Presentations

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Pioneering science delivers vital medicines™

NC3R Meeting

“Overcoming the barriers to wider uptake of human tissue for safety assessment”

Human Vascular Tissue in Safety Assessment

Hugo M. Vargas, PhD, DSP

Safety & Exploratory Pharmacology

Toxicology Sciences

Amgen, Inc

Outline

- **Human Vascular Tissue: Use in Safety Pharmacology**
- **Value: Case-Study**
  - Drug-induced Vasoconstriction and Hypertension Risk
- **Overcoming Barriers: Practical Issues**

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Vascular Tissue Models:
Use in Cardiovascular Safety Pharmacology

- **Animal derived: routine methods**
  - Extensive experience in scientific literature
  - Rodent and non-rodent species
  - Conduit and resistance vessels
  - Functional evaluation of smooth muscle:
    - contractility or relaxation protocols
  - Role of endothelium

- **Access to Tissues: relatively easy**
  - Timed tissue collection: planned
  - Optimum handling and viability
  - Number of vascular samples: not a primary limitation
  - Healthy (normal) tissues: prevalent

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Vascular Tissue Models:
Use in Cardiovascular Safety Pharmacology

- **Human: Methods similar to animal tissue protocols.**
  - Conduit and resistance vessels available
    - Chorionic artery (placenta); internal mammery artery (CABG), coronary artery (proximal/distal; CAG), resistance arteries; saphenous vein, etc
  - Functional evaluation of smooth muscle:
    - same as animal-based tissue protocols
  - Role of endothelium
    - same as animal-based tissues protocols
- **Access to Tissues: complicated**
  - Tissue collection: planned, but timing based on donor availability
  - Handling & viability: dependent of blood vessel; skill of surgeon; ease of dissection; tissue variability; donor variability; etc
  - Number of vascular samples: can be a limitation
  - Diseased versus Healthy donors:
    - Access to diseased specimens more likely

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Application to CV Safety Pharmacology: Drug-induced Changes in Vascular Tone

- **FTY720 (Gilenya®): sphingosine 1-phosphate (S1P) receptor agonist (treatment of MS)**

- **Blood Pressure elevation observed: clinical studies**
  - Warning and Precaution (Label; FDA)
    - ↑3 mm Hg systolic; ↑2 mm Hg diastolic (1 month of dosing)
    - Recommendation: Monitor BP during treatment

- **Blood pressure elevation observed: animal models**
  - Rat telemetry
    - Single dose
    - Repeat dose
  - Non-human primate telemetry
    - Single dose (unpublished; Amgen)

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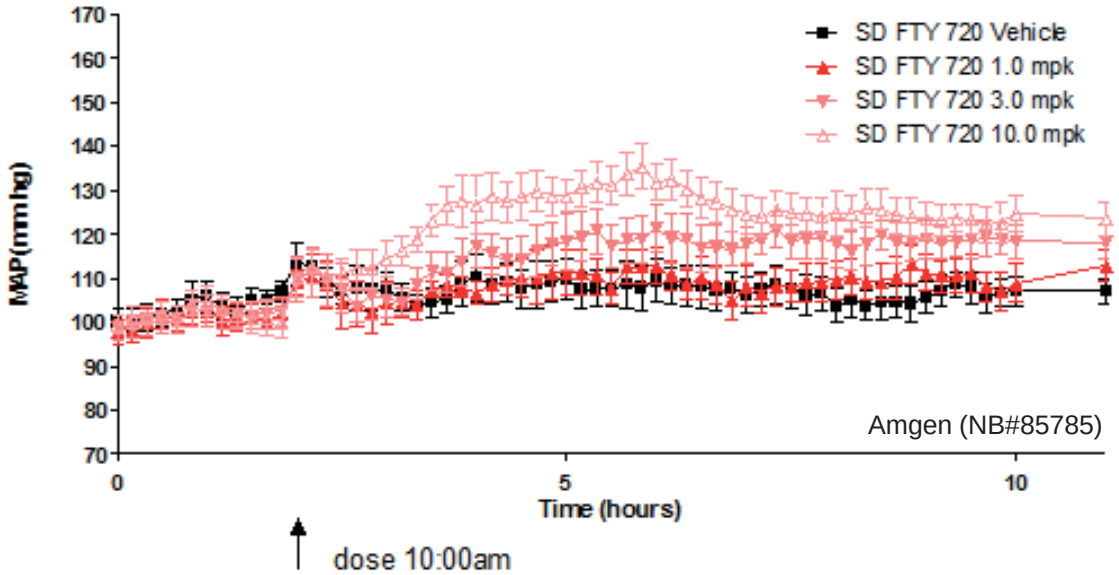
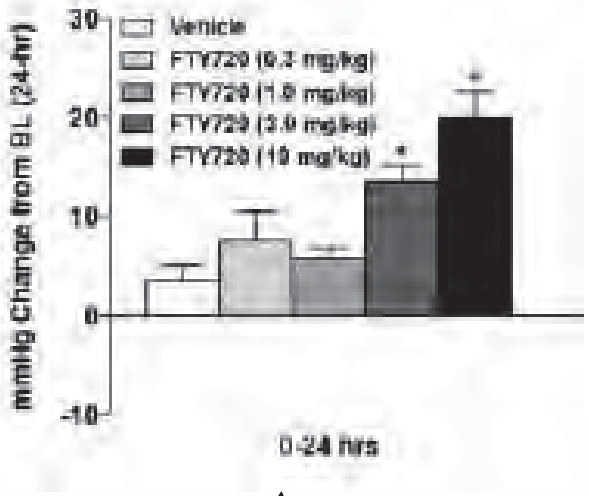
FTY-720 induced Arterial Pressure Elevation:
Rat Telemetry

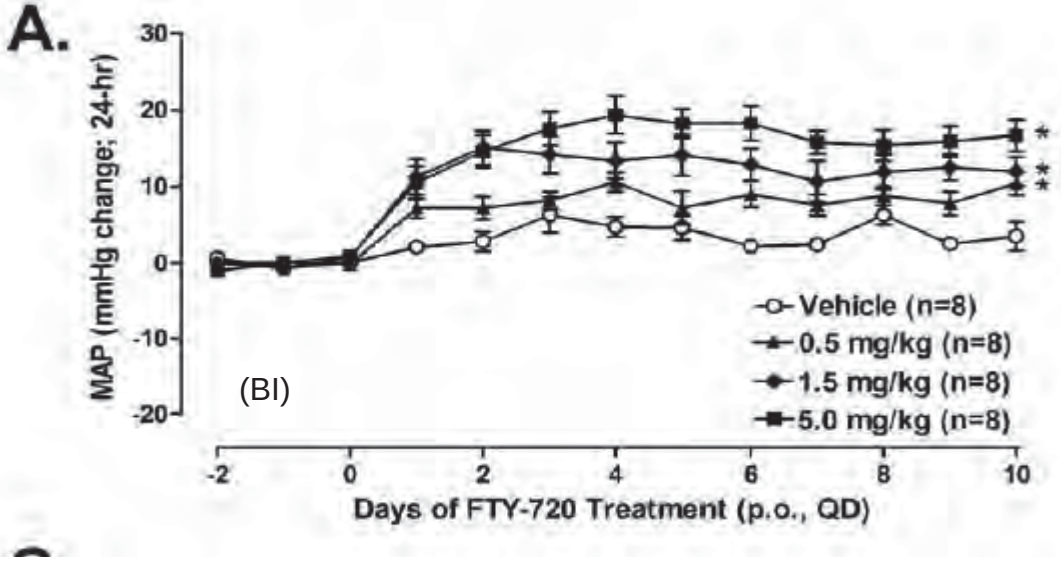




(single dose)

Fryer et al. PLOS ONE 7:1-9 (2012)
Boehringer-Ingelheim (BI)

(repeat dose)



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Mechanism of BP Elevation:
Does FTY-720 Contract Human Vascular Tissue?

- **Study: Evaluate FTY-720P on human subcutaneous arteries**
  - FTY-720P (phosphate) is the active metabolite of FTY720
- **Which S1P receptor subtype is responsible for vascular effect: S1P1 or S1P3?**
  - S1P1: thought to be target for immunosuppressive action
  - S1P3: potential off-target for mediating vascular effects
  - Note: FTY-7290P is a pan-agonist at multiple S1P receptors
- **Are effects of FTY-720P dependent upon endothelium?**
  - Intact
  - denuded

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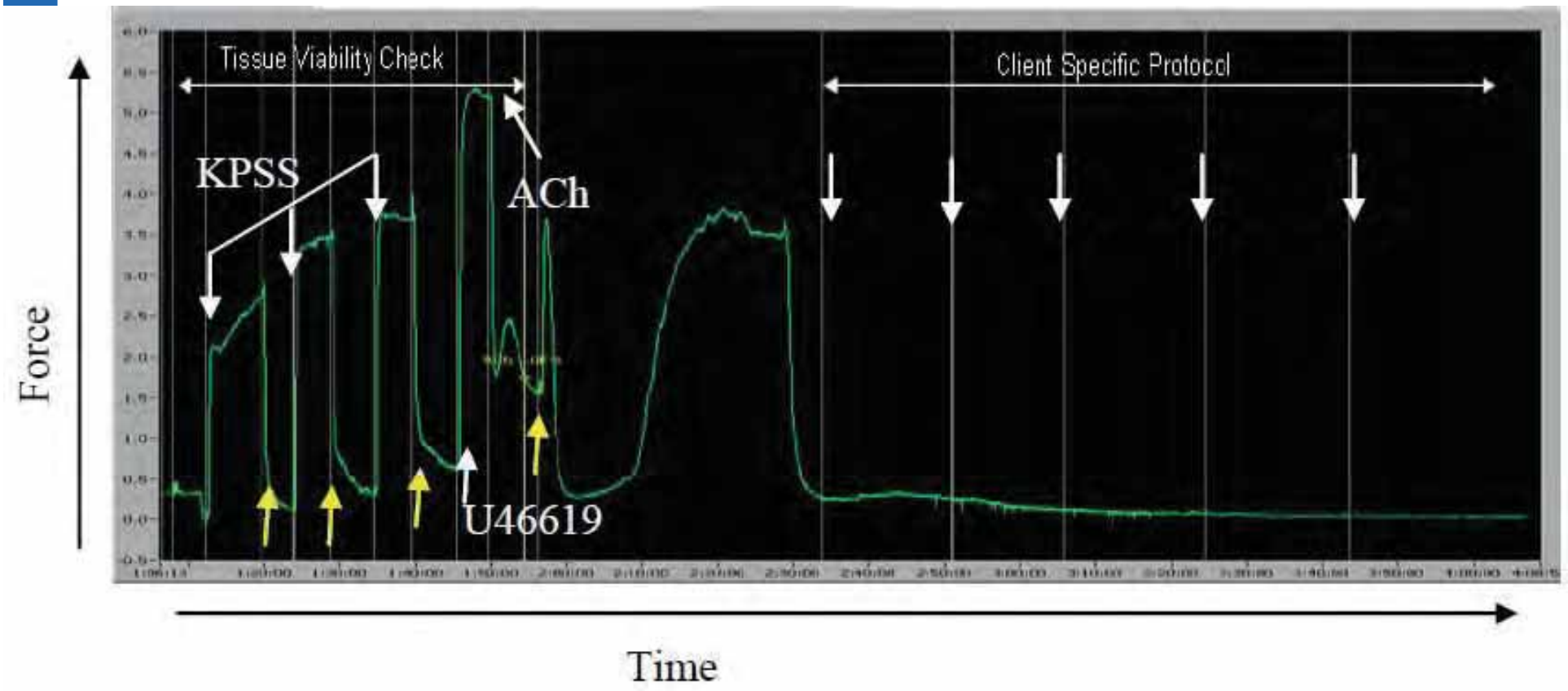
Vasoconstriction Protocol: Example



KPSS --High potassium (62.5 mM) physiological saline solution
U46619 – Thromboxane A2 mimetic (vasoconstrictor)
ACh: acetylcholine (endothelium-dependent vasodilator)

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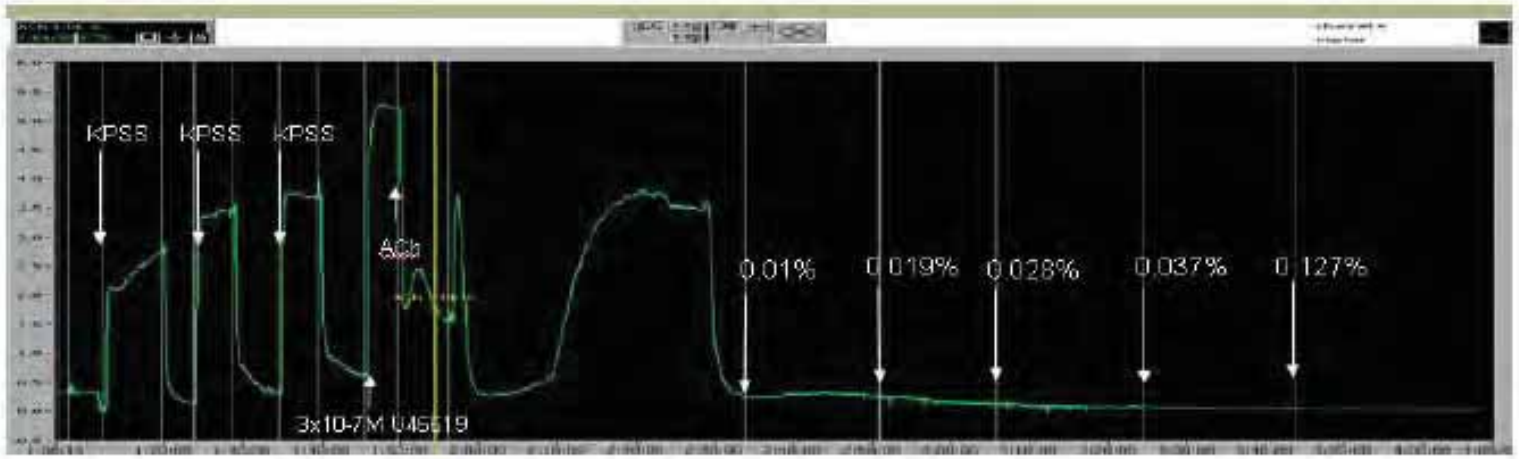
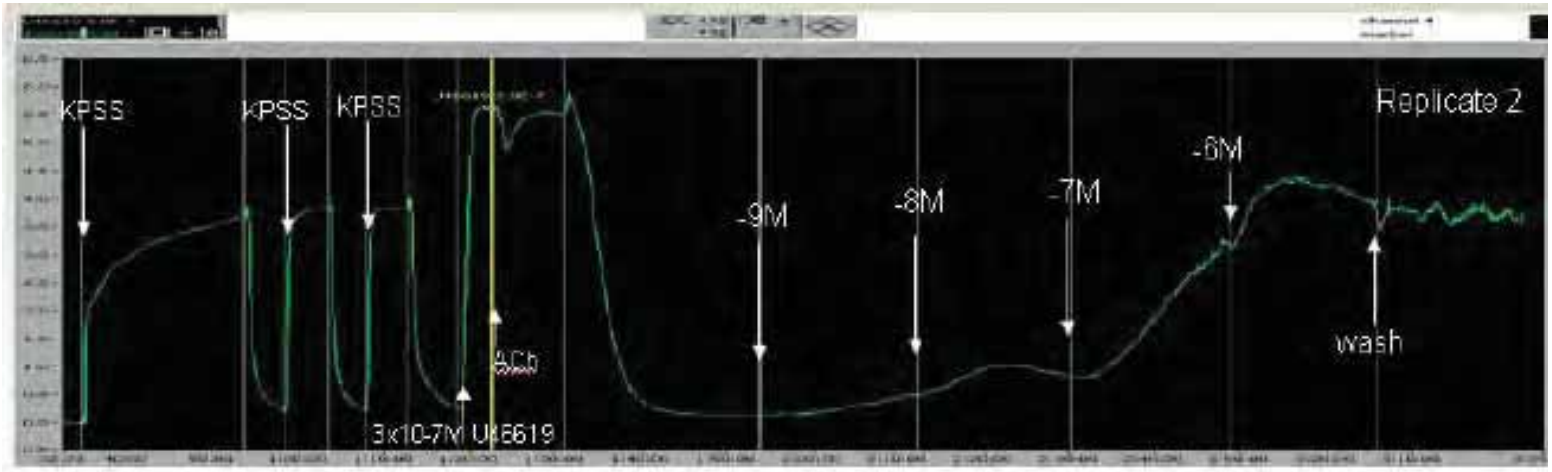
Vasoconstriction Protocol: FTY-720P

DMSO



FTY-720P



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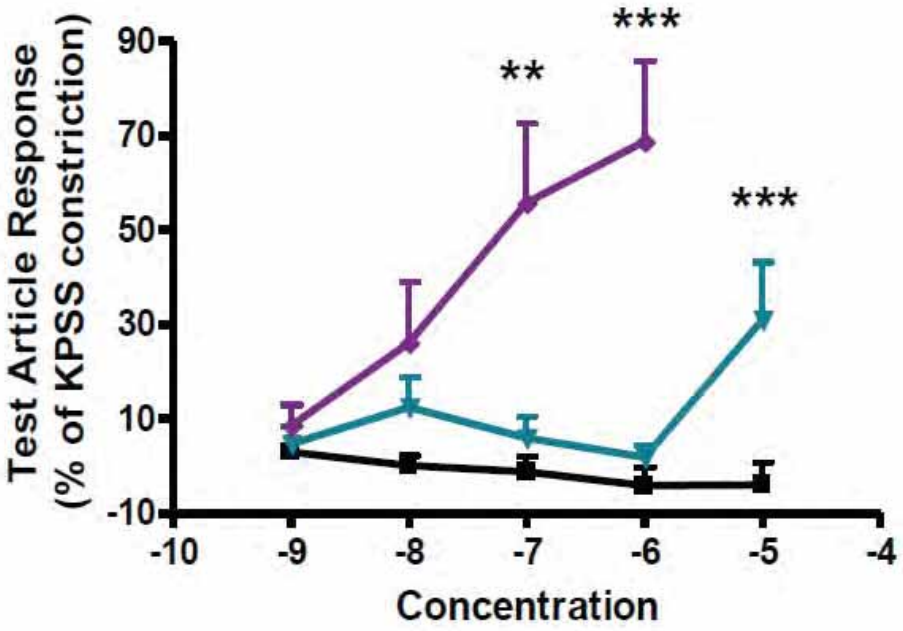
Summary 1: FTY-720P Contracts Human Resistance Arteries



S1P Agonist	hS1P1 (μM)	hS1P3 (μM)
FTY-720P	0.006	0.027
AMG1	0.005	> 25

- FTY-720P: constricted human resistance arteries in a concentration dependent manner
- AMG1: constriction only at 10 uM, but not lower concentrations
  - Conclusion: S1P1 receptors do not evoke vasoconstriction in this human vascular model.

