First published: 17 January 2017 | https://doi.org/10.1111/bph.13713 | Cited by: 2

Project I: Differentiating Cardiotoxicity Within HDAC Inhibitors



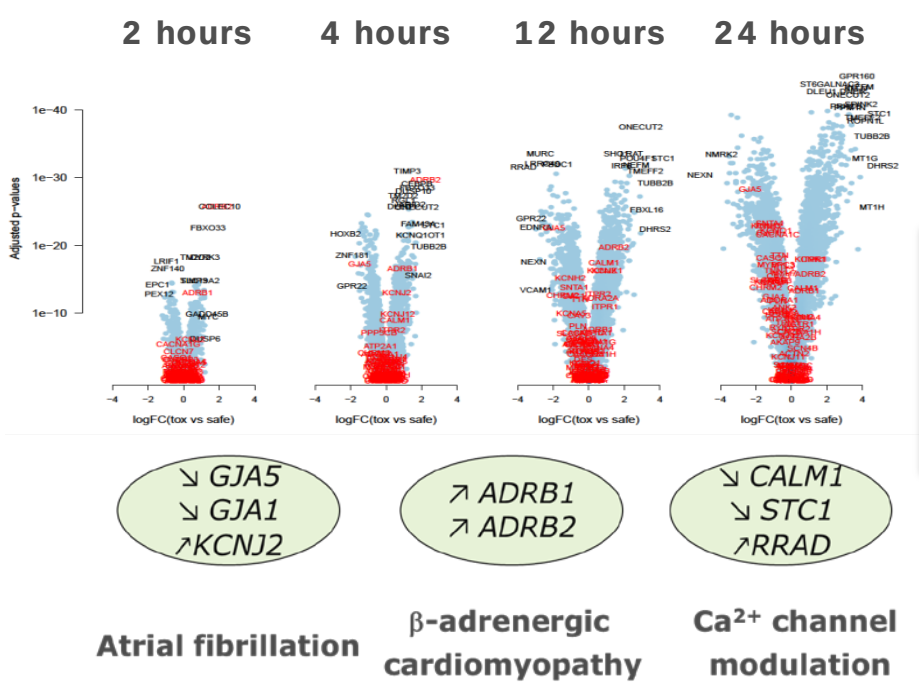
Project I: Differentiating cardiotoxicity Among HDAC inhibitors

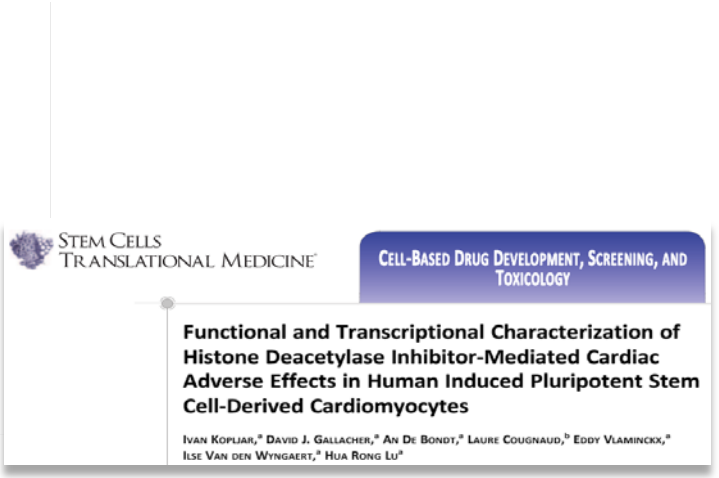




Good translation to dose-related (free C_{max}) cardiac adverse effects in the clinic

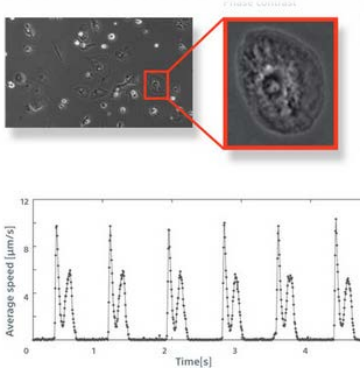
Project I: Transcriptional Characterization of Genes Related to Cardiac Toxicity Compared to Unrelated Genes in human stem cell-CMs