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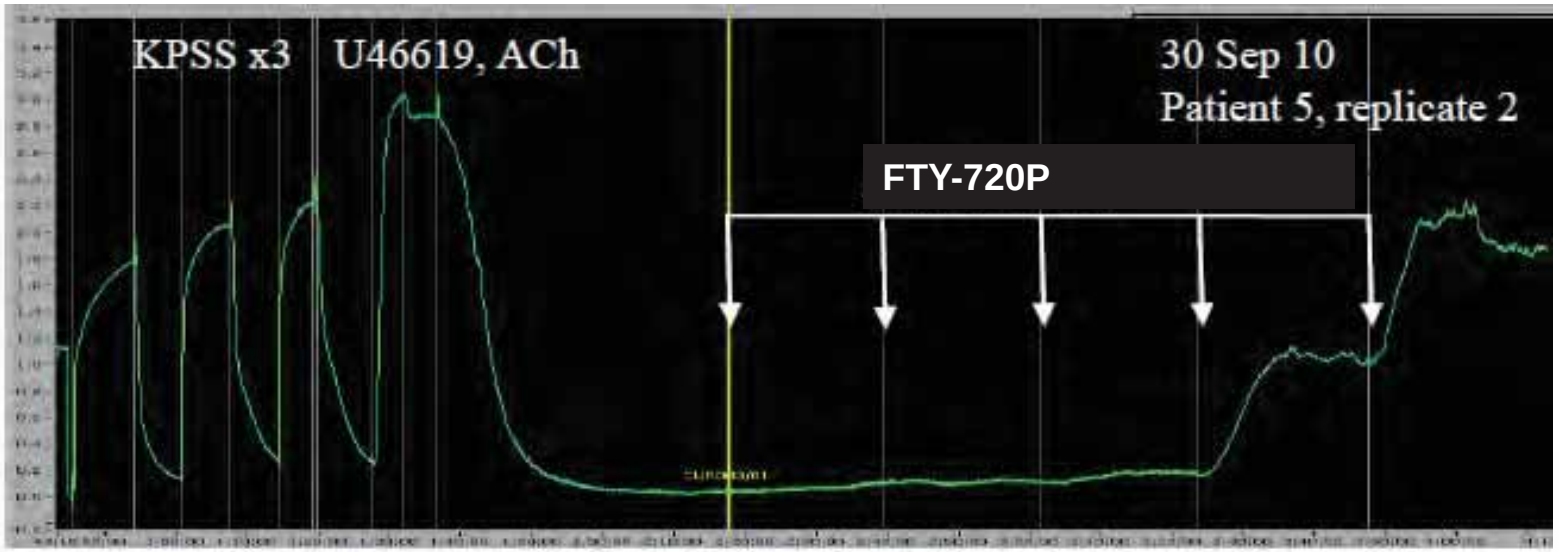
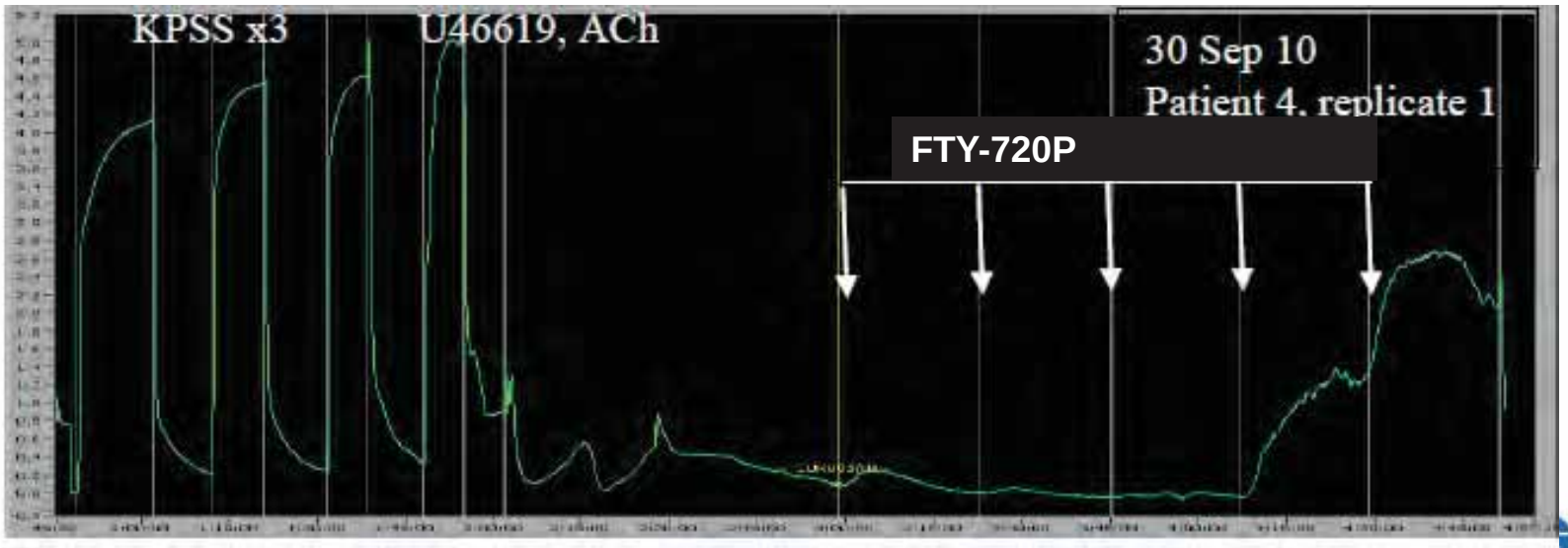
FTY-720P Contracts Endothelium-Intact *and* Denuded Human Resistance Arteries

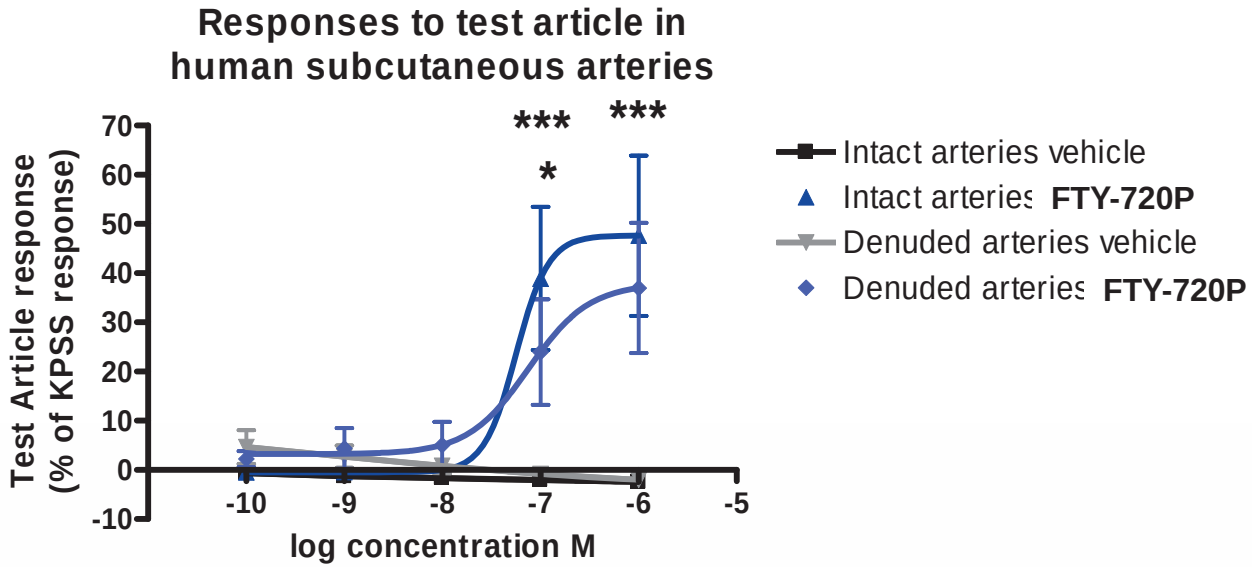
Denuded

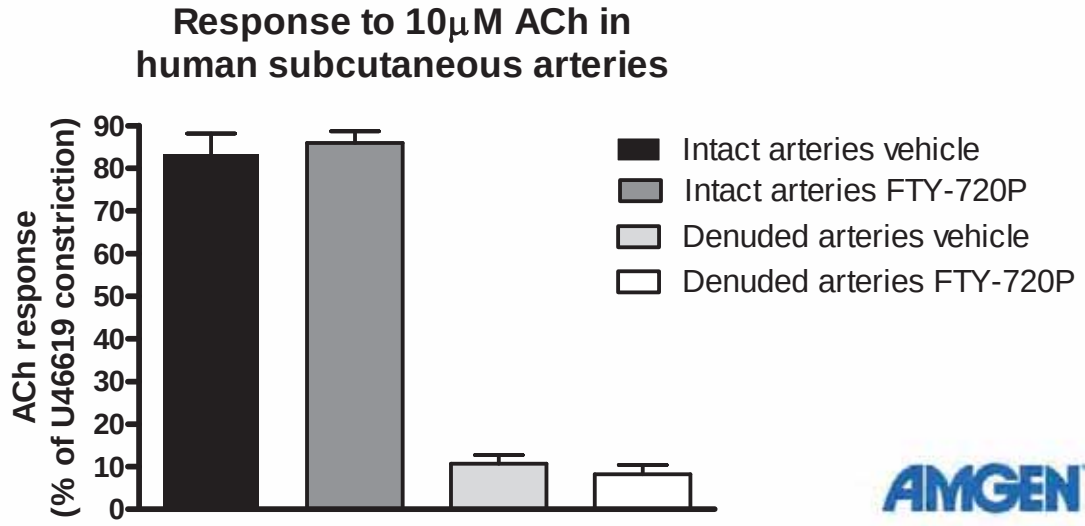


Intact



Summary 2: FTY-720P-induced vasoconstriction does not involve the endothelium





Barriers to Use of Isolated Human Tissues: Dealing with Issues (Real and Perceived)

Practical Consideration (e.g., barrier)	Issue	Comment
Logistics of accessing & using fresh tissue or cells	Y (T)	Procurement, Transport, Shelf-life, etc.
Specific expertise in methods	Y (T)	Requires experienced collaborators
Pharmacological characterization of tissue	Y (T/S)	Vessel dependent (right tissue?) Normal vs Disease tissue Variability of tissue response
Sources of variability: inter-individual versus preparation	Y (S)	Need to quantify effect (endpoint) in population of interest
Lack of relevant information (is there a model in the organ system of interest?)	Y (S)	Is drug target expressed in the available tissue? If not, can the "right tissue" be acquired?
Internal stakeholder acceptance (quality of data; interpretation of findings)	Y (S)	Confidence in model = pharmacological validation; value in decision-making
Operational Factors: Cost and Time (is "the juice" worth "the squeeze"?)	Y (O)	How much time for validation take? And cost? Will impact use of the assay
Regulatory acceptance	Y (R)	Use of data acquired in human *in vitro* surrogate systems, e.g, safety decision making

T: technical; S: scientific; O: operational; R: regulatory

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Conclusions

- **Human Vascular Tissue Preparations: Can Add Value to Risk Assessment of New Drugs**
  - FTY-720P: clinical BP effects studied mechanistically in isolated arteries obtained from human subjects
    - Assay: can be used to differentiate drug candidates

- **Barriers exists and must be overcome to use human tissues**
  - Is there a human-tissue based in vitro model to test the hypothesis?
  - Is it validated for use? If not, how much work is required to establish confidence in the test system, e.g., validation.

- **Need to define confidence needed for safety decision making**
  - Internal: can this new agent move forward into clinical testing?
  - Regulatory: will this new agent be safe in the target population?

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Acknowledgements

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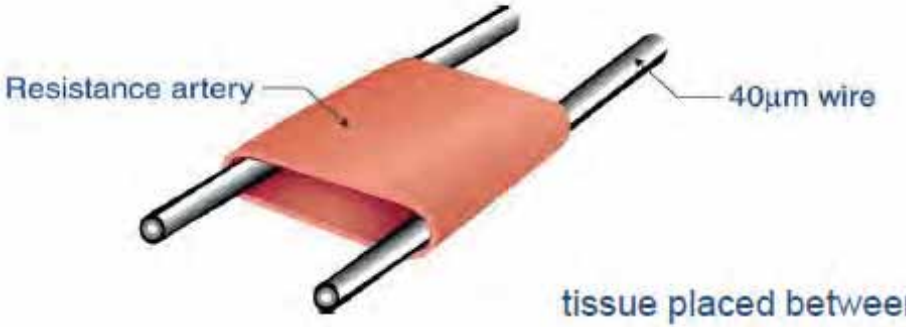
- Yusheng Qu, PhD

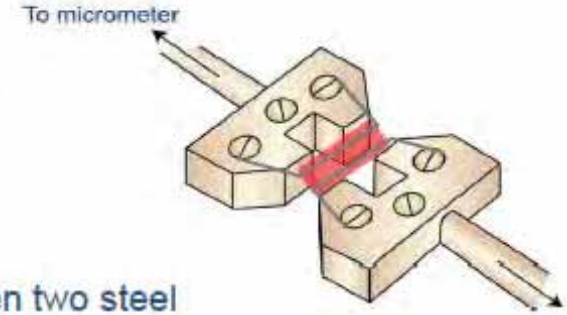
Biopta (UK)

Perceived or real barriers

- Inadequate supply and characterisation of tissues
- Perceived regulatory acceptance
- Practicalities/logistical issues of using fresh tissue/stem cells, e.g. transport, shelf-life
- Obtaining correct ethical consent documentation
- Inter-individual variability
- Lack of relevant information
- Internal stakeholder acceptance
- No appropriate models exist in the organ systems needed
- Lack of specific expertise in human tissue work
- Cost
- Licensing issues with cells
- Others?

Tension Measurement in Resistance Artery





tissue placed between two steel heads and attached to a micrometer and force transducer

biopta
human tissue experts

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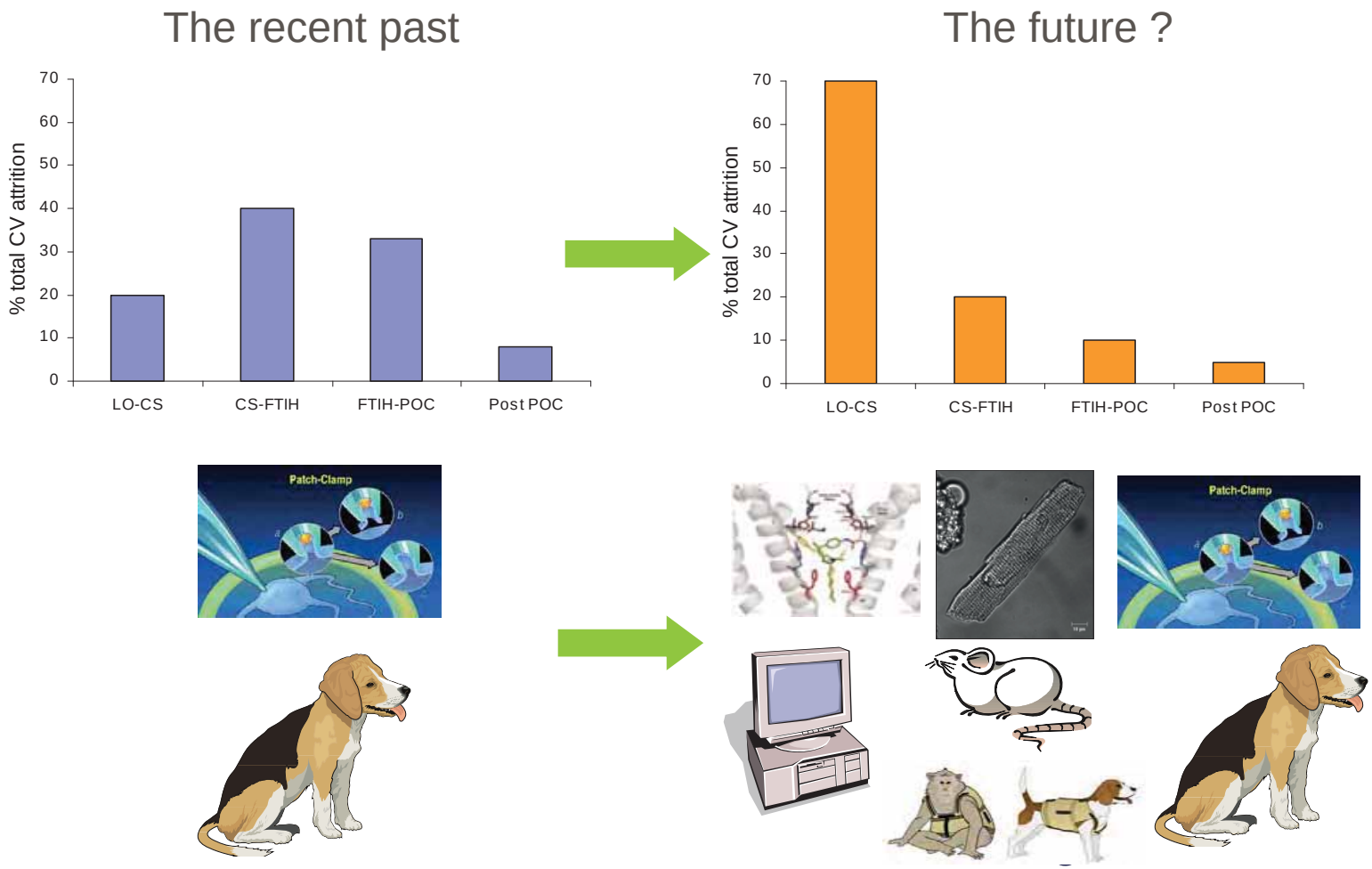
An iPSC cardiomyocyte MEA Model

Dr Jim Louttit

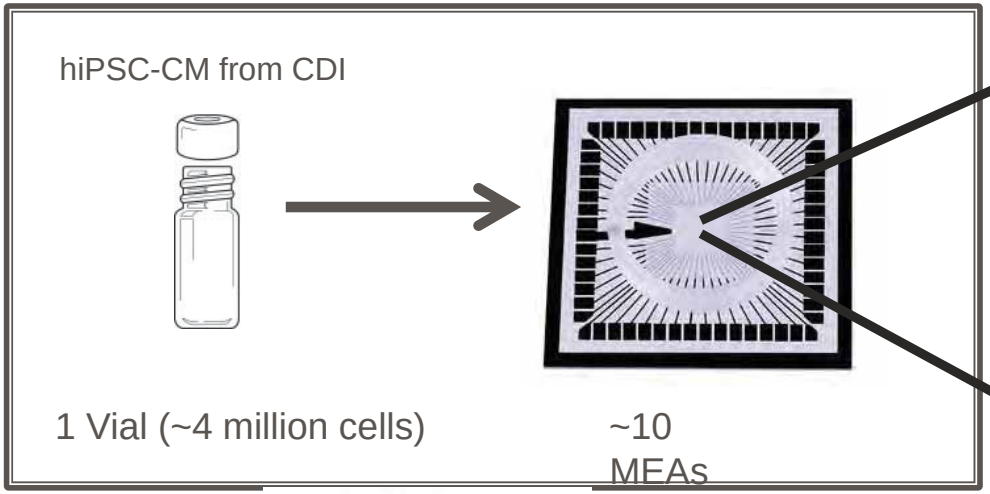
Introduction

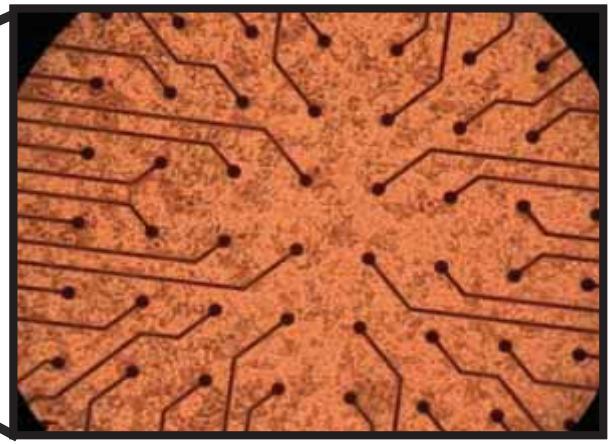
- **Background**
- Functional cardiotoxicity, notably QT prolongation and pro-arrhythmic risk, is a leading cause of drug attrition during development.
- Current pre-clinical cardiovascular electrophysiology safety assays within GSK include manual and automated patch clamp ion channel screening, the rabbit ventricular wedge assay and non-rodent telemetry studies.
- Can we move towards an in vitro system of pro-arrhythmia determination and away from just in vitro and in vivo QT measurement?
- **Approach**
- Develop a model to determine liability of compounds to induce pro-arrhythmia in human iPSC derived cardiomyocytes
- hiPSC-CMs screening offer the following advantages:
  - Reduction in animal use (3R's)
  - Reduced costs
  - May be a better predictor (pending validation and translation).
  - Although hiPSC-CMs have been pharmacologically characterised in multiple studies, there is limited translation of this data to existing pre-clinical assays.

Safety Pharmacology…..Responding to the Challenges of Safety Attrition

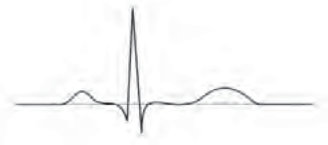


iPSC - Multi Electrode Array Process

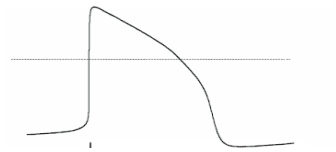




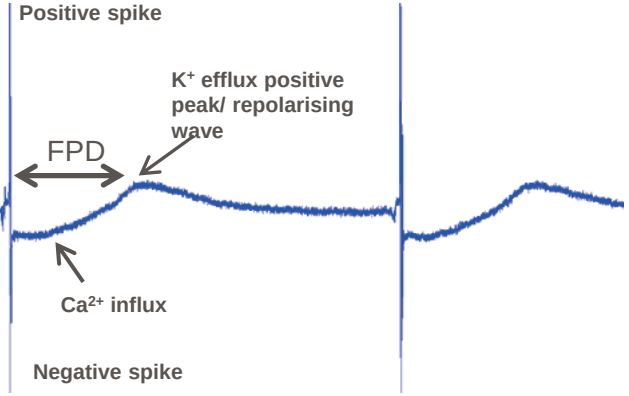
ECG



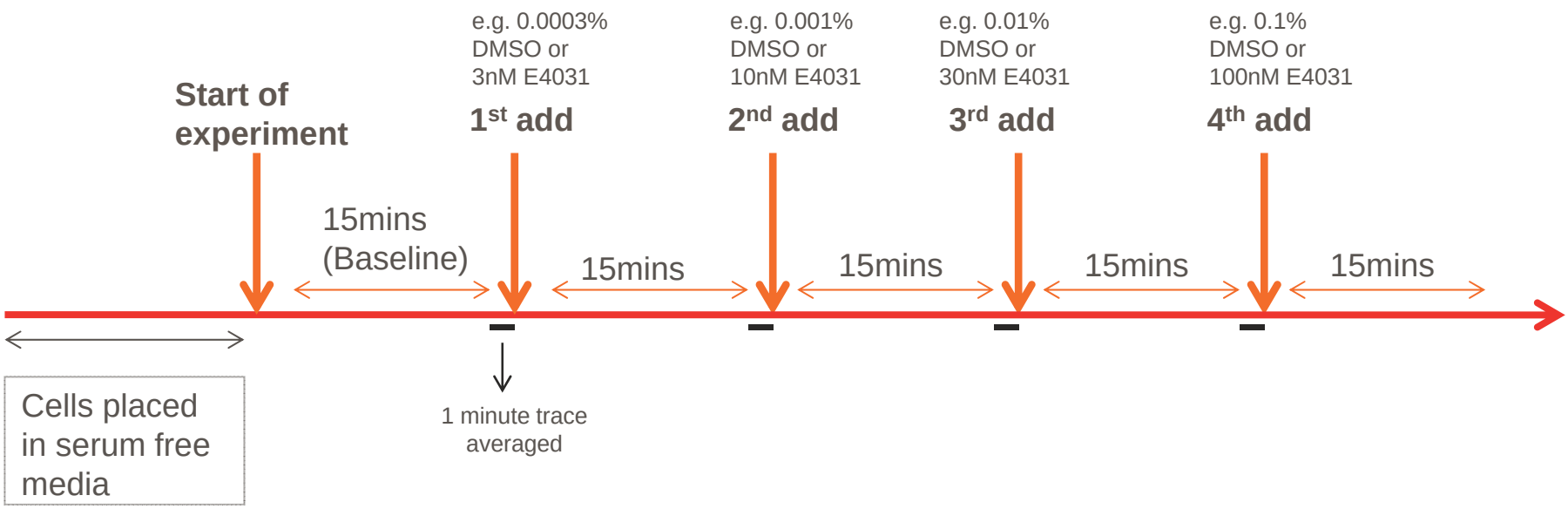
Action potential



Field potential

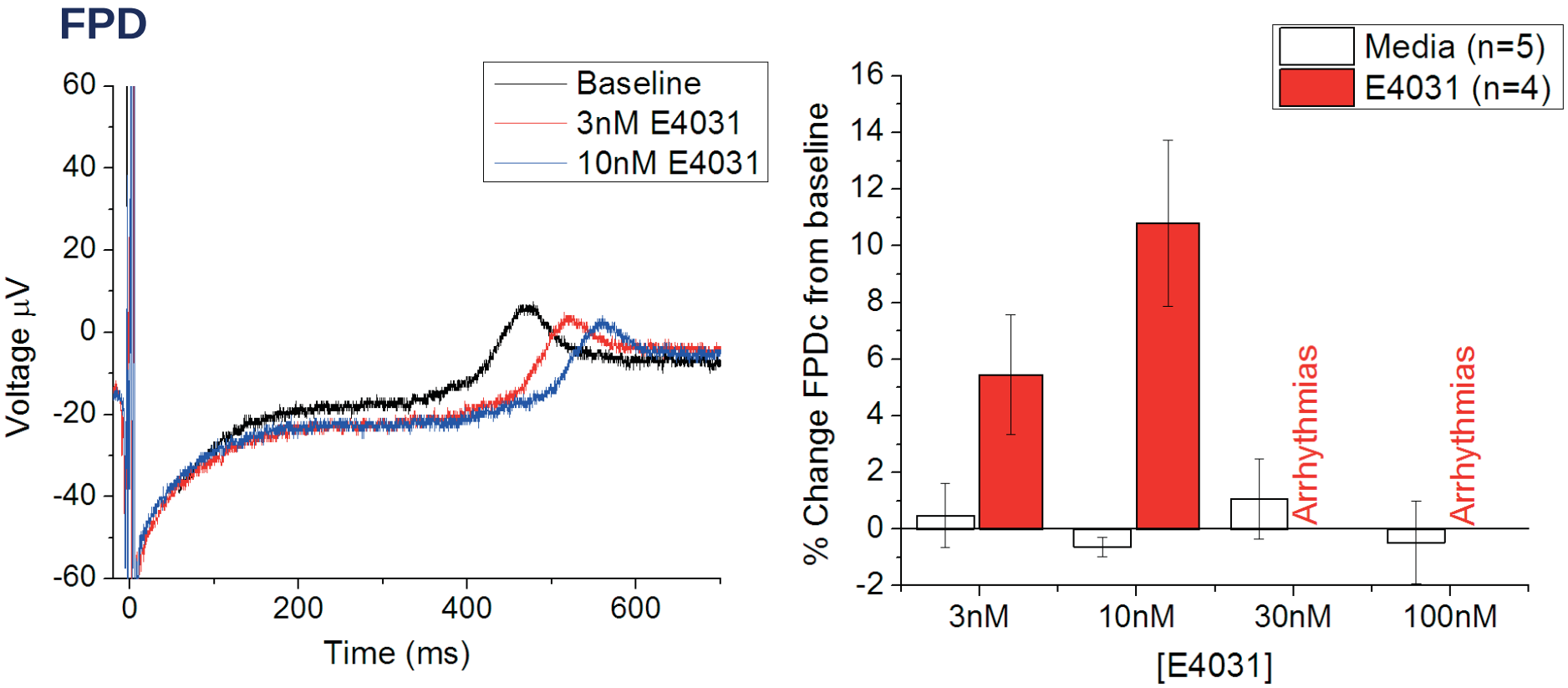


Experimental Protocol

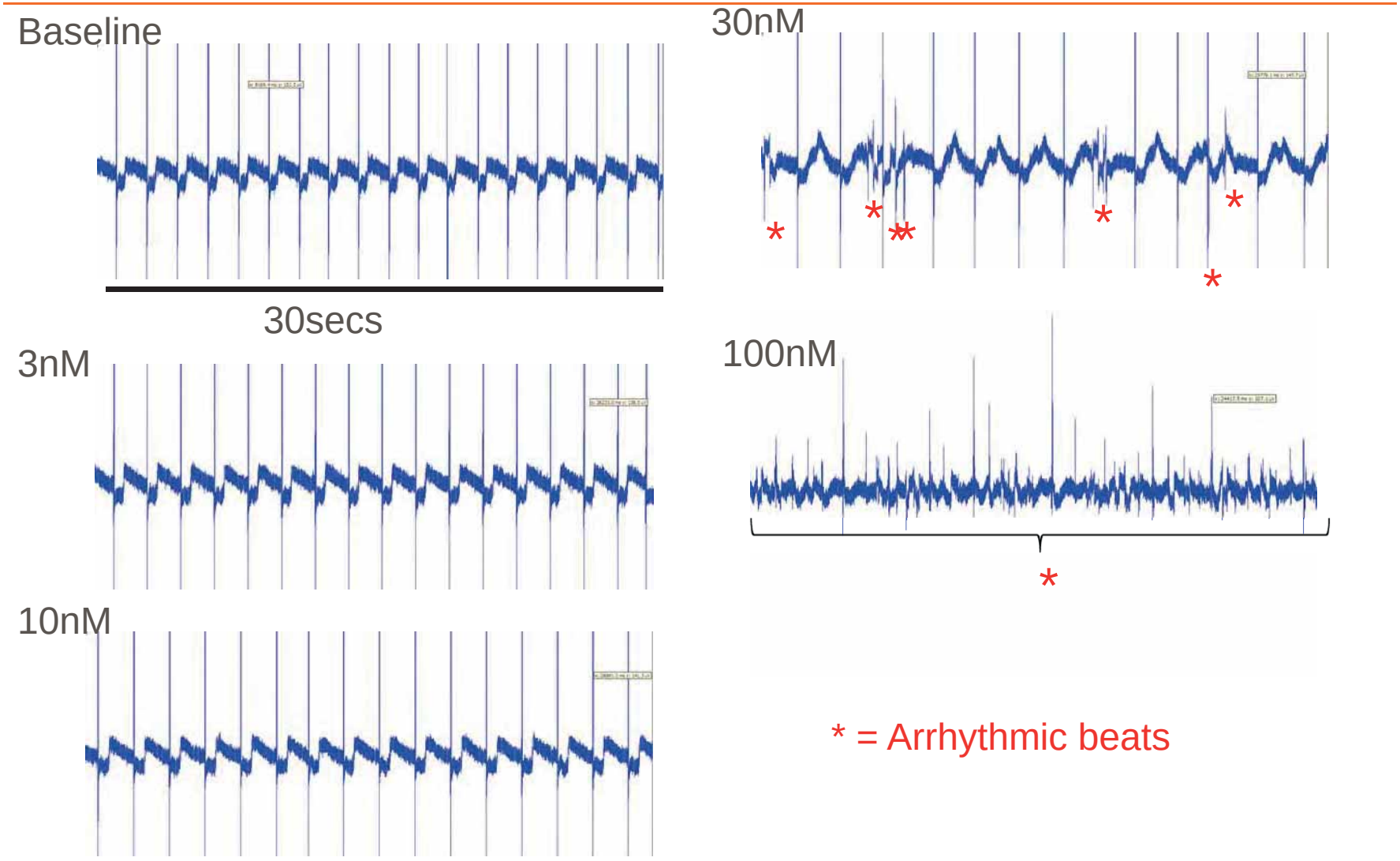


- Data Handling and Analysis
  - Acquired using Multichannel Systems MC_RACK
  - Generates large amounts of raw data
  - Analysed using Neural ID IWS software

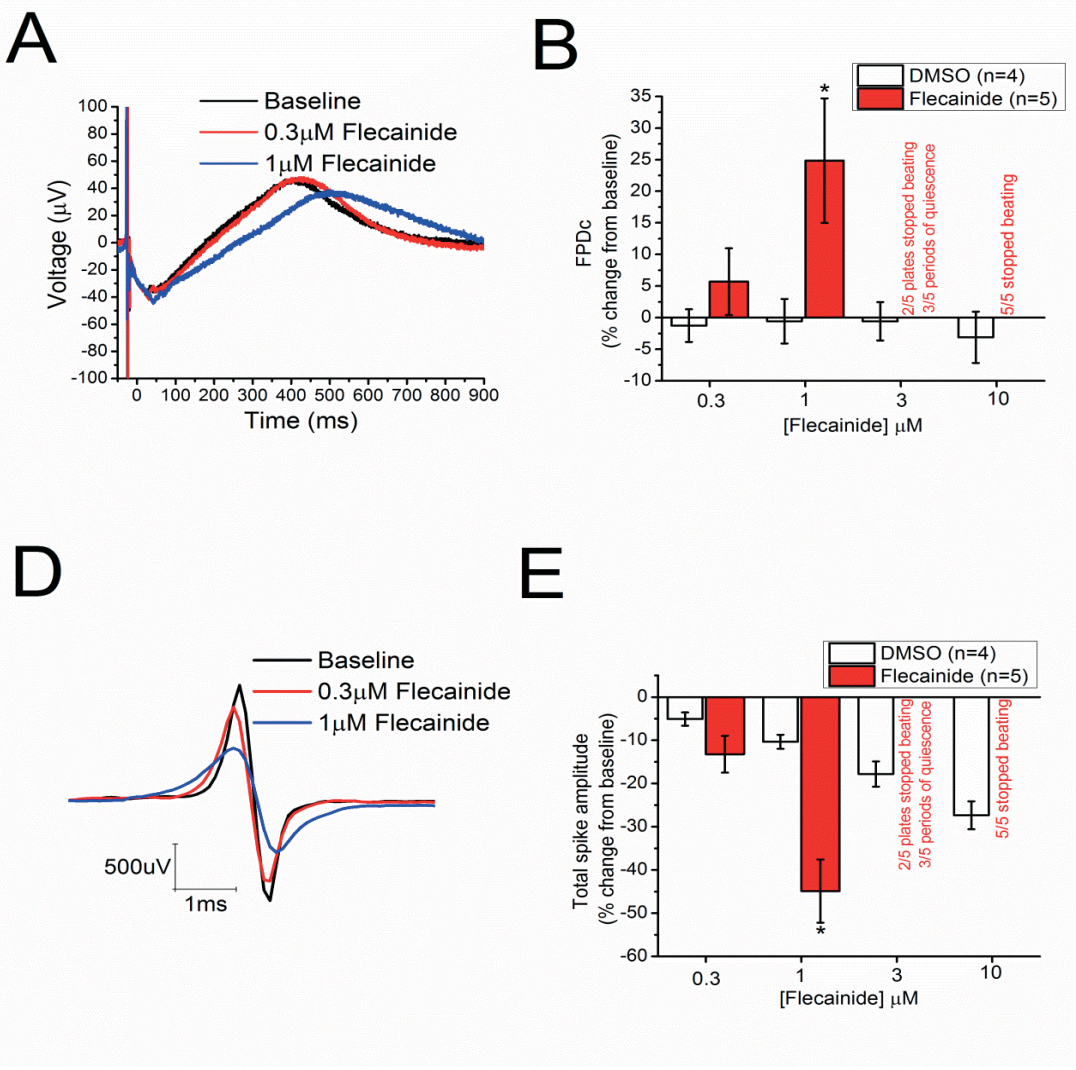
E4031 – Effect on FPD



Raw traces illustrating the effect of E-4031 on the FP and showing the development of arrhythmias at higher concentrations



Flecainide – Effects on FPD and Spike Amplitude



- FPDc prolongation at 1μM (consistent with hERG channel block).
- Decreased total spike amplitude. (Consistent with Nav1.5 block)

hERG:

IC_{25} = 1 uM

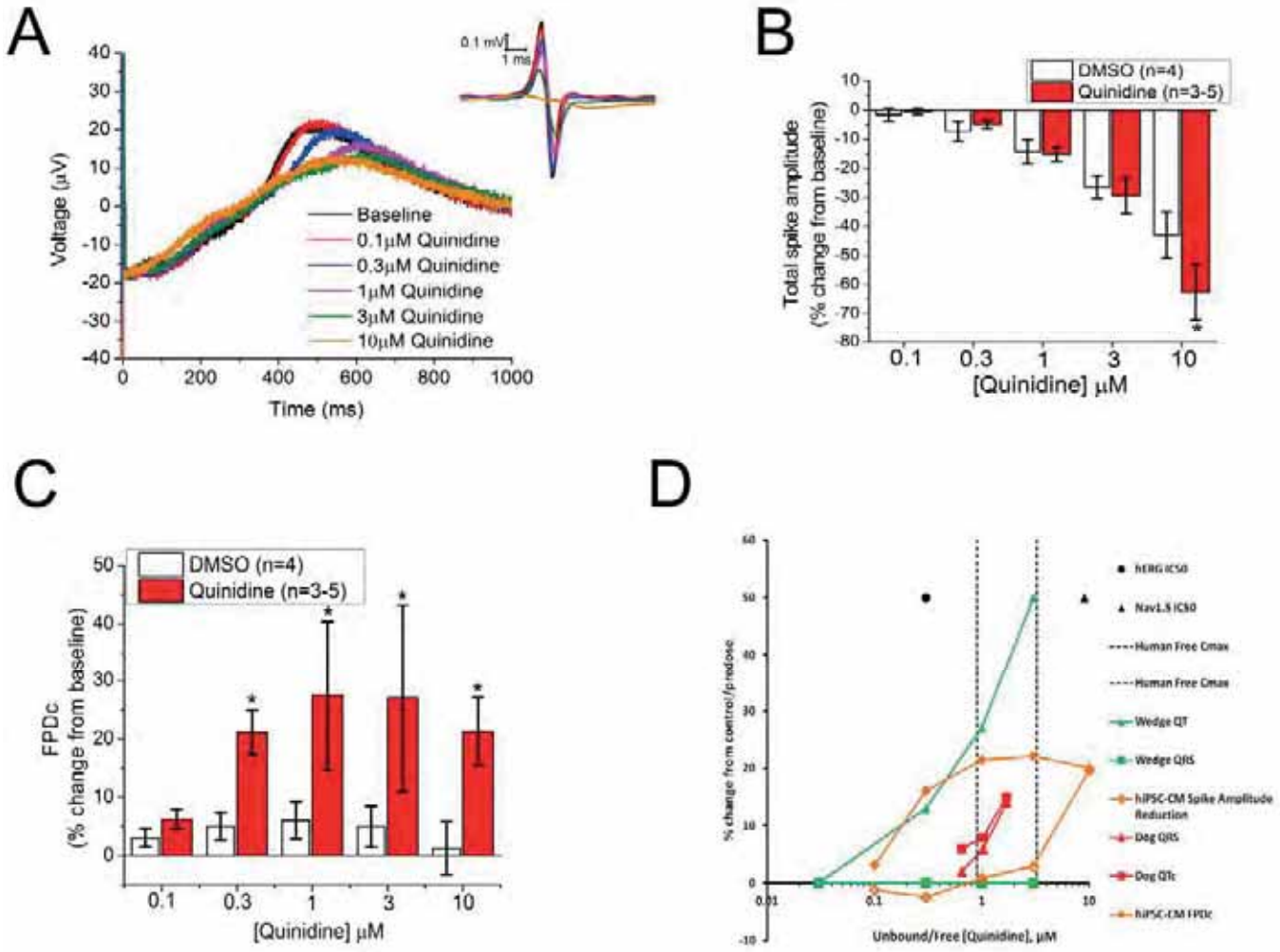
IC_{50} = 3.91 uM

$Na_v1.5$

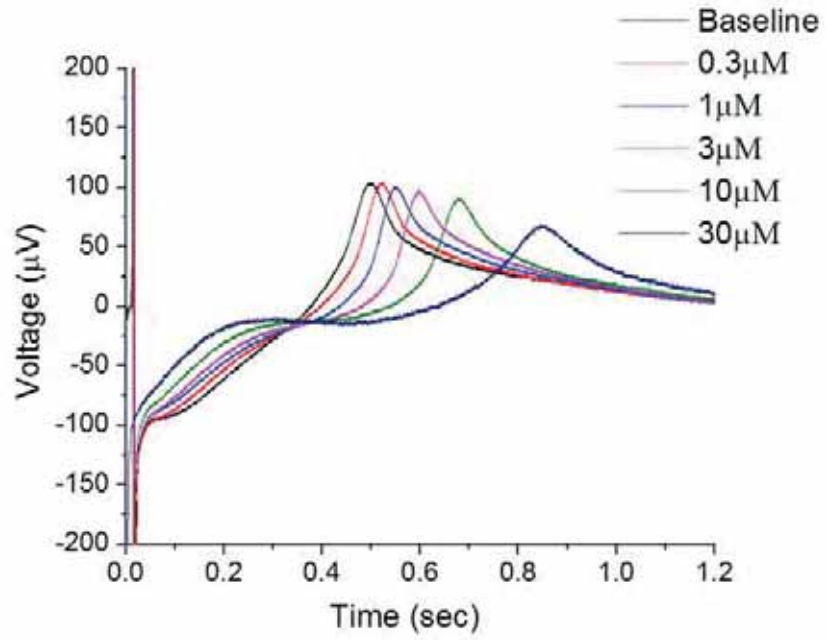
IC_{25} = 3 uM

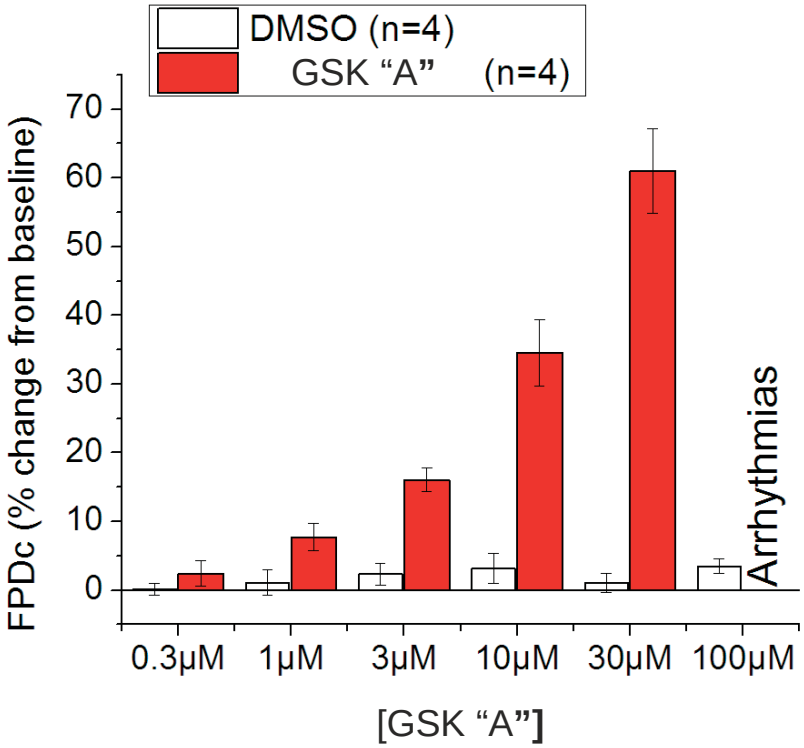
IC_{50} = 10 uM

Effects of Quinidine on FPD and Spike Amplitude

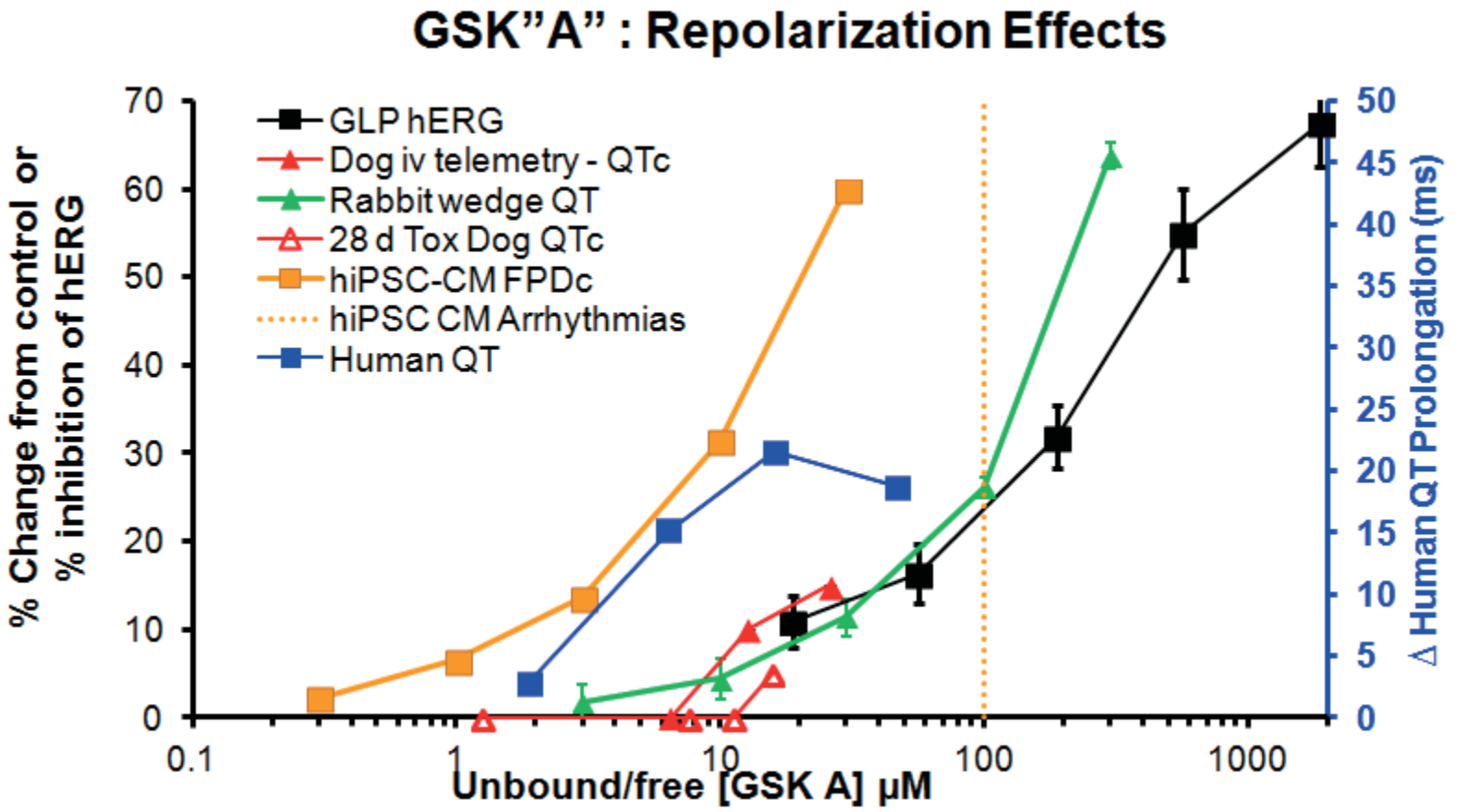


GSK “A” – Effect on FPD





Integrated Risk Assessment for GSK “A”