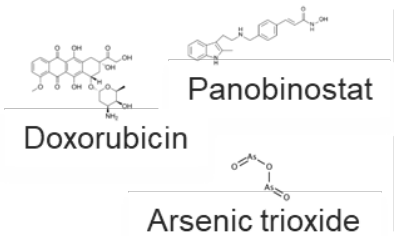


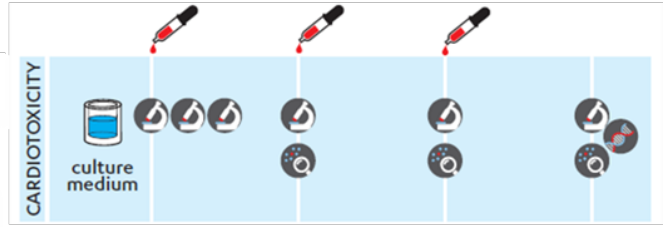


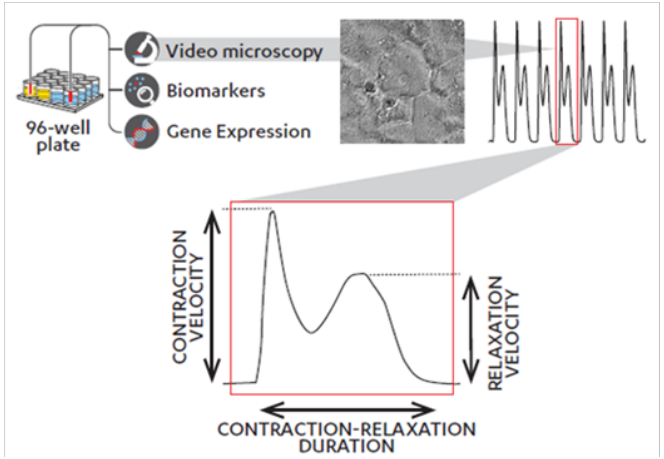
Project II: Detection of Various Mechanisms of Delayed Cardiac Liabilities

Video Microscopy (Sony)

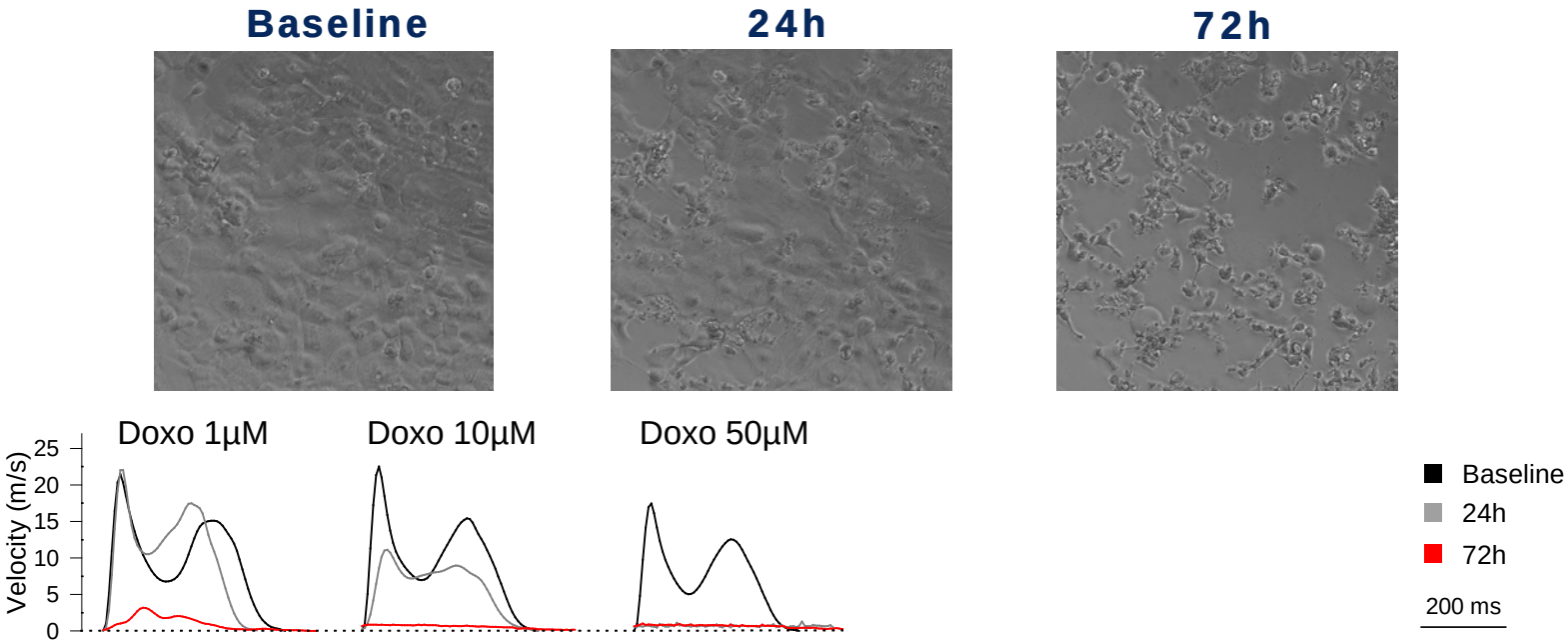








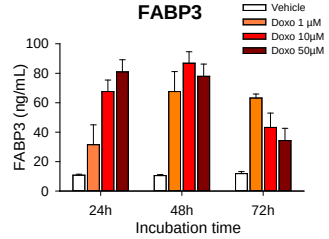
Project II: Detection of Various Mechanisms of Delayed Cardiac Liabilities



Video motion analysis can detect different mechanisms of cardiac liabilities in human stem cell-CMs

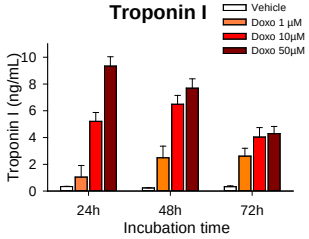
Project II: FABP3 is a Good Predictor of Diverse Cardiac Liabilities on this assay