Comparability of MEA with Rabbit Ventricular Wedge

Concentration at Which a Statistically Significant Change in QT/QRS Was Seen in the Wedge Compared With FPDc/Spike Amplitude change in the hiPSC-CM MEA Assay

Compound	Concentration for $p < .05$ in the rabbit wedge	Concentration for $p < .05$ in the MEA	MEA/wedge fold difference (Log units)
Nifedipine repolarization	0.03	0.03	0
Cisapride repolarization	0.1	0.1	0
Terfenadine repolarization	1	0.1	−1[a]
Terfenadine conduction	10	1	−1[a]
Verapamil repolarization	1	0.03	−1.52[a]
Flecainide conduction	3	1	−0.47[a]
Flecainide repolarization	3	1	−0.47[a]
Quinidine repolarization	0.1	0.3	0.47[b]
GSK A repolarization	10	1	−1[a]
GSK B repolarization	0.6	n/a due to beat rate changes	n/a

Note. The logarithmic scale difference is shown in the last column. In most cases, MEA fell within half a logarithmic unit of the wedge data and in certain cases was better predictor of an effect. 0, no difference; n/a, not applicable.

[a]MEA > Wedge.

[b]Wedge > MEA.

MEA as good or better than RVW

MEA worse than RVW

– Current Validation Set
  – Now have 20 compounds, standards, active and inactive in house compounds
  – Greater focus on inactive and weakly active compounds and physiological interventions

– Harris, et al. (2013) Tox. Sci.;134(2):412-426

Perceived or real barriers I

- Internal stakeholder acceptance
  - Inherently conservative environment
    - MEA models generate less data than in vitro ventricular wedge models (no data for dispersion of refractoriness, currently no contractility data)
    - Projects with pre-existing data for groups of compounds in chemical series
  - Limited validation datasets
    - Validation sets focus largely on “potent” ion channel blockers
    - Published work with weak inhibitors/inactives is very limited
  - Experience with current models
    - Current models have been in place for 6-7 years with large database of standard and test compounds (~350 compounds)
    - Translation data available from in vitro to in vivo models
  - Sensitivity of model
    - Good comparability for FPD/QT prolongation within limited datasets
    - Much higher incidence of arrhythmias in iPSC-CMs than in other models

Perceived or real barriers II

- Internal stakeholder acceptance
  - Differences between iPSC and ventricular myocyte phenotypes
    - iPSC-CMs are a mixed population of pacemaker, atrial and ventricular like cells
    - Focus has previously been on ventricular preps only
    - iPSC-CM have an immature phenotype
      - Ion channel populations differ between iPSC-CMs and mature ventricular myocytes
- Practicalities/logistical issues of using fresh tissue/stem cells, e.g. transport, shelf-life
- Inter-individual variability
  - Batch to batch variability
  - Acceptance criteria
  - Screening against 1 individual/limited population
- Cost
  - Research based MEA systems do not represent a financial saving over current models
  - Move to “high” throughput assays

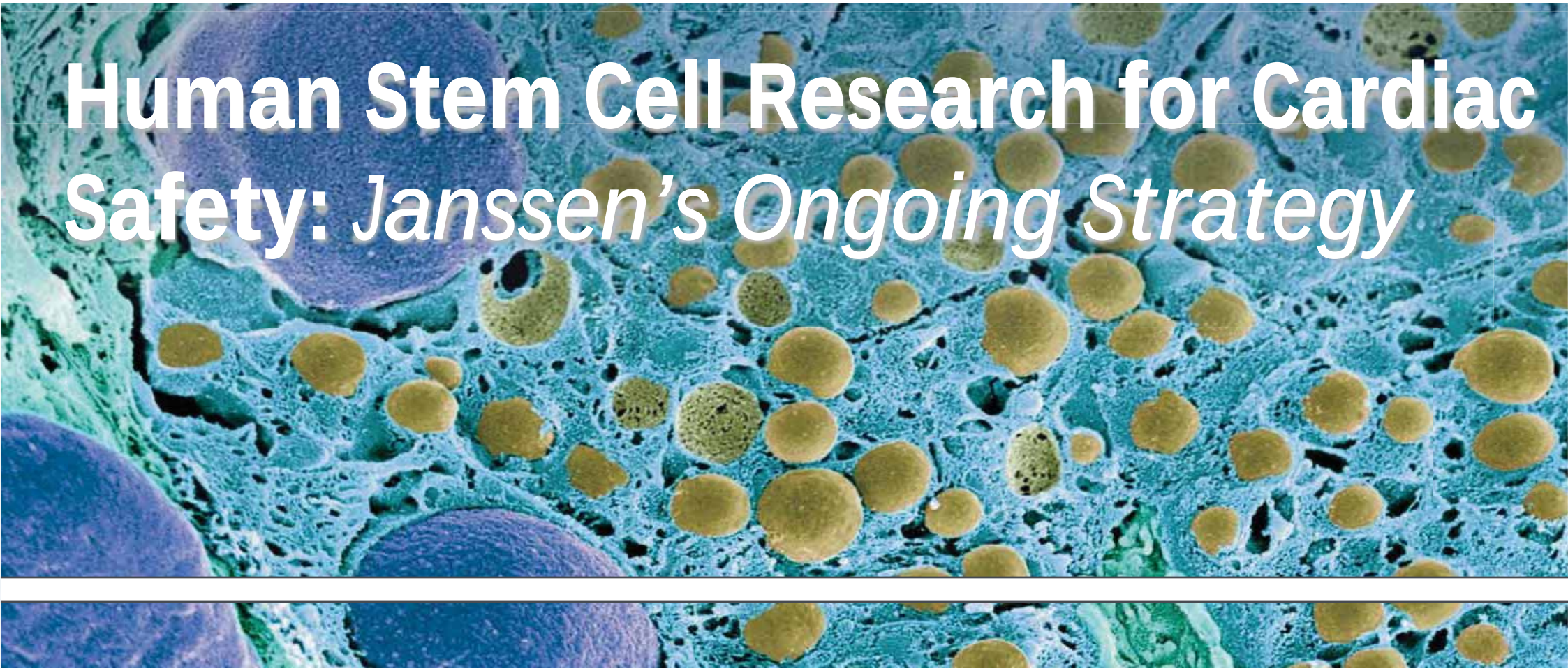
CiPA – An evolving landscape?

Rechanneling the Current Cardiac Risk Paradigm: Arrhythmia Risk Assessment During Drug Development Without the Thorough QT Study
FDA White Oak Facility July 23, 2013

Assay component	Description
Voltage clamp studies	Detailed analysis of a compound's effects on five or more functional cardiac ion channel currents, including HERG, Na1.5, CaV1.2, KvLQT1 and Kir2.1
Stem cell-derived cardiomyocytes	Cell-based studies that look at the repolarization effects of a compound on fully functional adult human ventricular cardiomyocytes
Computational modeling of cardiomyocytes	Reconstruction of a compound's effects on the electrical activity of a human cardiomyocyte. The model takes as input data from the voltage clamp and cell studies and outputs a prediction of a compound's early afterdepolarization (EAD) and/or action potential duration (APD), both of which predict the risk of a compound triggering cardiac arrhythmias such as TdP.

Acknowledgments

– GSK Safety Pharmacology
– Arun Sridhar, Kate Harris, Sara Graham, Mike Aylott, Yi Cui, Nick McMahon,



Human Stem Cell Research for Cardiac Safety: *Janssen's Ongoing Strategy*

David Gallacher, PhD

Global Head of Safety Pharmacology, Preclinical Development & Safety, Discovery Sciences, Janssen R&D

NC3Rs Workshop: 'Overcoming the barriers to wider uptake of human tissues for safety assessment'

July 15th, 2014

janssen

Agenda

I. The Janssen strategic approach
II. mRNA-gene expression on hIPS/hES-CMS
III. HTS Ca^{2+} transients : 3 cell providers & 60 reference compounds
IV. Multi-Electrode Arrays (MEAs): 4 cell providers & 20 reference compounds
V. Effects of 20 compounds in Optical action potentials (iCELLs)-CellOPTIQ system
VI. Comparison between Ca^{2+} transient, MEAs, Optical APD & the isolated rabbit wedge: 20 reference compounds
VII. Conclusions

I. The Janssen strategic approach

Our Stem Cell History

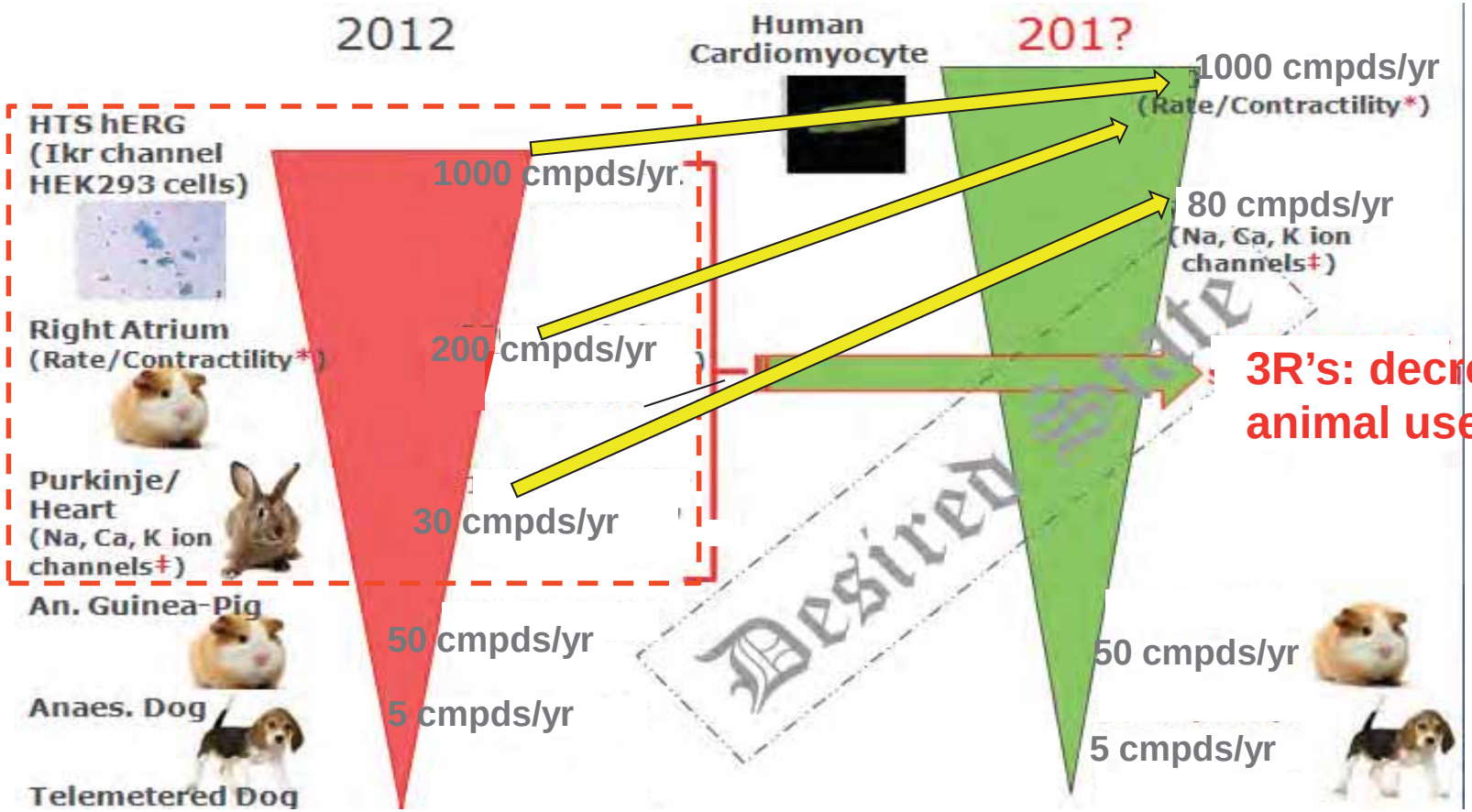
Companies/institutes engaged with…

- VivoMedica/Midas MediScience *(rat (2005-9), human (2009-10))*
- Axiogenesis *(mouse (2008), now in human)*
- ReproCell *(human (2009/2012))*
- Leiden University *(now Pluriomics) (human from 2009)*
- ACEA (2012: xCELLigence) and Axiogenesis *(2012: human iPS-CMs)*
- *2012-14: many others: CDI, NMI, Vala Sciences, Clyde Bioscience, Oxford university, Israel Institute of Technology, Cellectis……..*

Our initial "*Wish-list*" for a viable system: 2005

1. Human cells (ventricular)
   - Possibly other cells (atrial, pacemaker, etc) ??
2. Scientifically validated & accepted
3. Possibility of "disease models" in iPSC's *(e.g., LQT1, LQT2)*
4. Fast, reproducible, quantitative, easy higher throughput?
   a) Reasonable throughput for 'in depth" MEA studies
   b) HTS for Ca^{2+} influx-dye technologies
   c) Reasonable throughput for xCelligence technologies
5. Picks up *othe*r cardiotoxic effects/cytotoxic *(e.g., troponin, FABP3….)*
6. Replace currently used models *(i.e. binding, patch-express, isolated g-p right atrium, isolated cardiac tissue/heart etc..)*
7. Accepted by Regulators (FDA) -July 2013-CIPA ? ?

Cardiovascular Safety Goals: Potential replacement of preclinical in vitro work with hIPS/ES –CMs platforms



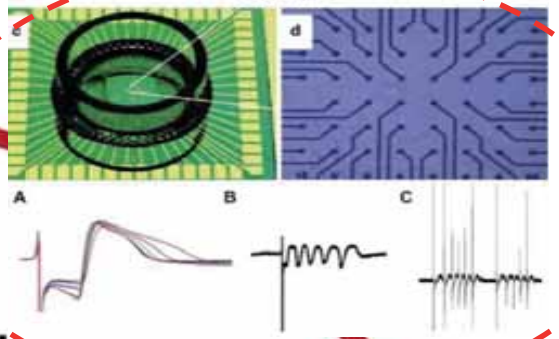
Stem cell technologies?

3R's: decreased animal use and cost

The Promise of Stem Cell Research

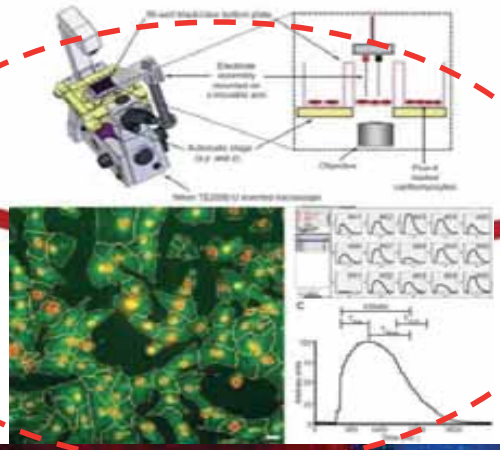
Technologies to be used in hiP5/ES-CMs

1. MEAs

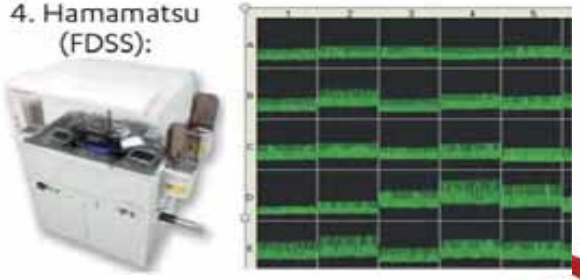


2. XCelligence

3. Kinetic Image Cytometry (Vala)

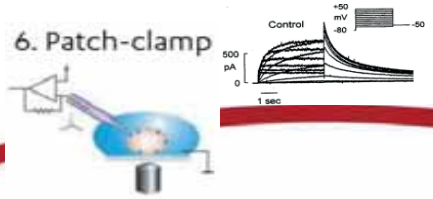


4. Hamamatsu (FDSS):



5. CellOPTIQ instrument (Clyde Bio):

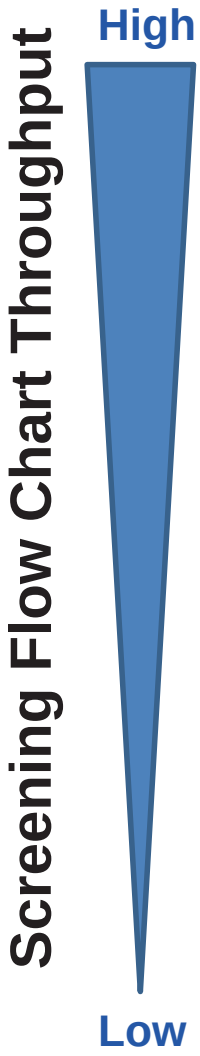
6. Patch-clamp



Drug Testing on Human Heart Cells

mRNA profile

Validation Strategy
J&J Corporate ITS Funding (2012-14)



1. Human Cardiomyocyte (iPSc/ES) – Ca^{2+}transients — ~1000 cmpds/yr (Rate/Contractility Index)

2. MEA –FP *vs* Voltage Sensing — ~80 cmpds/yr ePhys

3. Human ***Native*** Cardiomyocyte — ~5 cmpds/yr ePhys

4. Human ***in silico***

External *versus* Internal Technology

60 drugs -various MOA validation:
Valasciences *vs* Internal FDSS

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20 drugs of various MOA validation:
MEA *vs* Clyde Biosciences vs wedge