Doxorubicin

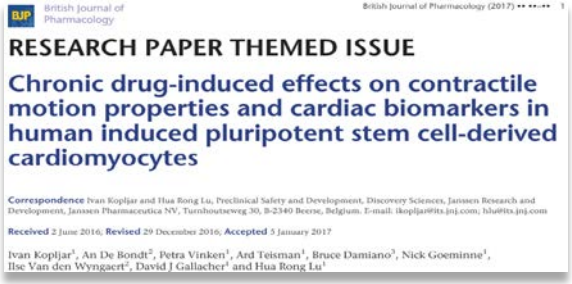


FABP3 ↗

cTnI ↗



FABP3 is a better predictor of cardiotoxicity in human stem cell-CMs



British Journal of Pharmacology

RESEARCH PAPER THEMED ISSUE

Chronic drug-induced effects on contractile motion properties and cardiac biomarkers in human induced pluripotent stem cell-derived cardiomyocytes

Correspondence Ivan Kopljar and Hua Rong Lu, Preclinical Safety and Development, Discovery Sciences, Janssen Research and Development, Janssen Pharmaceutica NV, Turnhoutseweg 30, B-2340 Beerse, Belgium. E-mail: ikopljar@its.jnj.com; hlu@its.jnj.com

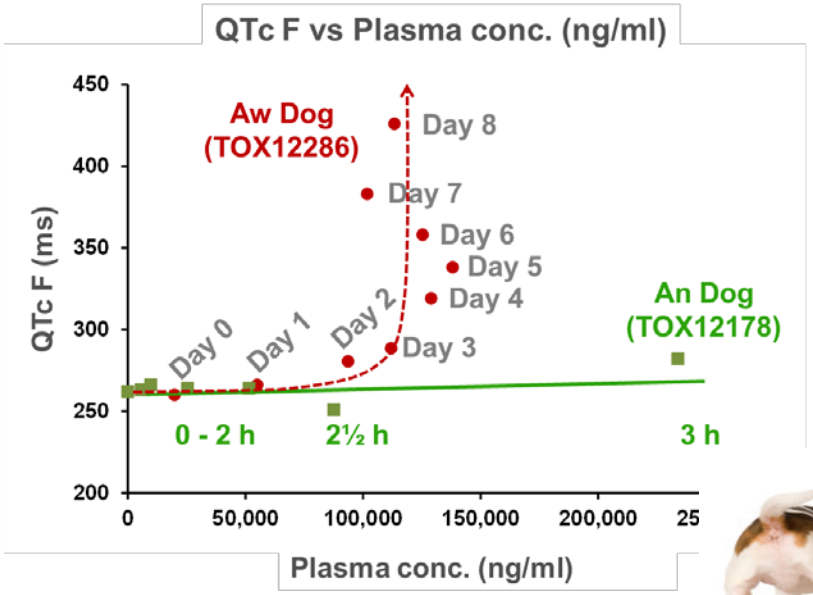
Received 2 June 2016; Revised 29 December 2016; Accepted 5 January 2017

Ivan Kopljar[1], An De Bondt[2], Petra Vinken[1], Ard Teisman[1], Bruce Damiano[3], Nick Goeminne[1], Ilse Van den Wyngaert[2], David J Gallacher[1] and Hua Rong Lu[1]

Example: Infectious Disease Therapeutic Area

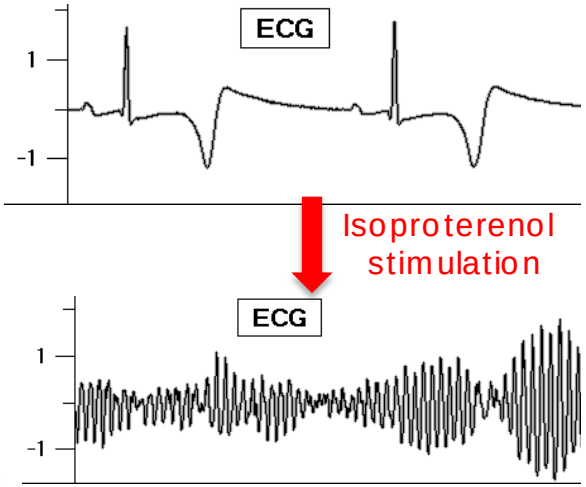
JNJ-X

Delayed QTc prolongation in dogs



Free C_{max}: 0.2-0.3 µM

SCD & Induced TdPs in dogs leading to death



An Dog

Profile Summary

JNJ-X

- ✓ Massive QT and QTc interval prolongation
- ✓ Large negative T-wave (ECG *LQT1 - like*)
- ✓ Two dogs died suddenly, SCD
- ✓ No concentration/effect relationship, but delayed
- ✓ Induction of Torsades de Pointes after ISO

Delayed HERG/ I_{Ks} channel effect?

JNJ-X Electrophysiological Profile

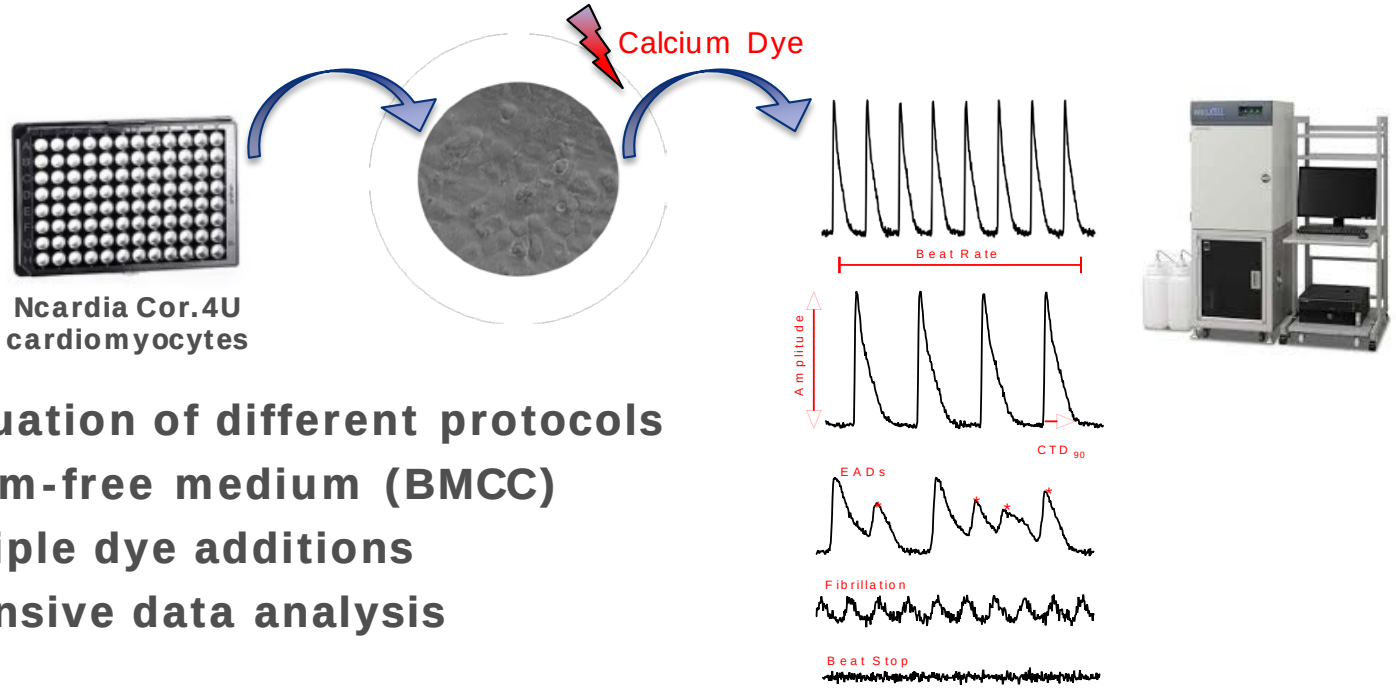
In vitro: Acute Ion channel effects : IC50 HERG=2 µM, Iks= 1 µM

In vivo: no acute ECG effects

Free C_{max} in dogs: 0.2-0.3 µM where observed marked prolongation in 3 days

QTc prolongation in dogs cannot be explained by (acute) effects on cardiac ionic currents

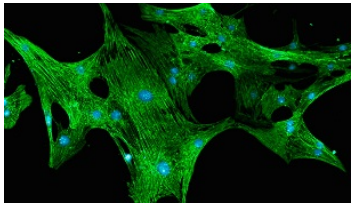
Could repeated calcium dye exposure be used to assess delayed cardiac effects in CTCM?



- Evaluation of different protocols
- Serum-free medium (BMCC)
- Multiple dye additions
- Extensive data analysis

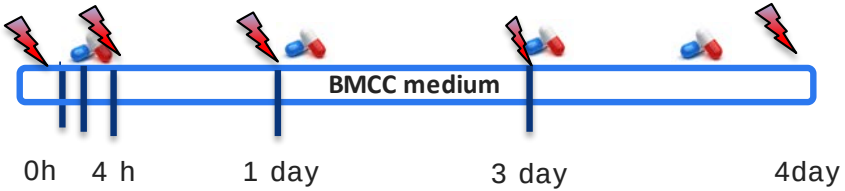
CTD_{90}: Calcium Transient Duration

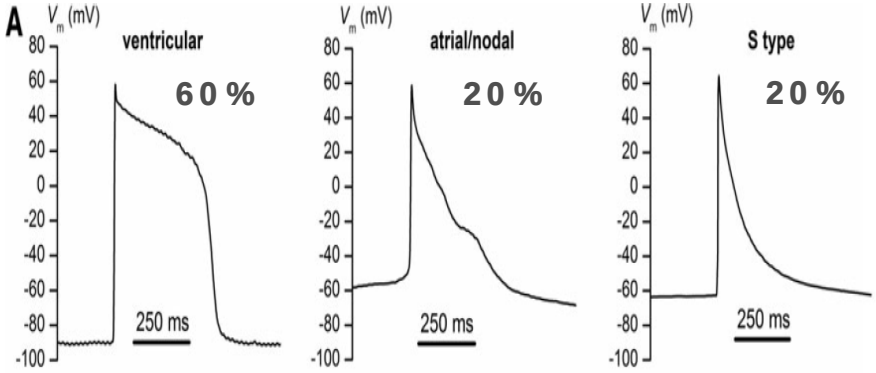
Delayed CTCM Assay in human stem cell-CMs (4-day Exposure)