NMI achieving results

multichannel systems

CLYDE BIOSCIENCES

5 drugs of various MOA validation:
Patchclamp Study

5 compound of various MOA validation:
In silico

UNIVERSITY OF OXFORD
DEPARTMENT OF COMPUTER SCIENCE

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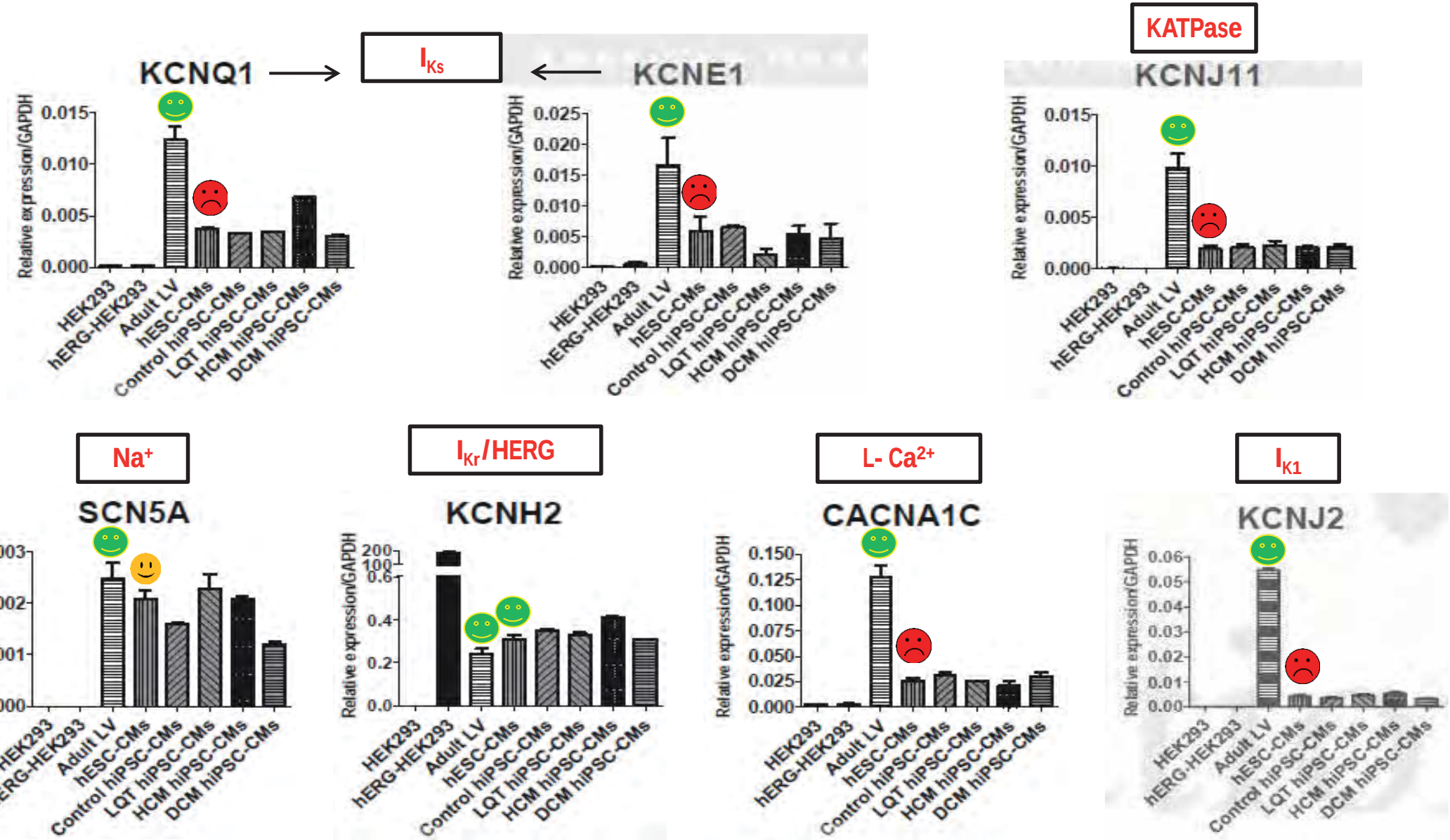
II. mRNA-gene expression on hIPS/hES-CMS:

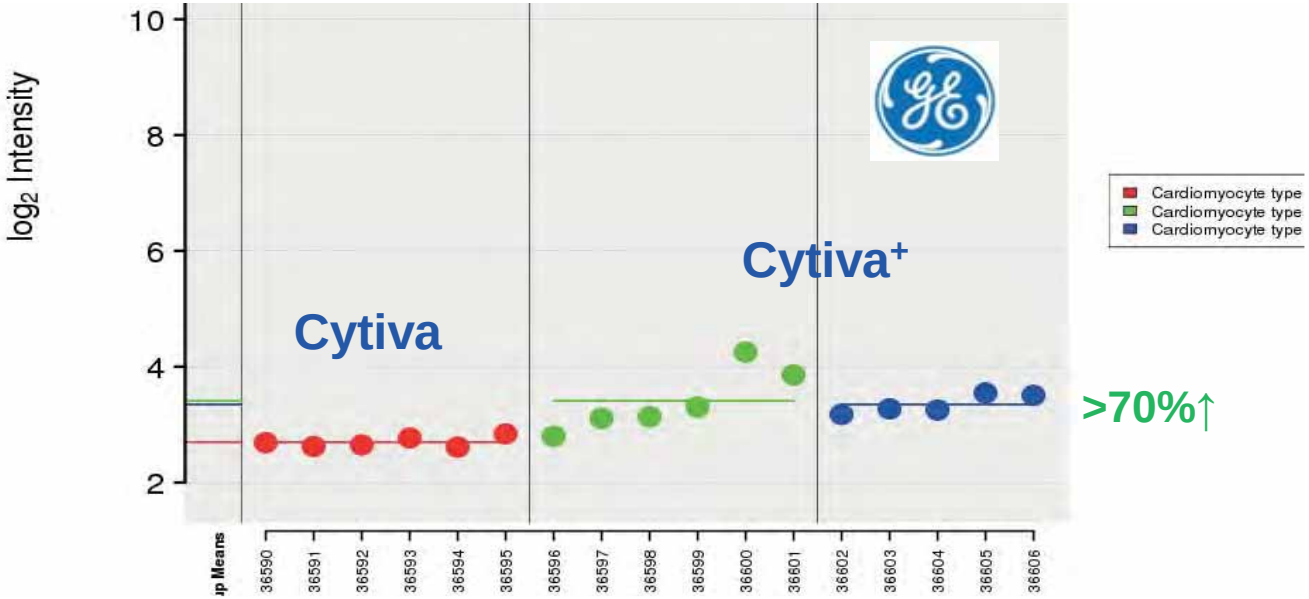
Do cells express genes of cardiac ionic channels?

Dr. Liang (*Circulation; 127(16), 2013*) shows that gene expression of some cardiac ionic channels are different between adult CMs and hiPS/ES-CMs

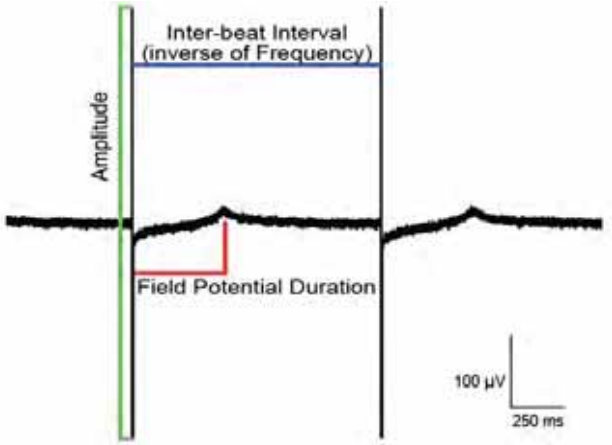


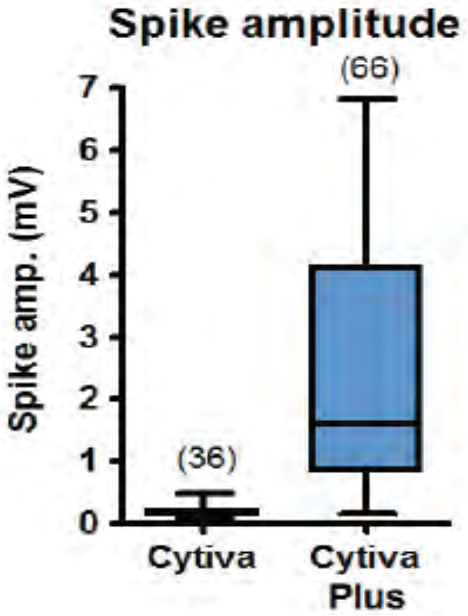
SCN5A (TTX-fast Na^+ channel): higher ion channel expression with higher Na^+ peak in MEA – *(Improvements made by GE)*

SCN5A: Na^+ channel



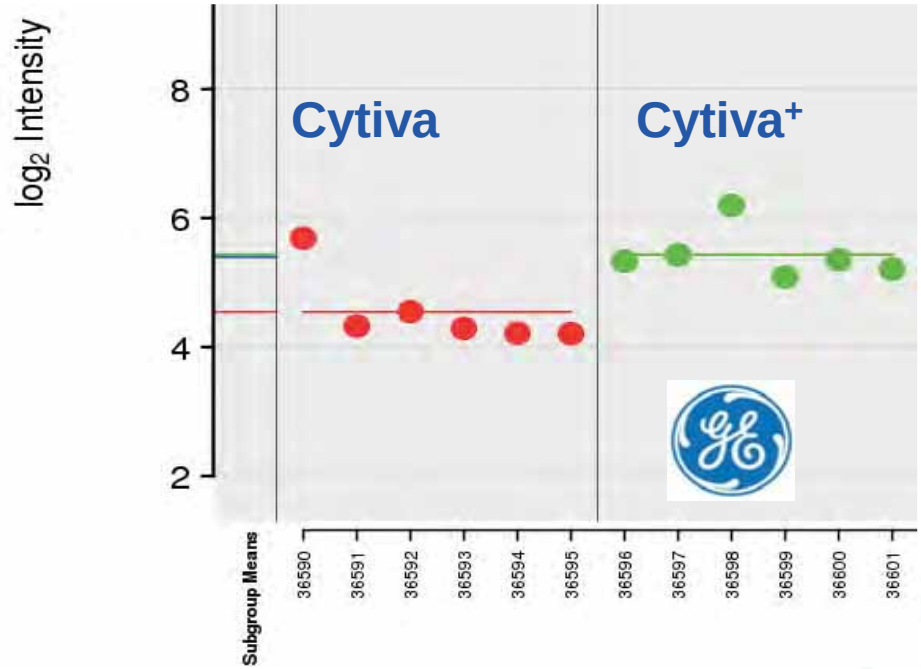
>70%↑



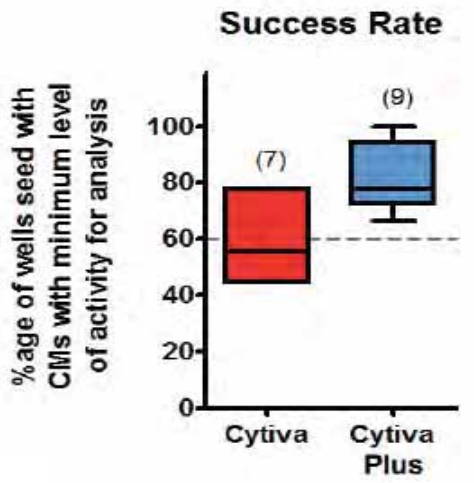


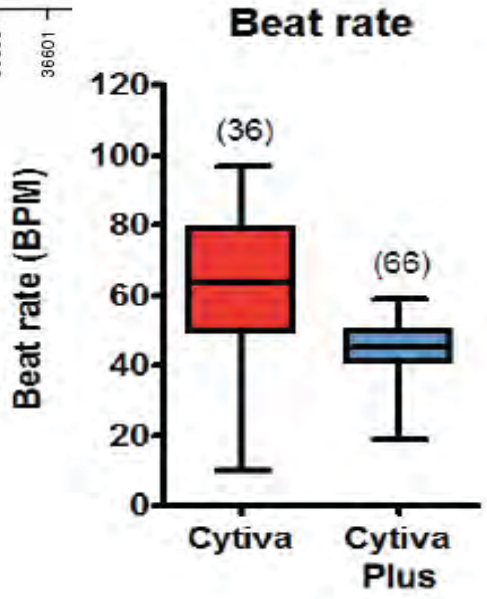
>70% ↑ in Na^+ Expression

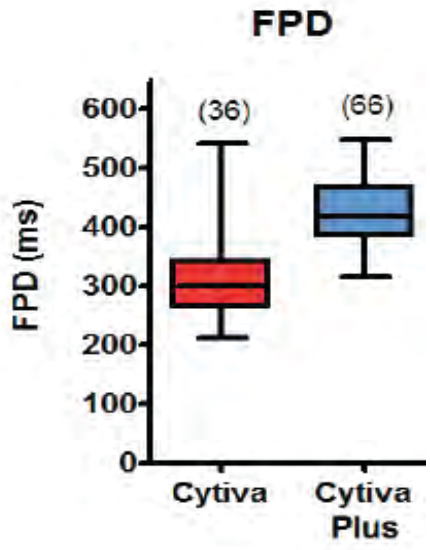
HCN4 (pacemaker channel): higher ion channel expression and better cardiac function – *(Improvements made by GE)*



>100% ↑



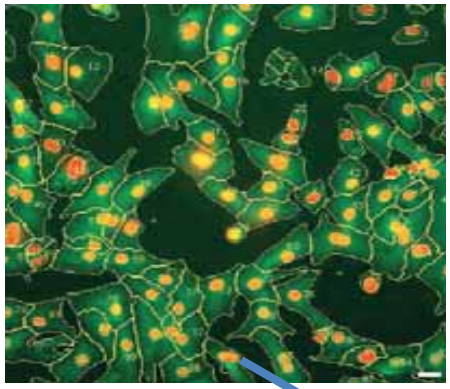


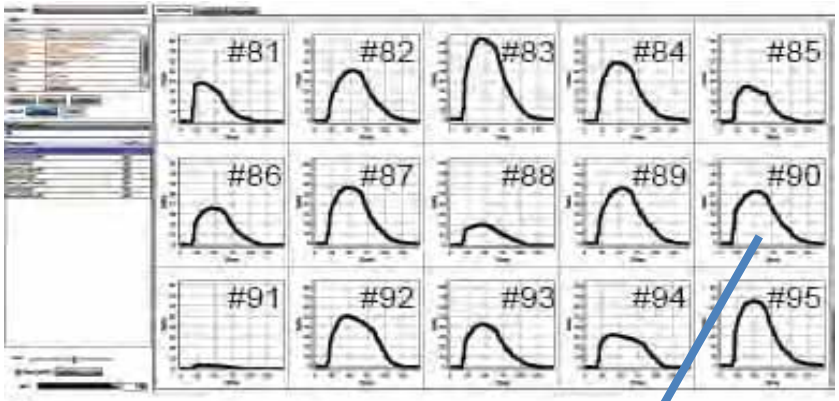


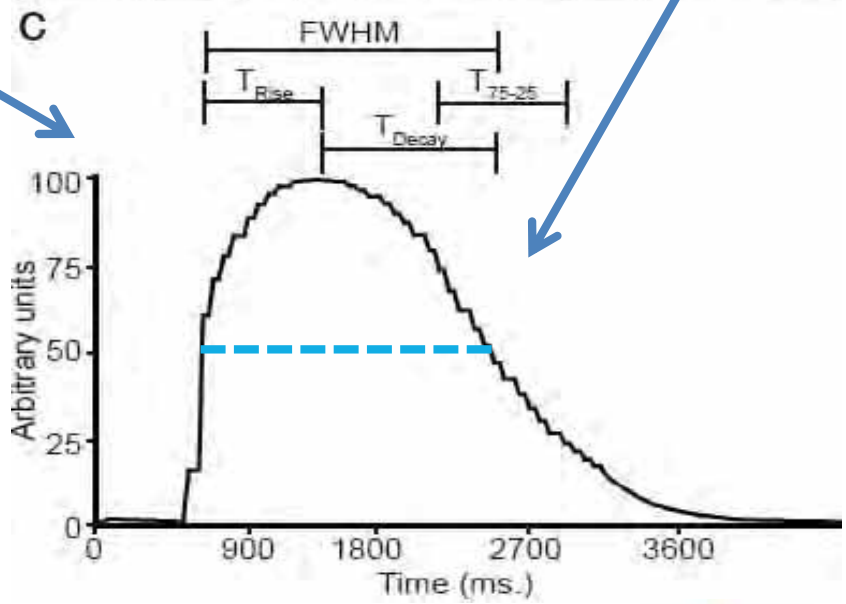
III. Ca^{2+} transients HTS: 3 cell providers & 60 reference compounds

KIC Cyteseer® Calcium transient: Full Width Half-Maximum (FWHM) ≈ CTD50 ≈ QT-interval

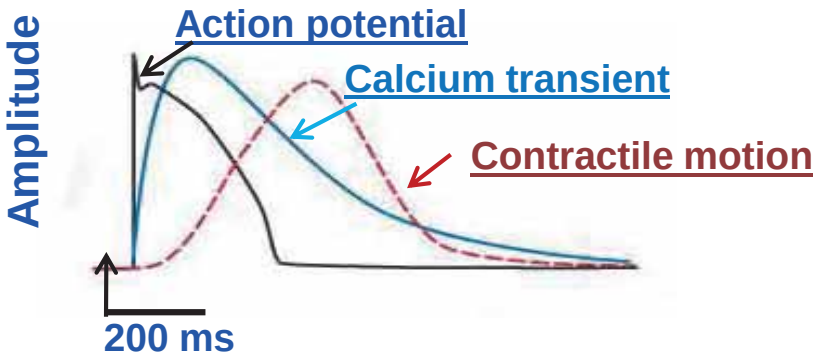
1. Cell loading (for 1 hour at 37°C) with Hoechst 33342 & Fluo-4
2. Cells incubated with compounds at 37°C for 30 mins before imaging
3. Negative & positive controls distributed throughout each plate.
4. Kinetic Image Cytometer (KIC) used to capture movies of Ca^{2+} transients
5. KIC fitted with a 20X objective and captured an image of the nuclei (Hoechst), then recorded 10s of spontaneous Ca^{2+} transient activity at 30fps from each well.
6. Cultures with abnormalities eg. low cell number, abnormal morphology or contamination were not used
7. Sotalol: CTD50 Time ≥ 115% of control,
   Verapamil: CTD50 Time ≤ of control,
   BayK 8644: CTD50 Time ≥ 125% of control,







Journal of Pharmacological and Toxicological Methods 66 (2012) 246–256



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Results: HCS-Ca^{2+} transients: 60 reference compounds

	iCEll-CDI (Drug/T drug) (%)	Cellectis (Drug/T drug) (%)	Cor.4 U-Axgiogenesis (Drug/T Drug) (%)
QT-interval ↑ drugs	23/23 (100%)	17/23 (74%)	20/23 (87%)
QT-interval ↓ drugs	6/6 (100%)	2/6 (33%)	5/6 (83%)
Ca^{2+} Antagonists	5/5 (100%)	3/5 (60%)	5/5 (100%)
Na^{+} blocking drugs	7/10 (70%)	5/10 (50%)	7/10(70%)
HR ↑ drugs	6/6 (100%)	1/6 (17%)	6/6 (100%)
Negative control drugs	0/4 (100%)	0/4 (100%)	0/4 (100%)

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cellectis

axioGENESIS

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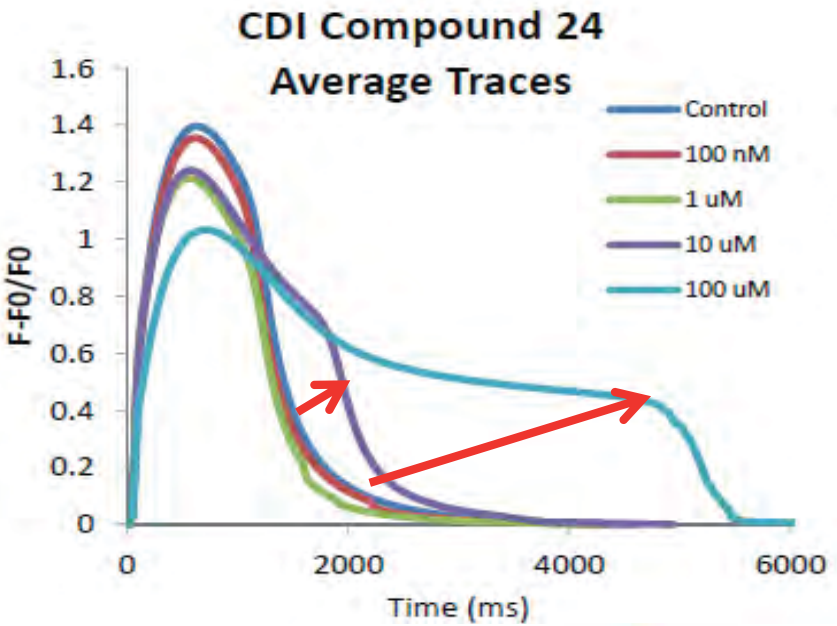
23 QT-prolonging drugs using Ca^{2+} transient

23 QT-prolonging drugs	
Name of compound (μM)	**iCell-CDI (μM)**
dofetilide	↑↑ Duration (≈APD/QT-interval) (10 nM)
Flecainide	↑↑ Duration (≈APD/QT-interval) (1μM)
Quinidine	↑↑ Duration (≈APD/QT-interval) (1μM)
Ranolazine	↑↑ Duration (≈APD/QT-interval) (1μM)
Ibutilide	↑↑ Duration (≈APD/QT-interval) (10nM)
Dimenhydrinate	↑ Duration (≈APD/QT-interval) (3μM)
Spafloxacin	↑↑ Duration (≈APD/QT-interval) (10μM)
Bepridil (1)	↑ Duration (≈APD/QT-interval) (0.1μM)
Bepridil (2)	↑ Duration (≈APD/QT-interval) (1μM)
Tedisamil	↑↑ Duration (≈APD/QT-interval) (1μM)
E4031	↑↑ Duration (≈APD/QT-interval) (50nM)
Mesoridazine	↑↑ Duration (≈APD/QT-interval) (1 μM)
Azithromycin	↑ Duration (≈APD/QT-interval) (30μM)
dl-sotalol	↑↑ Duration (≈APD/QT-interval) (30μM)
4-aminopyridine	↑↑ Duration (≈APD/QT-interval) (10μM)
BAYK8644 (1)	↑ Duration (≈APD/QT-interval) (10nM)
BAYK8644 (2)	↑↑ Duration (≈APD/QT-interval) (10nM)
Zatebradine	↑↑ Duration (≈APD/QT-interval) (1μM)
Ivabradine	↑↑ Duration (≈APD/QT-interval) (10nM)
Citalopram	↑↑ Duration (≈APD/QT-interval) (1μM)
Eliprodile	↑↑ Duration (≈APD/QT-interval) (0.1μM)
$BaCl_2$	↑ Duration (≈APD/QT-interval) (1μM)
Veratrine	↑↑ Duration (≈APD/QT-interval) (1μM)