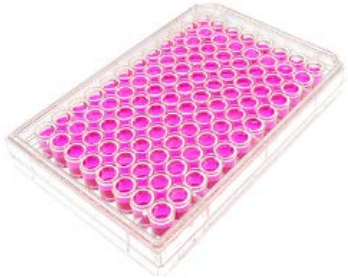


human Cor.4U® Cardiomyocytes

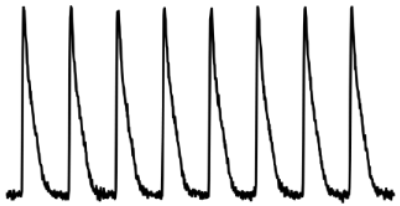
Multi-dyes additions:



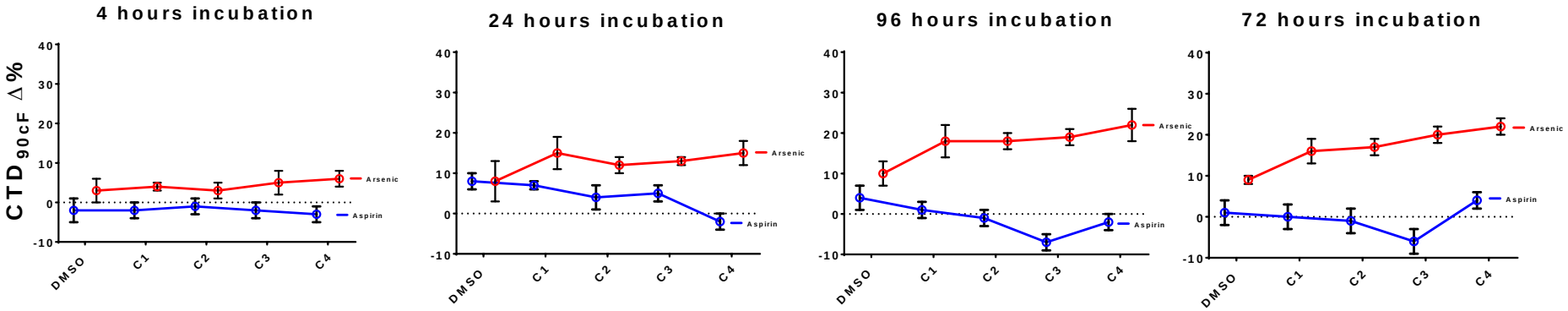




Measurement



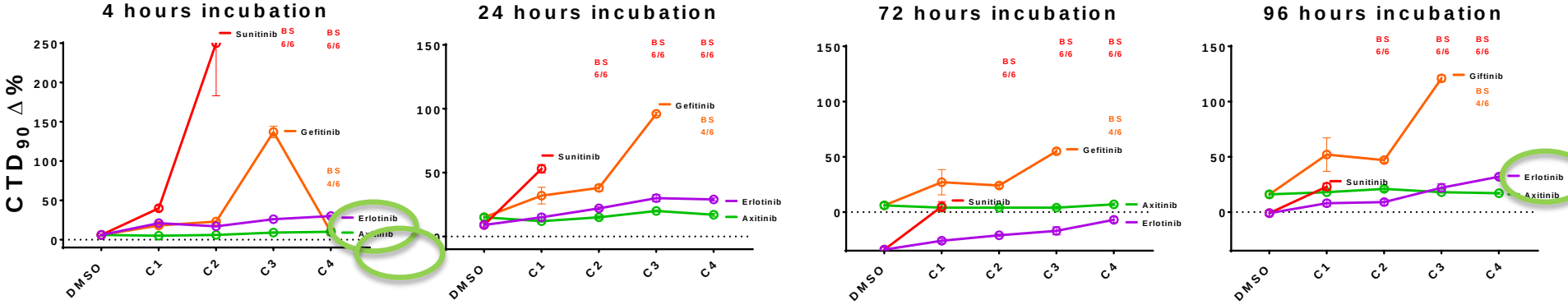
Effects of 4-day exposure to arsenic on human stem cell-CMs



Aspirin: C1: 10 µM; C2: 30 µM; C3: 100 µM; C4: 300 µM / **Free Cmax:** 20 µM

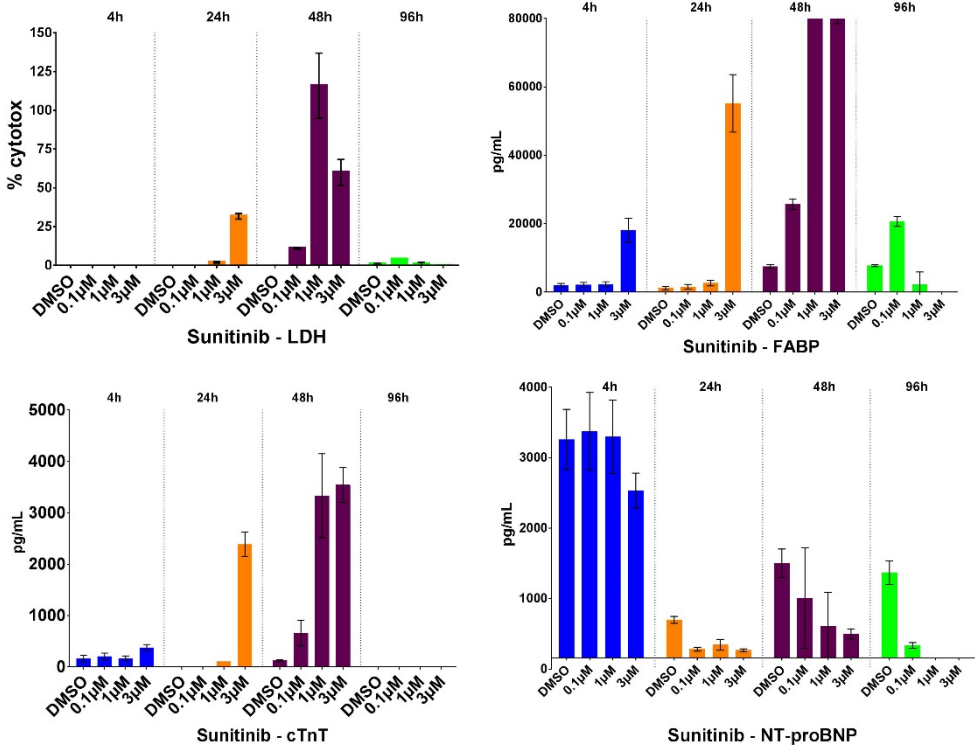
Arsenic: C1: 0.1 µM; C2: 0.3 µM; C3: 1 µM; C4: 3 µM / **Free Cmax:** 0.2 - 10 µM

Effects of 4-day exposure to Tyrosine kinase inhibitors (TKI) on stem cell-CMs

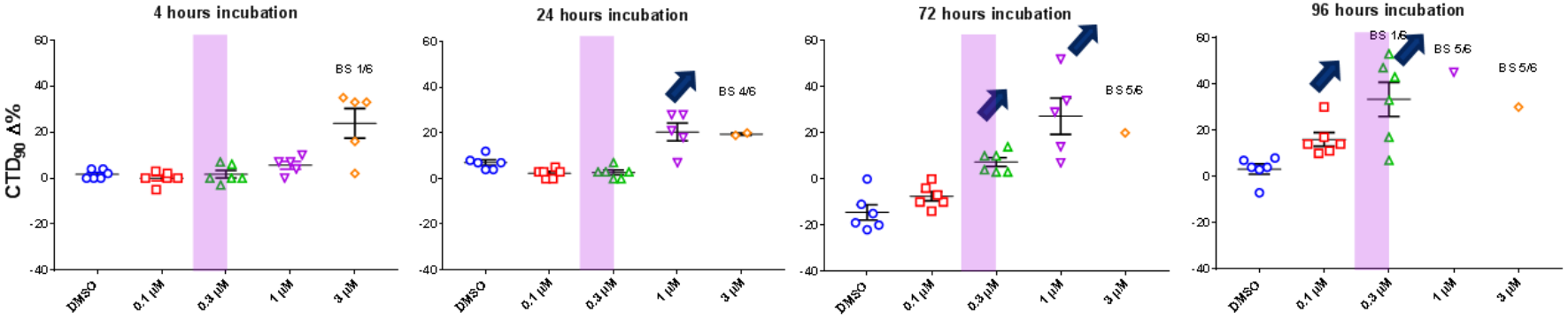


- **Giftinib:** C1: 0.1 µM; C2: 0.3 µM; C3: 1 µM; C4: 3 µM / **Free Cmax:** 0.045 µM
- **Axitinib:** C1: 0.03 µM; C2: 0.1 µM; C3: 0.3 µM; C4: 1 µM / **Free Cmax:** 0.007 µM
- **Sunitinib:** C1: 0.1 µM; C2: 1 µM; C3: 3 µM; C4: 10 µM / **Free Cmax:** 0.125 - 5 µM
- **Erlotinib:** C1: 1 µM; C2: 3 µM; C3: 10 µM; C4: 30 µM / **Free Cmax:** 1.5 - 2 µM

Effects of 4-day exposure to Tyrosine kinase inhibitors (TKI) on biomarkers in stem cell-CMs

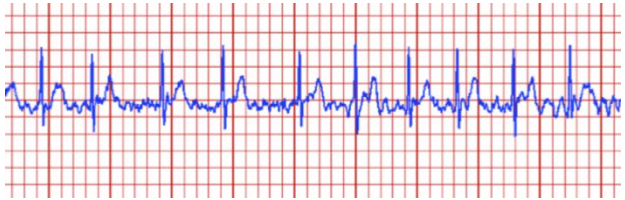


Effects of 4-day exposure to JNJ-X on human stem cell-CMs

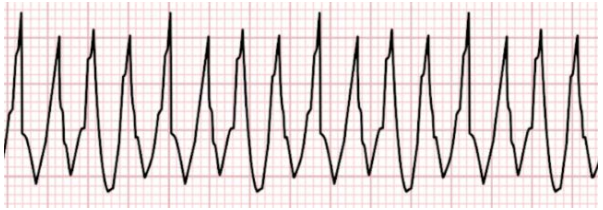


dog free Cmax 0.2-0.3 µM

Some delayed Drug-induced cardiac effects occur a few months after treatment: could prolong study period on dish?