Sparfloxacin



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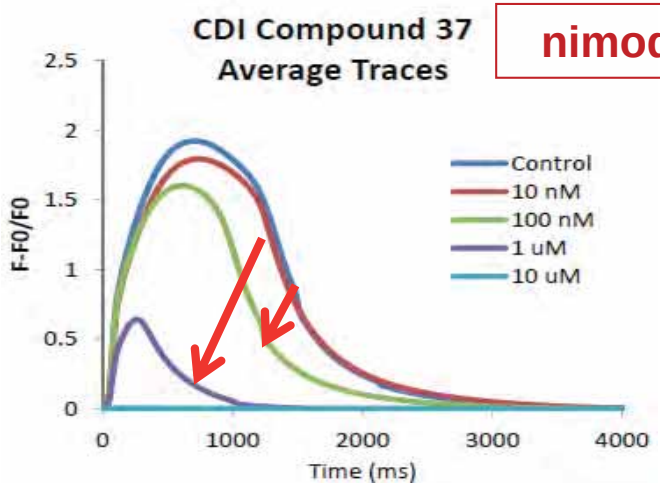
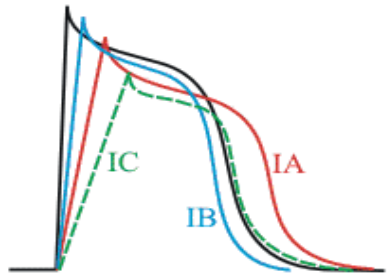
5 Ca^{2+} antagonists using Ca^{2+} transients

	5 Ca^{2+} antagonists	CTD50
27	Diltiazem	Duration↓↓ (10), Duration ↑ (10nM); BR↑ (10)
37	nimodipine	Duration↓↓ (1), BR ↑ (1)/stop(10); Others↓ (1)
42	verapamil (1)	Duration ↓↓ (1), BR↑, beating stops (5)
52	Verapamil (2)	Duration ↓↓(1), BR↑ (1 /5)
59	Nitrendipine	Duration↓↓(100nM), BR↑ (100nM)
SQT: QT-interval/APD/duration ↓		



10 Na^+ blockers using Ca^{2+} transients

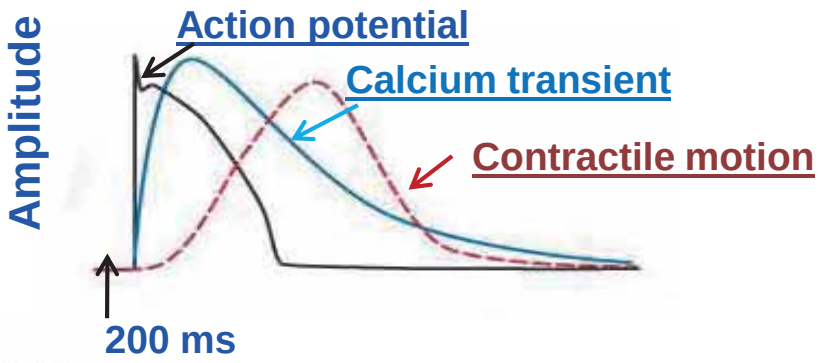
10 Na^+ channel blockers		
Nr.	Name of compound	Parameters (µM)
9	Quinidine (Class **Ia**)	Duration↑ (1), Peak Value ↓(0.1), Rise T (0.01)↓
60	Procainamide (Class **Ia**)	Duration↑, Peak value (+/-), Rise T (+/-)(100),
19	Primidone (I_B)	No clear effects
20	Phenytoin (I_B)	3 µM: BR↑, mostly paramters ↓ , stop 70% (30)
21	Mexiletine (**I_B**)	Duration ↑, Rise T↑ (10µM), stop 100% (100)
5	Flecainide (**Ic**)	Duration ↑↑, Peak Value/downstroke↓ (10), stop (100)
17	Propoxyphene (Ic)	Peak value/decay T/ T75-25 ↓ (10); stop (100)
6	Terfenadine 1 (**Ic**)	Duration ↑ (10nM), Decay T/T75-25 ↓ Peak V; stop (10)
23	Terfenadine 2 (**Ic**)	Beating stops (10)
40	Terfenadine 3 (**Ic**)	Beating stops (10)



Ventricular Action Potential

- Class IA: e.g., quinidine
  - Moderate Na^+-channel blockade
  - ↑ ERP
- Class IB: e.g., lidocaine
  - Weak Na^+-channel blockade
  - ↓ ERP
- Class IC: e.g., flecainide
  - Strong Na^+-channel blockade
  - → ERP

Singh Vaughan Williams -1970's



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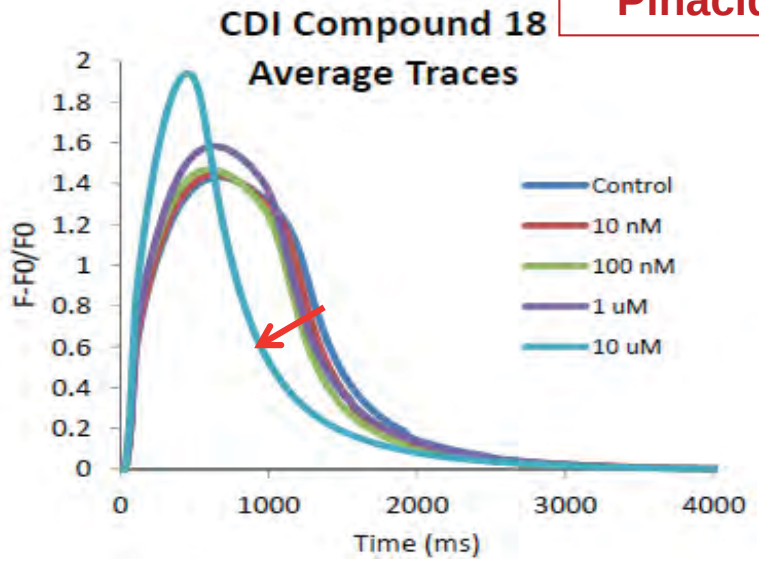
6 QT-shortening drugs using Ca^{2+} transients

	6 QT-shortening drugs	
1	NS1463	Duration ↓↓ (10μM)
2	Mallotoxin	Duration ↓↓(0.1μM) Beating stops at 10μM
3	Levcromakalim	Duration ↓↓1μM, Beating stops (5μM)
12	Nicorandil	No Effect
18	pinacidil	Duration ↓↓ (0.1μM)
11	Digotoxin	Duration ↓ (5μM)

Pinacidil



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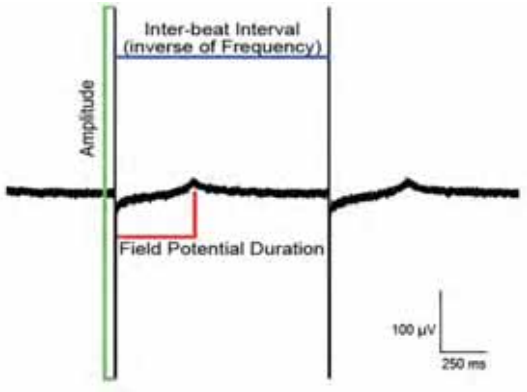
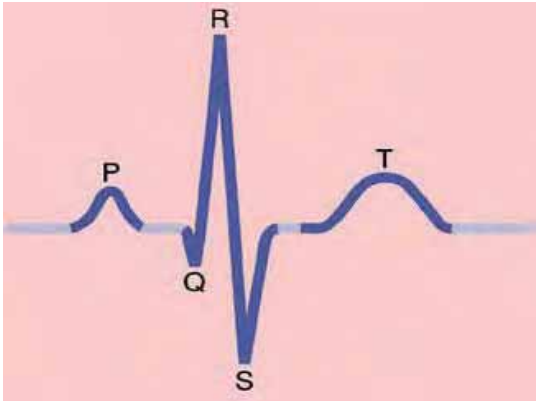
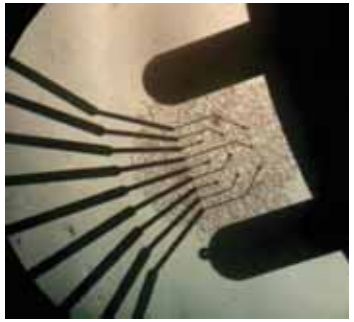
IV. Multi-Electrode Arrays (MEAs) – *"ECG-Like"* : 4 cell providers and 20 reference compounds

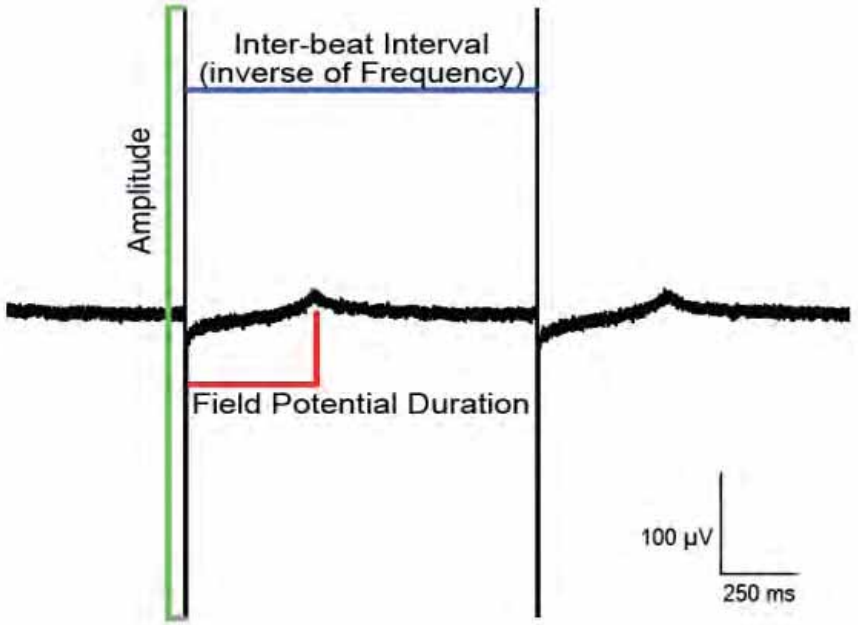




MEA Method

- Cells plated on fibronectin coated MEA chips and kept in maintenance medium until usage day 5-7 - *culturing of cells according to commercial provider instructions*
- Plating density ~ 4,000-7,000 cells/well
- At 5 days usability of cells inspected (*shape, beat rate, amplitude of fAP and variability of parameters between wells. If not suitable they stay in culture 1-2 days extra*).
- On experiment day, 50% of media is exchanged & cells settle for 1hr in incubator
- Experiments performed in moisturised 5% CO_2 air
- Cells settle for 10 min after placement in MEA amplifier
- Single drug concentration/well. 2 min data samples at 5, 15 and 30 min (*15 and 30 min was a separate study*) were compared to pre-dose 2 min sample. Positive reference 30nM E4031 (*well accepted if fAP >30%).*

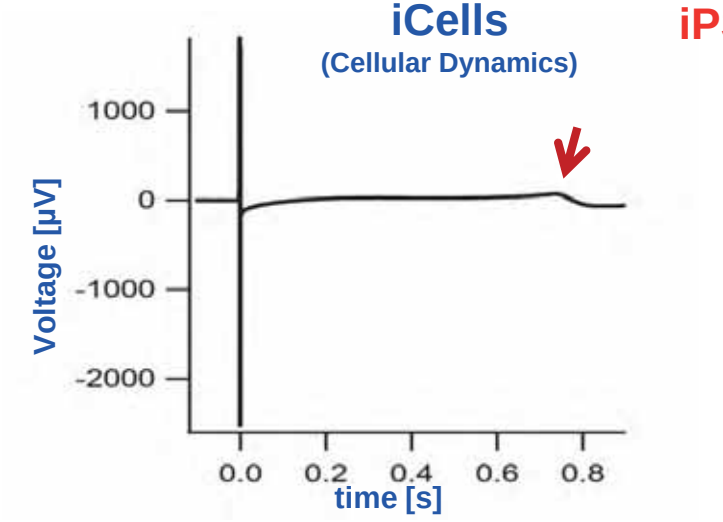




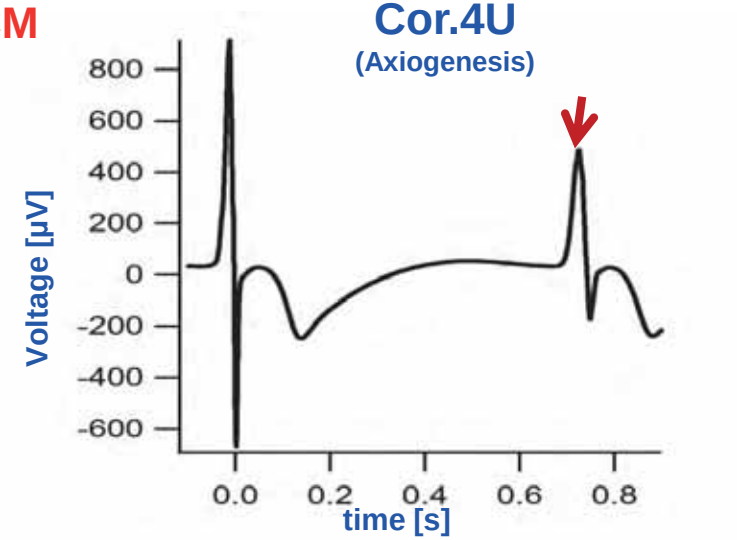
Example fAP waveforms from different cell providers:

iCells
(Cellular Dynamics)

iPSC-Derived CM

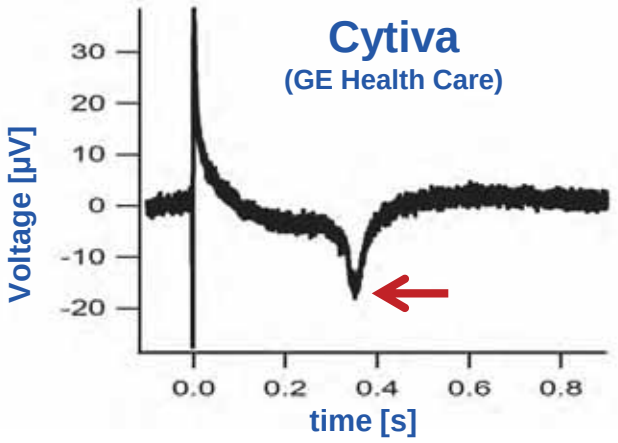


Cor.4U
(Axiogenesis)

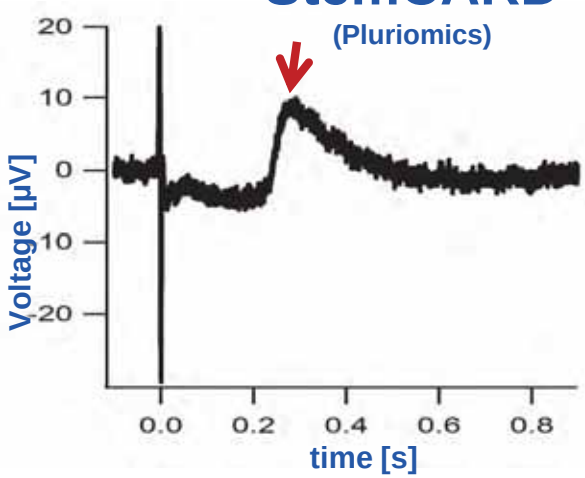


Cytiva
(GE Health Care)

ES-Derived CM



StemCARD
(Pluriomics)



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NMI achieving results

Results of 20-reference compounds with MEA measurements using 4 different cell providers (5 min/per dose)

	Cor.4U(Axio)	iCells(CDI)	Cytiva (GE)	StemCARD(Pluriomics)
Compounds	**hiPS-CM1**	**HiPS-CM2**	**HES-CM 1**	**HES-CM2**
BAYK8644	fAPD↑	fADP↑;BR↑	fAPD↑↑	fAPD↑↑; BR↑↑
ivabradine	fAPD↑ BR↓	fAPD↑↑ BR↓	fAPD↑↑ BR↓↓	fAPD↑BR↓
Dofetilide	fAPD (+/-)	fAPD(+/-) Amp↓↓BR↓↓	fAPD↑↑ BR↓;	fAPD↑↑; BR↑↑
Spafloxacin	fAPD↑ BR↓	fAPD↓; Amp↓↓BR↓↓	fAPD↑↑;BR↑	fAPD↑↑; BR↑↑
moxifloxacin	fAPD↑ Am↓	fAPD↑;BR↓	fAPD↑↑; BR↓	fAPD↑↑
Bepridil	fAPD(+/-) BR↓↓	fAPD↑;BR↓	fAPD(+/-);BR↓ BS	fAPD↑;BS
Ranolazine	fAPD(+/-) Amp↑↑	BS	fAPD↑;BS	fAPD↑↑
Flecainide	fAPD↑/Amp↓↓/BS	Amp↓↓/fAPD↑	fAPD(+/-); BS	fAPD↑; Amp↑; BS
Terfenadine	fAPD↑↑Amp/BR↓	BS	BS	fAPD↓;BS
Procainamide*	fAPD↑/Amp(+/-)	fAPD↑↑; Amp↓↓;BR↓	fAPD↑↑;Amp↓	fAPD↑Amp↓
Mexiletine	Amp↓	BS	BS	BS
Diltiazem	fAPD/BS	fAPD/Amp↓↓BR↑↑	BS	fAPD↓;Amp↓↓
Nimodipine	fAPD↓BS	fAPD↓↓;BR↑↑	fADP↓;BS	fAPD↓;BS
Levcromakalim	fAPD↓ /(+/-)/ BS	fAPD↓↓(2) (+/-) (1);BR↓BS	fAPD ↓;BR↓	BS(1) fAPD↓BS (2)
NS1463	fAPD↓ or no	BS	fAPD↓	fAPD↓
JNJ303	fAPD(+/-)	fAPD↑Amp/BR↓	fAPD(+/-)	fAPD↑
SEA400 (Na/Ca Ex In)	fAPD/Amp/BR↓	fAPD/Amp↓	fAPD↓↓;Amp/BR↓	fAPD↓;Amp↓
DPO-1 (Ikυ blocker)	fAPD↑	fAPD/Amp↓↓BR↑	fAPD↑ BR↑	fAPD↑;BR↑
Carbenoxolone (Gap G In)	fAPD(+/-)	fAPD(+/-)	fAPD(+/-)	fAPD(+/-)
Tegaserod	fAPD↑	fAPD↓Amp↓	BS	fAPD↓.BR↑

BS: beating stopped
Amp: amplitude
fAPD: Field potential duration
BR: Beat Rate

Differences between MEAs and Ca^{2+} -transient using iCELL (hiPS-CMS) with 20 reference compounds (30 min/dose)

Compounds	MEAs (NMI) 30 min (μM)	Ca^{2+} Transient (Vala Sci) 30min (μM)
Dofetilide	fAPD↑↑ (10nM)	Duration ↑↑ (0.01)
Sparfloxacin	fAPD↑ (10)	Duration ↑↑ (10)
Moxifloxacin	fAPD↑ (10)	NT
$BaCl_2$	fAPD (+/-), Slope ↓ (100)	Duration ↑ (10 nM)
BAYK 8644	fAPD↑ (1)	Duration ↑↑ (10nM)
Bepridil	+/-	Duration ↑ (0.1-1)
Ranolazine	fAPD↑ (10)	Duration ↑↑ (1)
Procainamide	+/-	Duration ↑ (100)
Flecainide	fAPD↑ (1) fAPD↑↑(10)	Duration ↑↑ (10)
Veratrine	fAPD↑↑(1)	Duration ↑↑ (1)
Ivabradine	fAPD↑ (1)	Duration ↑↑ (10nM)
Levcromakalim	+/-	Duration ↓ (1)
NS1463	+/-	Duration ↓ (10)
Nisoldipine	fAPD↓↓ (0.01)	NT
Nimodipine	fAPD↓↓ (0.1)	Duration ↓↓ (0.1)
Verapamil	fAPD↓↓ (0.1)	Duration ↓(1)
Mexiletine	Slope ↓(10)	Duration ↑ BS (100)
Pimidone	+/-	+/-
Phenytoin	+/-	+/- (10), BS (30)
Lidocaine	Slope ↓(10)	NT

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NMI achieving results

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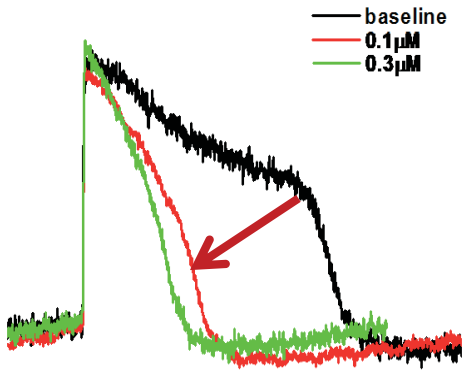
V. Effects of 20 compounds in Optical action potentials (iCELLs)-CellOPTIQ system

See poster 11 for details *HR Lu *, DJ Gallacher, M Hortigon-Vinagre, I Ghouri, MA Craig, GL Smith, G-X Yan*

Effects of 20 compounds in Optical action potentials using HiPS-CMs (iCELLs)- CellOPTIQ system



Effects of Nifedipine on action potentials recorded from human stem cells

CellOPTIQ System:

1.Cells seeded (40,000 cells/well) onto fibronectin-coated glass-bottom 96 well plates & maintained in culture for 10 days.
2.Experiments with testing drugs were carried out on days 10-15.
3.The cells washed in serum-free medium and exposed transiently to 6 µM Di-4-ANEPPS.
4.The plate was placed in a stage incubator & spontaneous electrical activity was recorded as the Di-4-ANEPPS fluorescence signal from areas of iPSCs in individual wells using a 40x (NA 0.6) objective.
5.Fluorescence signals (*optical action potentials*) were digitized at 10kHz and the records analyzed using a proprietary software:

1. Action Potential shape/activity;
2. AP duration (APD);
3. Upstroke-rate of rise of AP (Trise: rise time)

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Overview effects of 20 reference drugs in iCells using Optical AP measurements.

Compounds	Optical APD (Clyde BioSci) 30min (µM)
Dofetilide	APD↑↑ (0.1 nM)
Sparfloxacin	APD↑ (10), APD↓ (50)
Moxifloxacin	APD +/- (30)
$BaCl_2$	APD↑ (100)
BAYK 8644	APD↑ (0.01)
Bepridil	APD ↑ **(only at 0.01);**
Ranolazine	APD↑(50)
Procainamide	APD↑↑ (10)
Flecainide	APD ↑↑ (1), BS (50)
Veratrine	APD ↑↑ (1) BS (50)
Ivabradine	APD ↑ (10)
Levcromakalim	APD↓ (0.5), BS (1)
NS1463	APD↓ (10)
Nisoldipine	APD↓↓ (0.01), BR↑
Nimodipine	APD↓↓ (0.1), BR↑ (0.1)
Verapamil	APD↓ BR↑(0.1) BS (5)
Mexiletine	Trise↑/BR↓(10), APD↑
Pimidone	Triase/APD↑ BR ↓(0.01)
Phenytoin	+/-
Lidocaine	BR↓/APD90 ↑ (1) BS (50)

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VI. Comparison between Ca^{2+} transient, MEAs, Optical APD & the isolated rabbit wedge: 20 reference compounds

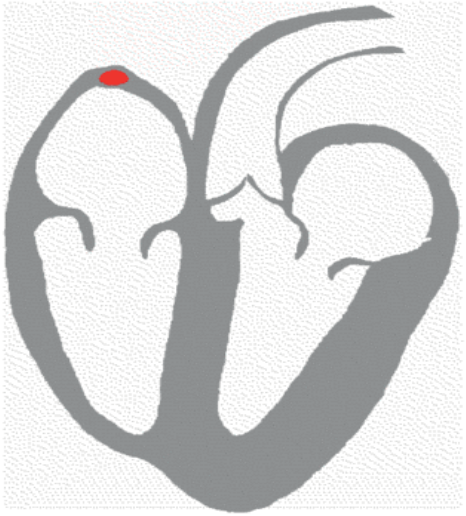
Comparison between Ca^{2+} transient, MEAs, Optical APD & the isolated rabbit wedge: 20 reference compounds

Compounds	IC50 (μM): Ion Channel/Receptor	IWP (HRS) 30 min (μM)	Optical APD (Clyde BioSci) 30min (μM)	MEAs (NMI) 30 min (μM)	Ca^{2+} Transient -FDSS 30min (μM)	Ca^{2+} Transient (Vala Sci) 30min (μM)
Dofetilide	hERG↓ (5-32 nM)	QT↑↑ (1 nM)	APD↑↑ (0.1 nM)	fAPD↑↑ (10nM)	Duration ↑↑ (0.01)	Duration ↑↑ (0.01)
Sparfloxacin	hERG↓ (0.23)	QT↑↑ (1)	APD↑ (10), APD↓ (50)	fAPD↑ (10)	Duration ↑↑ (10 0)	Duration ↑↑ (10)
Moxifloxacin	hERG↓ (29)	QT↑ (10) ↑↑(20), QRS↑ (20)	APD +/- (30)	fAPD↑ (10)	Duration ↑↑ (30 0)	NT
$BaCl_2$	I_{K1} ↓ (3.9)	QT ↑↑ (10)	APD↑ (100)	fAPD (+/-), Slope ↓ (100)	Duration ↑ (100)	Duration ↑ (10 nM)
BAYK 8644	I_{Ca} ↑ (10)	QT↑↑ FC ↑↑ (0.1)	APD↑ (0.01)	fAPD↑ (1)	Duration ↑↑ (10)	Duration ↑↑ (10nM)
Bepridil	hERG↓ (0.6) I_{Ca} (0.5)	QT ↑(0.3), QRS↑↑ (10) FC↓ (10)	APD ↑ **(only at 0.01);**	+/-	Duration ↑ (1)	Duration ↑ (0.1-1)
Ranolazine	hERG↓ (12), Late I_{Na}↑ (2.2)	QT ↑ (0.01) QT ↑↑, QRS↑ (10)	APD↑(50)	fAPD↑ (10)	Duration ↑↑ (100)	Duration ↑↑ (1)
Procainamide	hERG↓ (310) I_{Na} ↓ (811)	QT ↑ (10) QRS↑↑ (100)	APD↑↑ (10)	+/-	Duration ↑ (100)	Duration ↑ (100)
Flec						↑ (10)
Ver						↑ (1)
Ivab						(10nM)
Levcro						↓ (1)
NS1643	hERG ↑ (10)	QT ↓, QRS ↑ (10)	APD↓ (10)	+/-	NT	Duration ↓ (10)
Nisoldipine	Ica ↓(0.1-3.9) , hER ↓ (23)	QT↓ (1) FC↓ (0.1)	APD↓↓ (0.01), BR↑	fAPD↓↓ (0.01)	Duration ↓ (1)	NT
Nimodipine	Ica ↓(0.14) , hER ↓ (NA)	QT↓ (1) FC↓ (0.1)	APD↓↓ (0.1), BR↑ (0.1)	fAPD↓↓ (0.1)	Duration ↓↓ (0.1)	Duration ↓↓ (0.1)
Verapamil	Ica (L) ↓(0.34) , hER ↓ (0.2)	FC ↓ (0.1), QRS↑ (0.5)	APD↓ BR↑(0.1) BS (5)	fAPD↓↓ (0.1)	Duration ↓↓(0.1)	Duration ↓(1)
Mexiletine	I_{NA}↓ (18), hERG ↓ (57)	QRS ↑ (10) ↑↑ (100)	Trise↑/BR↓(10), APD↑	Slope ↓(10)	Duration ↑↑ BR/Am ↓ (10)	Duration ↑ BS (100)
Pimidone	I_{NA}↓ (>10), hERG ↓ (10)	No effect (10)	Triase/APD↑ BR ↓(0.01)	+/-	+/-	+/-
Phenytoin	I_{NA}↓ (173), hERG ↓ (100)	QT↓ , QRS↑ (30)	+/-	+/-	Am ↓ (30)	+/- (10), BS (30)
Lidocaine	I_{NA}↓ (20), hERG ↓ (>100)	QT↓QRS↑ (10, 100)	BR↓/APD90 ↑ (1) BS (50)	Slope ↓(10)	NT	NT

	IWP Rabbit	Optical APD (Clyde BioSci)	MEAs (NMI)	Ca^{2+} transient
QT prolonging drugs	11/11 (100%)	10/11 (91%)	9/11 (82%)	11/11 (100%)
QT shorting drugs	5/5 (100%)	5/5(100%)	3/5 (60%)	4/4 (100%)
QRS widening drugs	5/5 (100%)	2/5 (40%)	3/5 (60%)	3/4 (75%)

↓: inhibition **↓: reduction**
↑: Activation **↑: increase/prolong**

VII. Conclusions

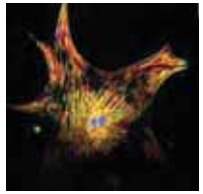


Conclusion

1. Using stem cell-CMs and Ca^{2+} transient technologies/optical AP could detect drug-induced QT prolonging/shortening, Ca^{2+} block and ↑ HR
2. Although current platforms do not detect Na channel blockers well - **good for HTS decision ???.**
3. We currently still need additional models to define the MOA and *in vivo* studies to look at drug-induced effects for cardiovascular safety assessment.

Future: Healthy Skepticism *meets* practical optimism

- **Gaps:**
  - **Selection of cell types:**
    Immature cells
    Batch to batch



  - **Low beating/variable frequency**
    - **Rate-dependence effects**

  - **Shape & Infrastructure,**
    Stress Tension
  - **'Ultimate solution' or HTS replacement?: user unfriendly software**

- **Compromise:**
  - **Cell sorting is improving**
    Genetic Profile (More Ik_1 etc.)
    Batch check before use
  - **Photorhodopsin (Optogenetics)**
    - **Combined voltage sensing/photopacing**
  - **Micropatterning (eg. Cytoo…)**
    Cytostretch Platform; 3-D
  - **Replace HTS hERG & other models ?:**
    Recording & analysis tools improving *(eg. Neural ID, Notocord Cardioxpress)*

Thanks to all contributors to our stem cell research

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Epithelix

3D human airway tissues for safety assessment: overcoming the barriers

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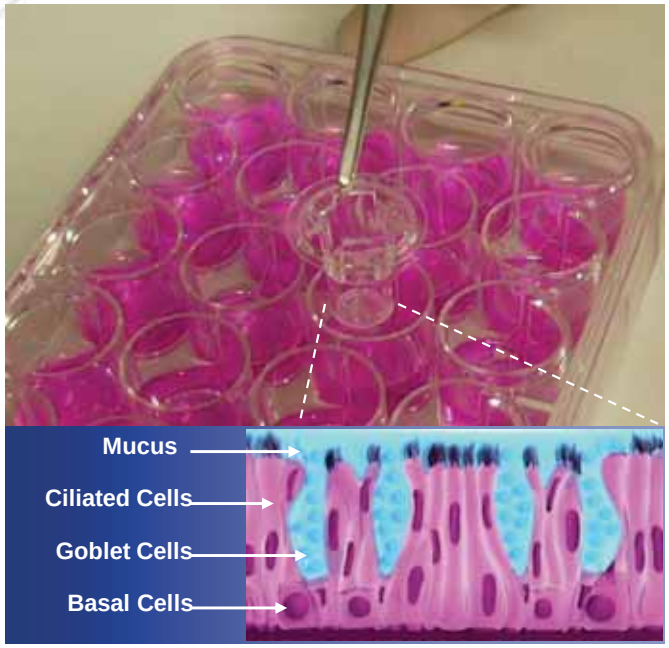
Epithelix

Part 1

Currently Availabe *in vitro* Airway Models and their Characteristics

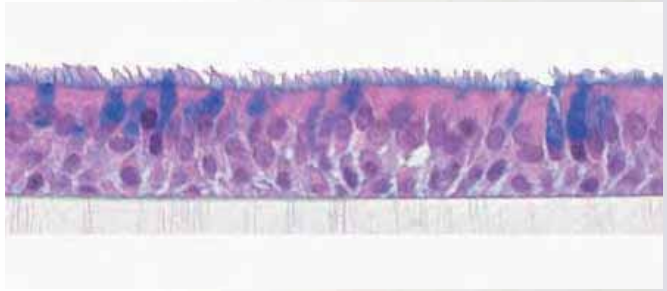
MucilAir™: Long shelf life *in vitro* Airway Tissues

Primary Human Cells
Isolation, amplification & seeding



Air-Liquid interface, differentiation

Ready-to-use
Fully differentiated
Airway Epithelium

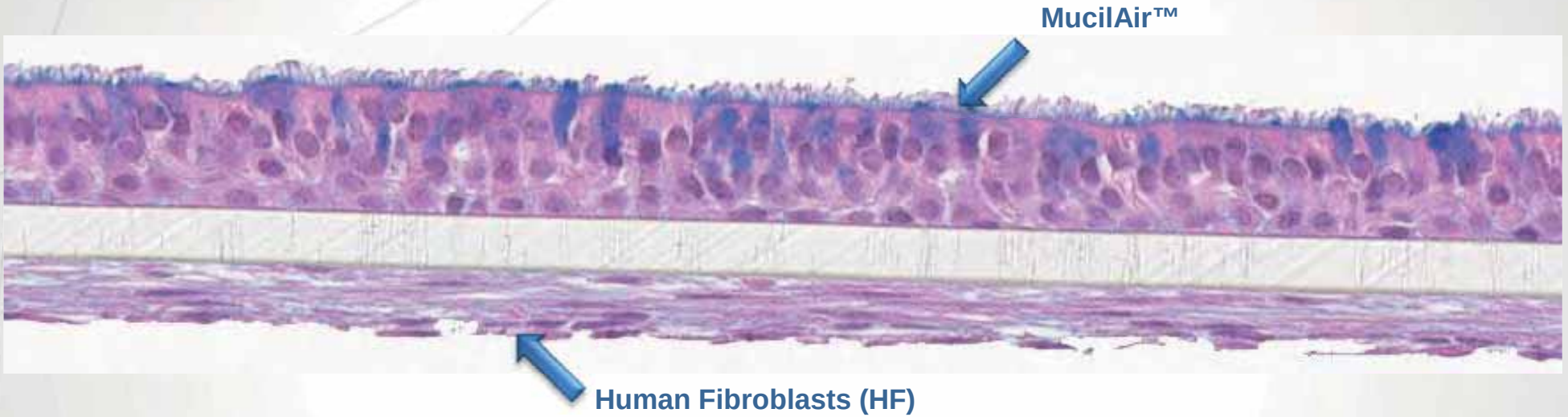


MucilAir™

Shelf-life of **1 year**

MucilAir™-HF

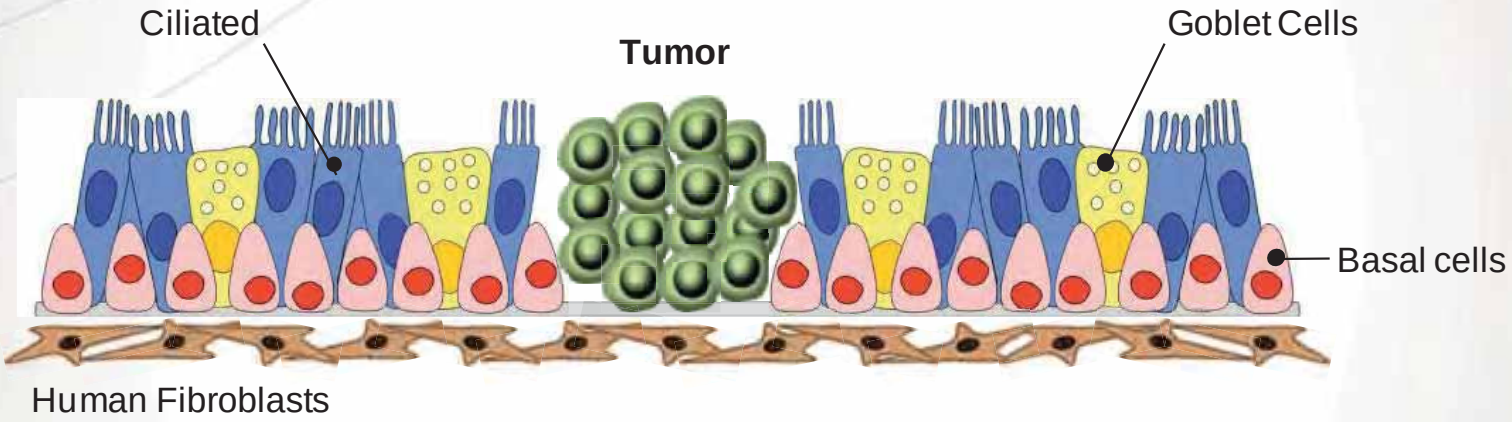
MucilAir™-HF: Long shelf-life co-culture model



Several versions are available:

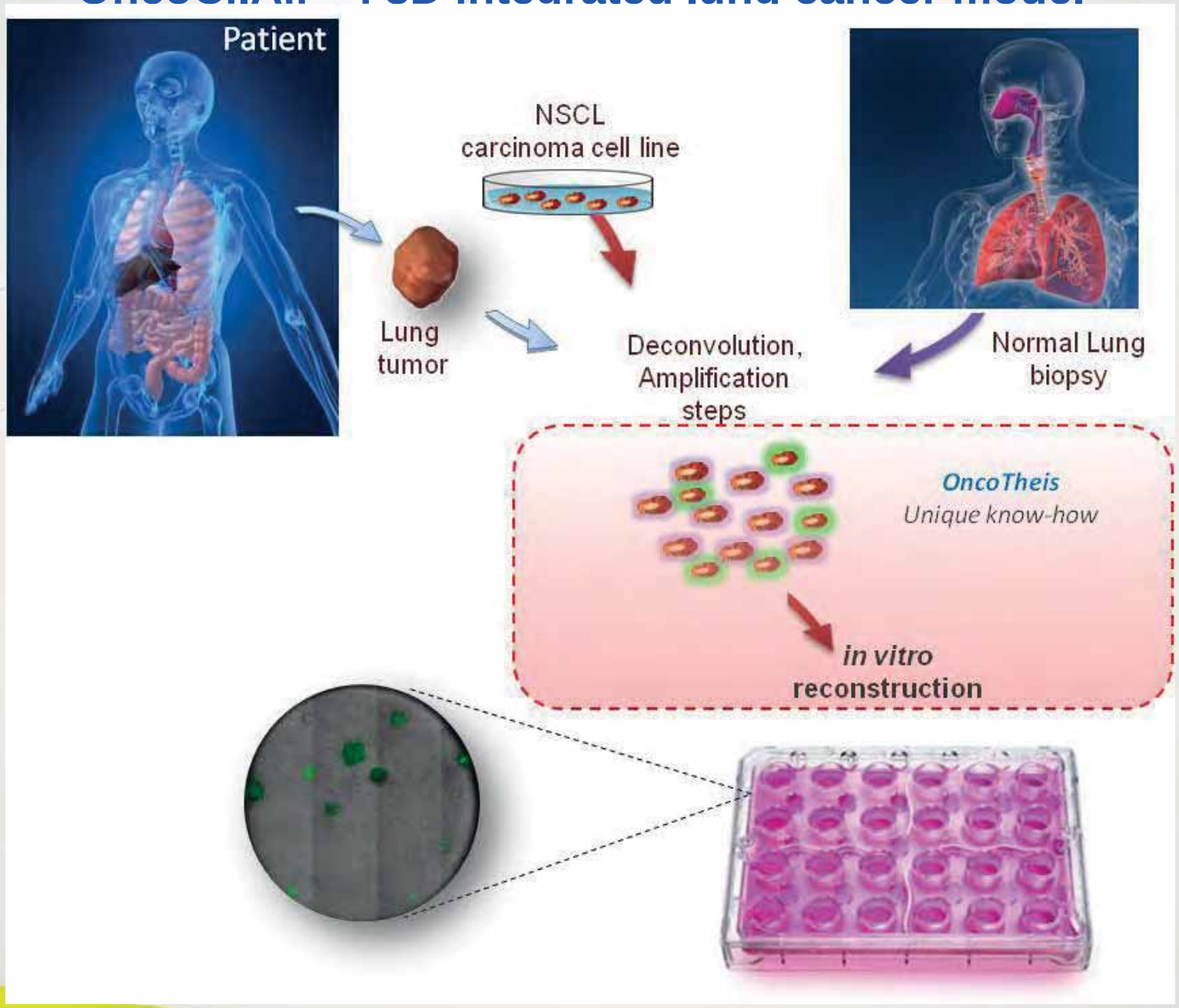
- ✓ MucilAir™-HF-Normal
- ✓ MucilAir™-HF-Asthma
- ✓ MucilAir™-HF-COPD (Chronic Obstructive Pulmonary Disease)
- ✓ MucilAir™-HF-CF (Cystic Fibrosis)
- ✓ MucilAir™-HF-Allergic Rhinitis

OncoCilAir™: 3D integrated lung cancer model



Oncology Department of Epithelix

OncoCilAir™: 3D integrated lung cancer model



Production: Overcoming the barriers

Access to starting materials from consent donors:

- ✓ Different legislations from countries to countries (i.e. easier in UK and France than in Germany or Switzerland)
- ✓ Involvement of collection centers (extra work for surgeons who don't have time)
- ✓ Paperwork for obtaining approval from ethical committees
- ✓ Need of fresh tissues (within 24h): Needs of fast logistics from collection center to laboratory
- ✓ Needs of sterility: Some tissues are strongly infected (i.e. CF donors) – caution to cross-contamination