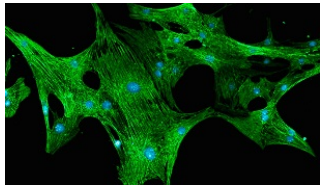


Atrial fibrillation

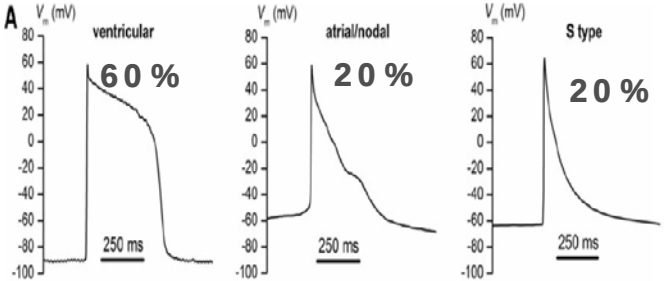


Ventricular tachycardia, HF

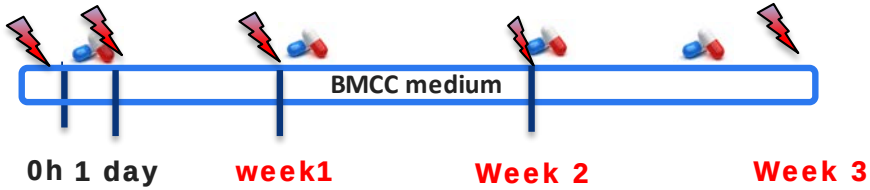
We plan a long-term calcium transient assay in human stem cell-CMs (up to week 3) to identify potential MOA?



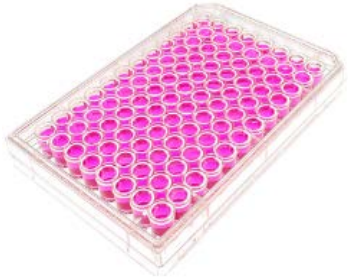
Human Cor.4U® Cardiomyocytes

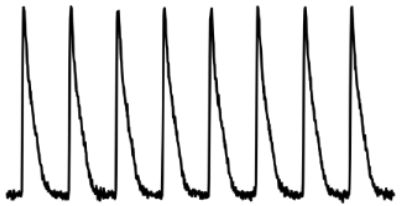


Multi-dyes addition:



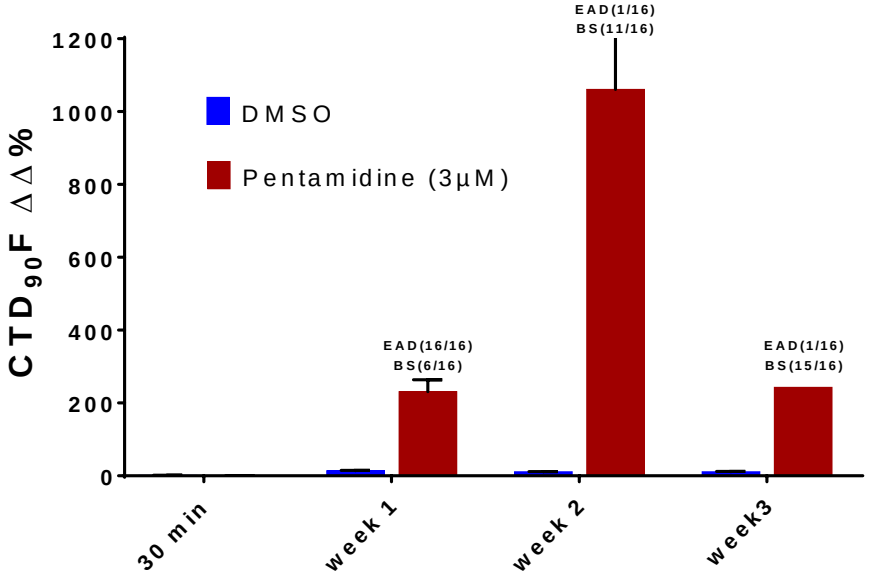
Measurement

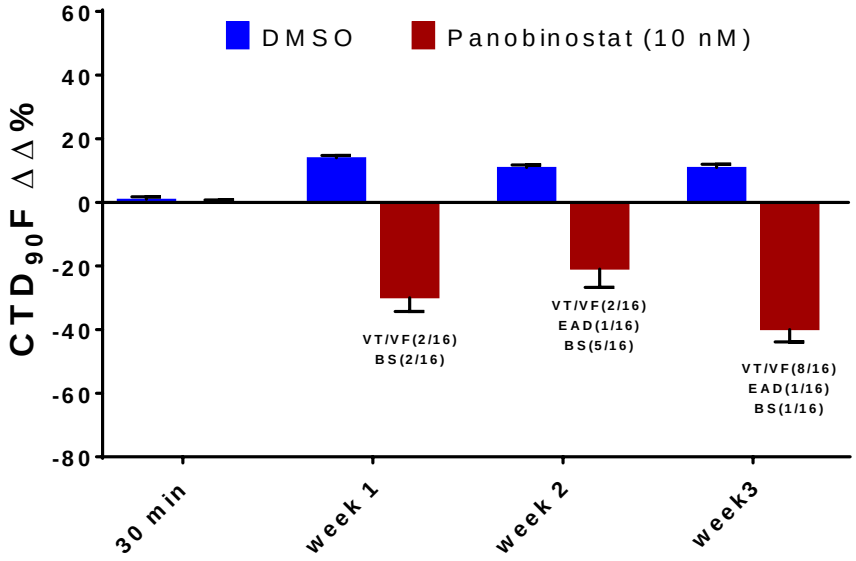




*These data are pilot data and still to be confirmed

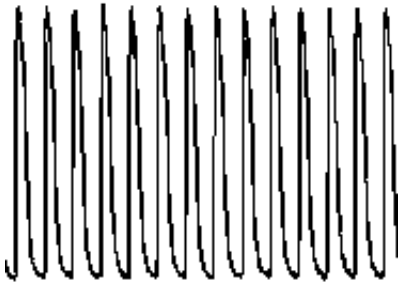
Effects of pentamidine and panobinostat on stem cell-CMs up to week 3; pilot/pre- study



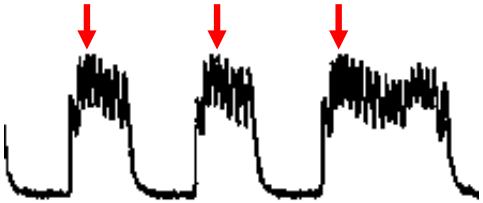


Delayed cardiac effects: incubation with drug up to week 3 on stem cell-CMs: pilot/pre- study

DMSO Control

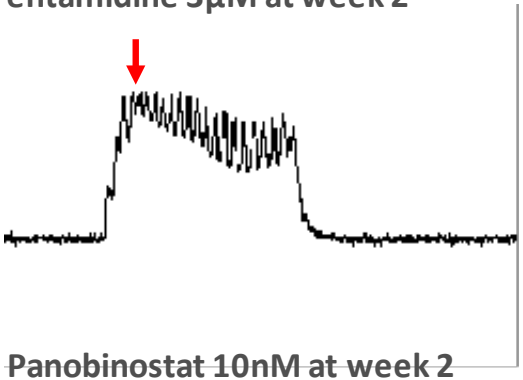


Pentamidine 3µM at week 1

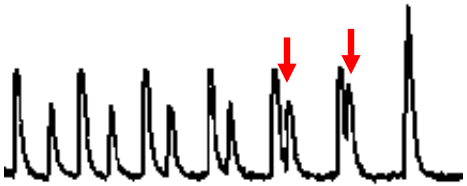


EADs

Pentamidine 3µM at week 2



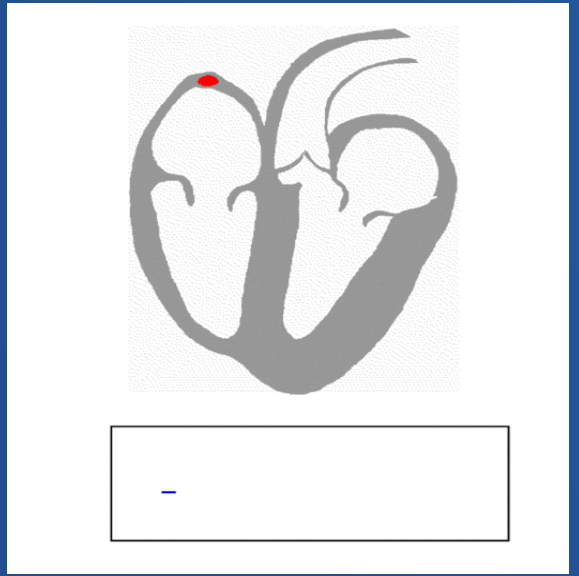
Panobinostat 10nM at week 1



Panobinostat 10nM at week 2

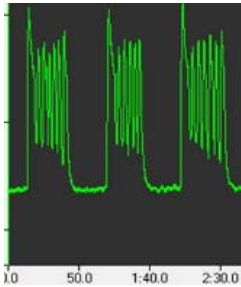
VT/VF like

Conclusions



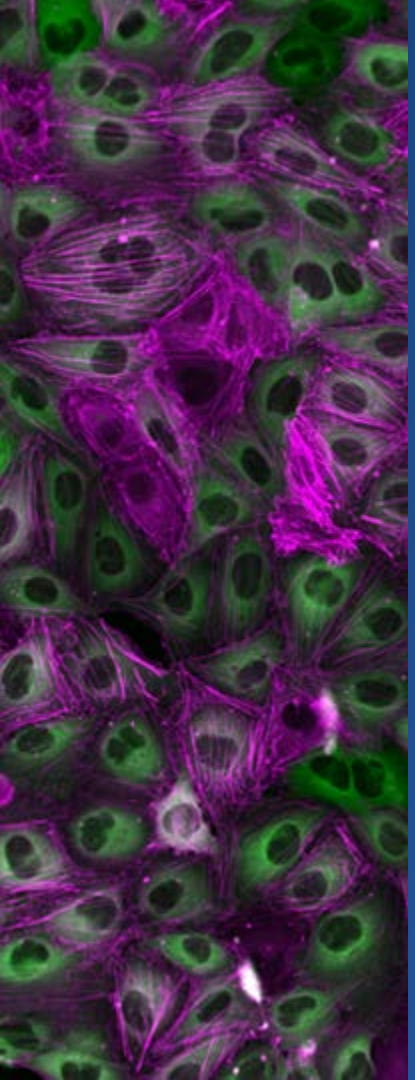
Conclusions

I. Human stem cell-CMs could be applied for potential drug-induced delayed cardiac effects



II. Different detection technologies in stem cell-CMs can be used for identification and mechanistic understanding of drug-induced delayed cardiac toxicities.

III. MOAs of drug-induced cardiac toxicities are complex, still….



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