Electron Microscopy

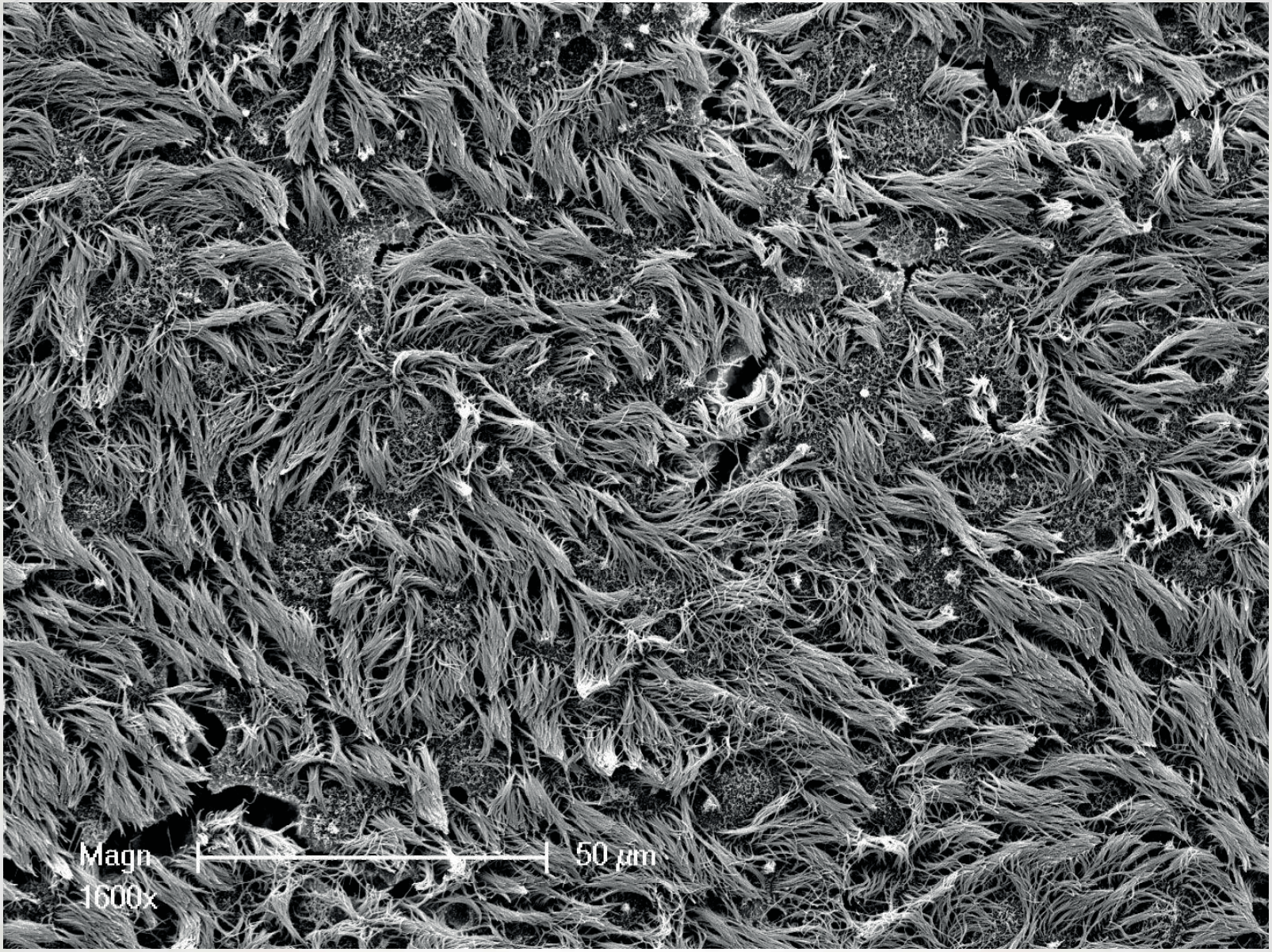


Photo Courtesy of Charles River Laboratories (www.criver.com)

The Main Characteristics of MucilAir™

- It has a unique shelf-life of **one year**
- It **mimics** the morphology and functions of the normal human airway epithelium.
  - ✓ Active Ion transport
  - ✓ Metabolic activity
- Epithelia from several **different pathologies** are available (Asthma, COPD, Cystic Fibrosis, Allergic Rhinitis, etc.)
- It is **easy** to handle and maintain
- **Serum free** medium
- **Worldwide Shipping**

Facing Throughput and Variability

MucilAir™ made of cells from a single donor:

- ✓ Specific donor response
- ✓ Standard 24-well format
- ✓ Number of cultures generated from one single donor:

  100 to 1’500 inserts

MucilAir™ made of cells from “**a Pool of donors**”:

- ✓ Sub population response. Minimize inter donor variation
- ✓ Standard 24-well format or HTS 96-well format
- ✓ Number of inserts generated from “a pool of donors”:

  > **35’000** inserts

Epithelix

Part 2

Functionnal Assays using MucilAir

Functional *In Vitro* Assays using MucilAir™

- Toxicity of inhaled products
  - ✓ Repeated dose toxicity
  - ✓ Pro-Inflammatory profile
  - ✓ Cyps induction
  - ✓ Modulation of cytokines release, etc..
- Efficacy assessment of drug candidates
  - ✓ Anti virals
  - ✓ Anti inflammatory, etc…
- Intranasal /intrabronchial permeation of drugs/xenobiotics
- Electrophysiology (activity of ion channels, etc.)
- Mucolytic Activity assessment
  - ✓ Mucociliary clearance
  - ✓ Cilia Beating Frequency
  - ✓ Mucus secretion

Overcoming barriers: Exposure systems

Static Exposure Systems :

Liquids - Solutions

Solids (Tablets)

Dynamic Exposure Systems :

Gas or smoke

VITROCELL

BRITISH AMERICAN TOBACCO

Nebulizing chambers

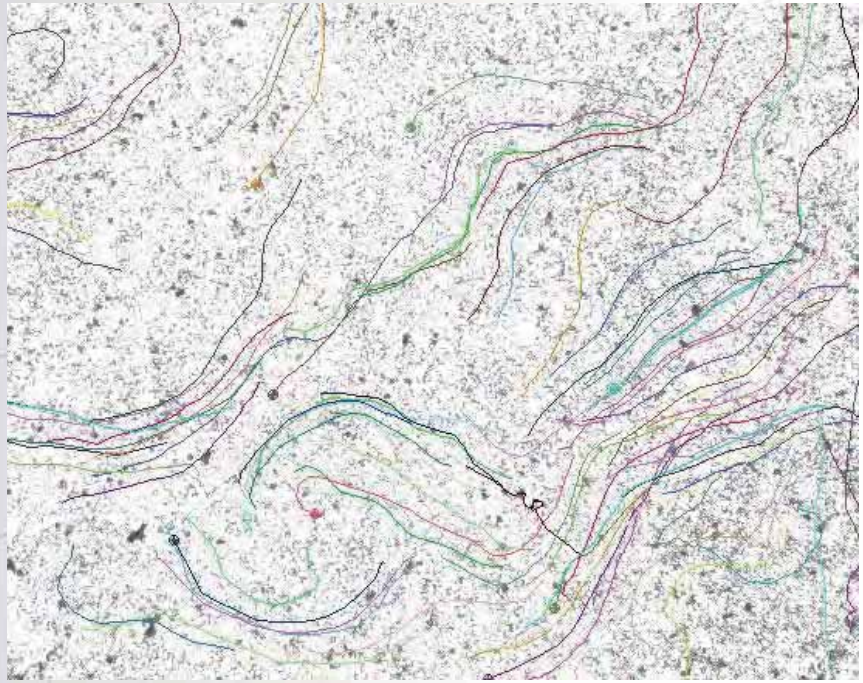
Overcoming Acceptance

Examples of **4** easily accepted assays

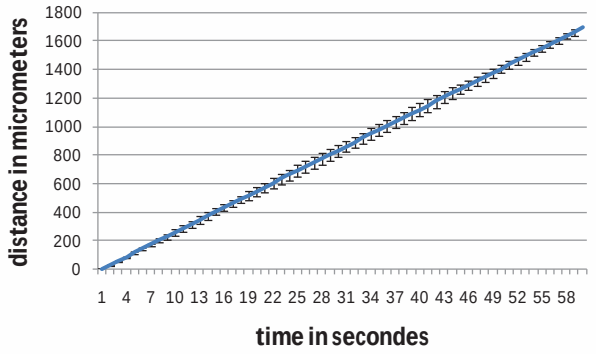
- Evaluation of mucolytic activity (mucociliary clearance)
- Ranking of anti-inflammatory compounds
- Evaluation of novel antivirals against hRV
- Efficacy assessment of corrector, activators or potentiators of CFTR (CF research)

Mucociliary Clearance Analysis

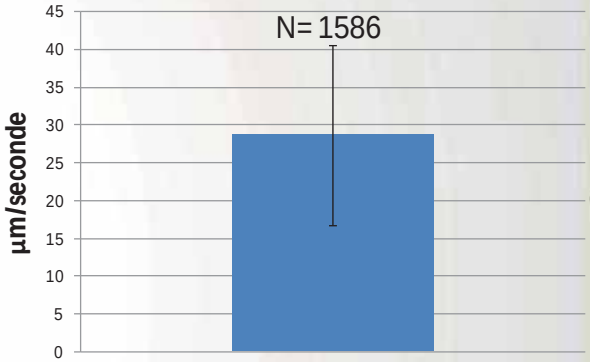
Particle tracking on MucilAir



(a)



(b)

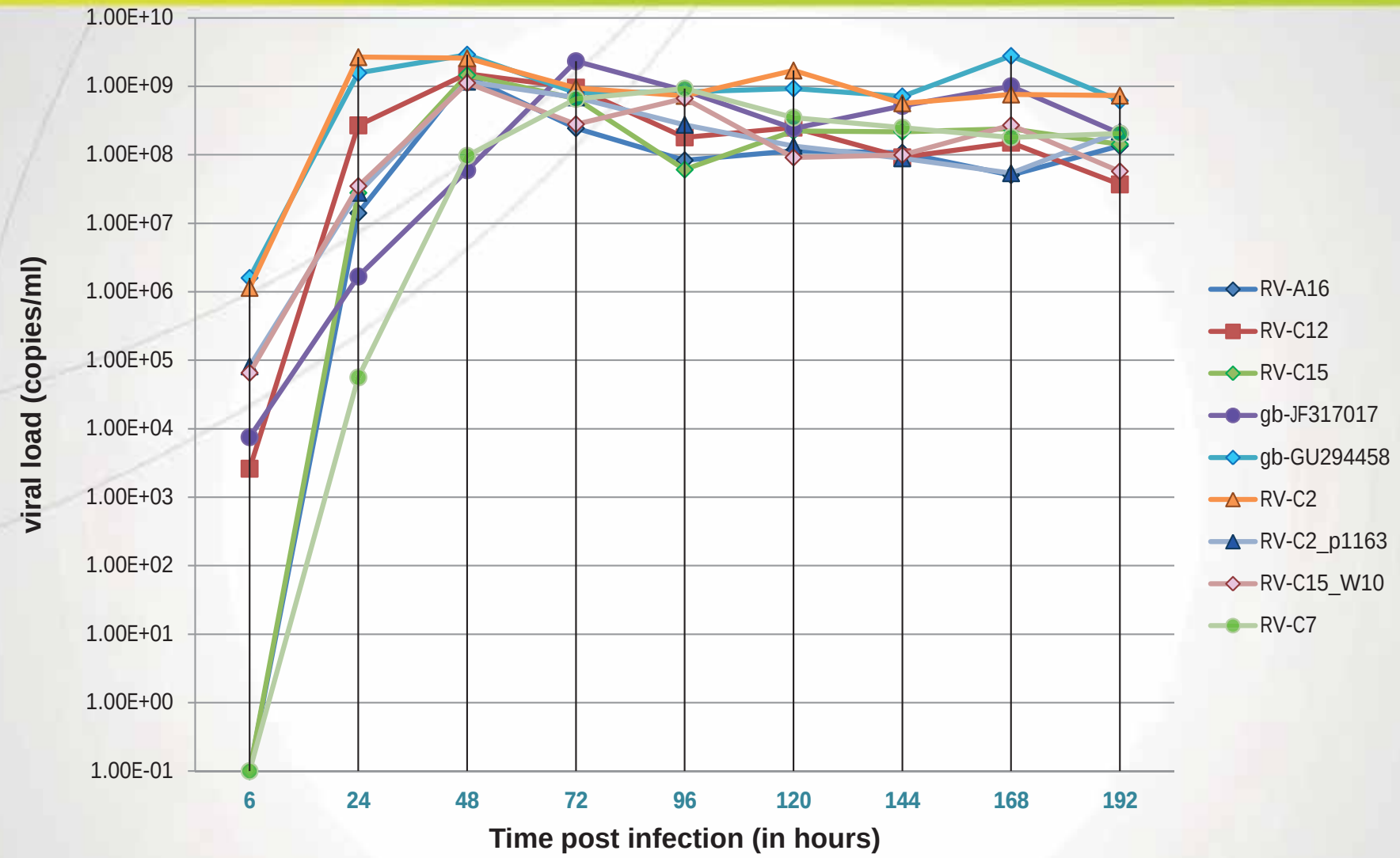


(c)

MCC (*in vivo* – human) ~ MCC (*in vitro* – MucilAir - human)

Human *in vitro* tissues can present quantifiable functions similar to the native tissue

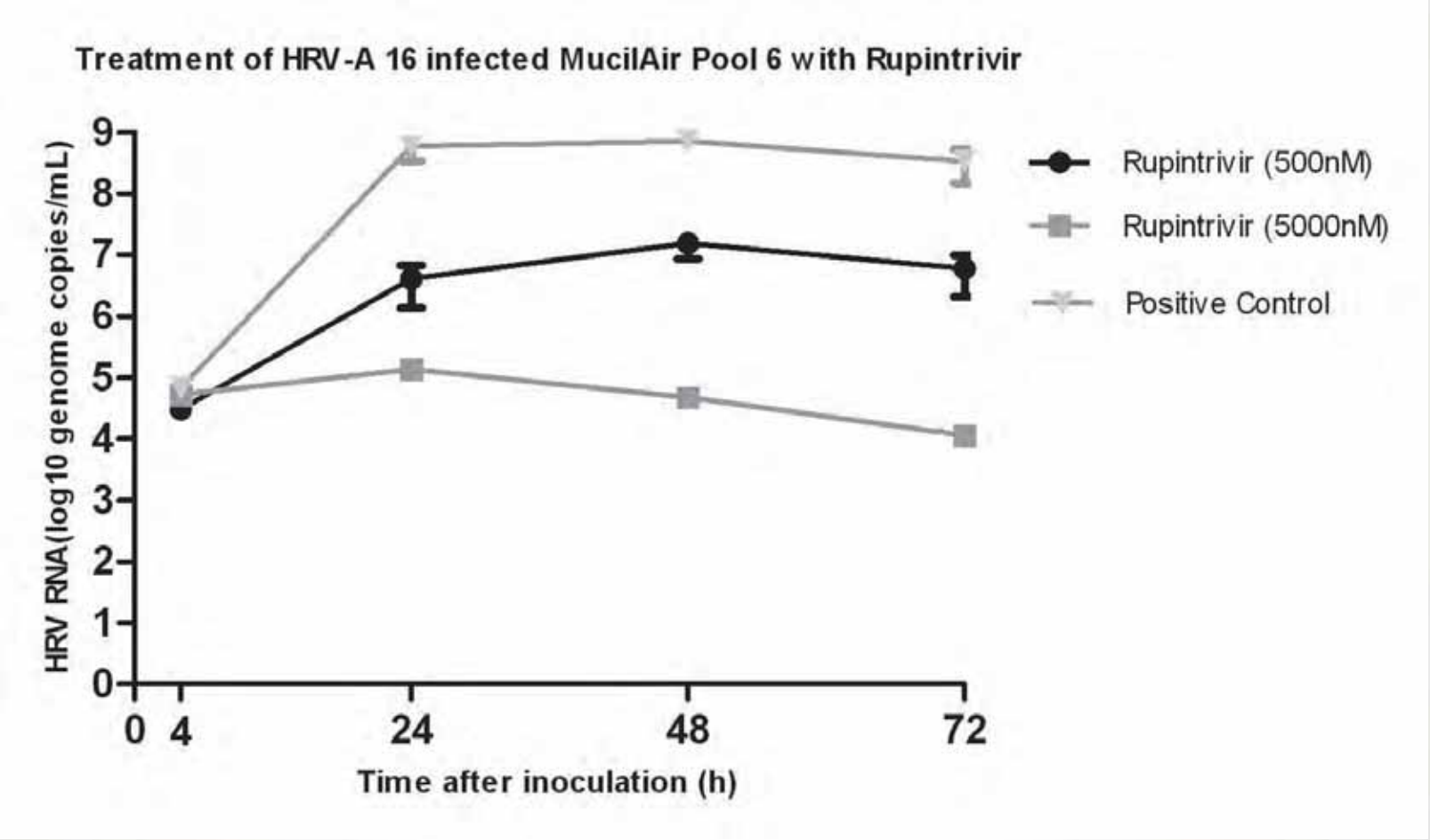
Viral Infection Studies



Rhinovirus growth after inoculation of infected clinical specimens on MucilAir Pool

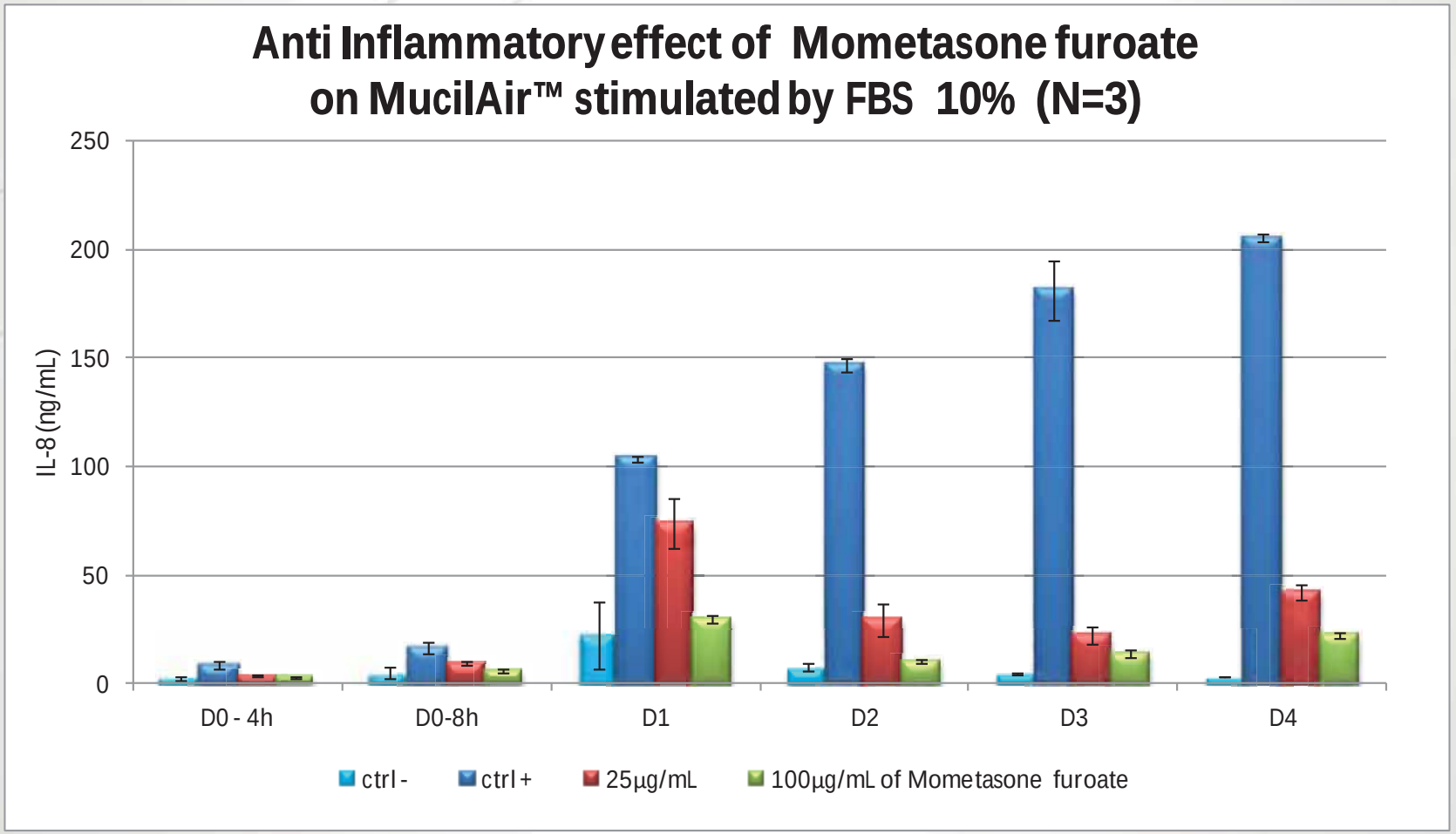
When available, the RV-C type is indicated (RV-Cxx) on the right. For untyped strains, the most similar entry in Genbank (found by blast analysis of the sequenced strain) is indicated (gb-xxxx). RV-A16 was used as a control. Viral load in cell supernatants was quantified with « Enterhino/Ge/08 » one step real-time RT-PCR. UV-irradiated clinical specimen presented undetectable ct values (not shown).

Evaluation of antivirals – HRV-A 16



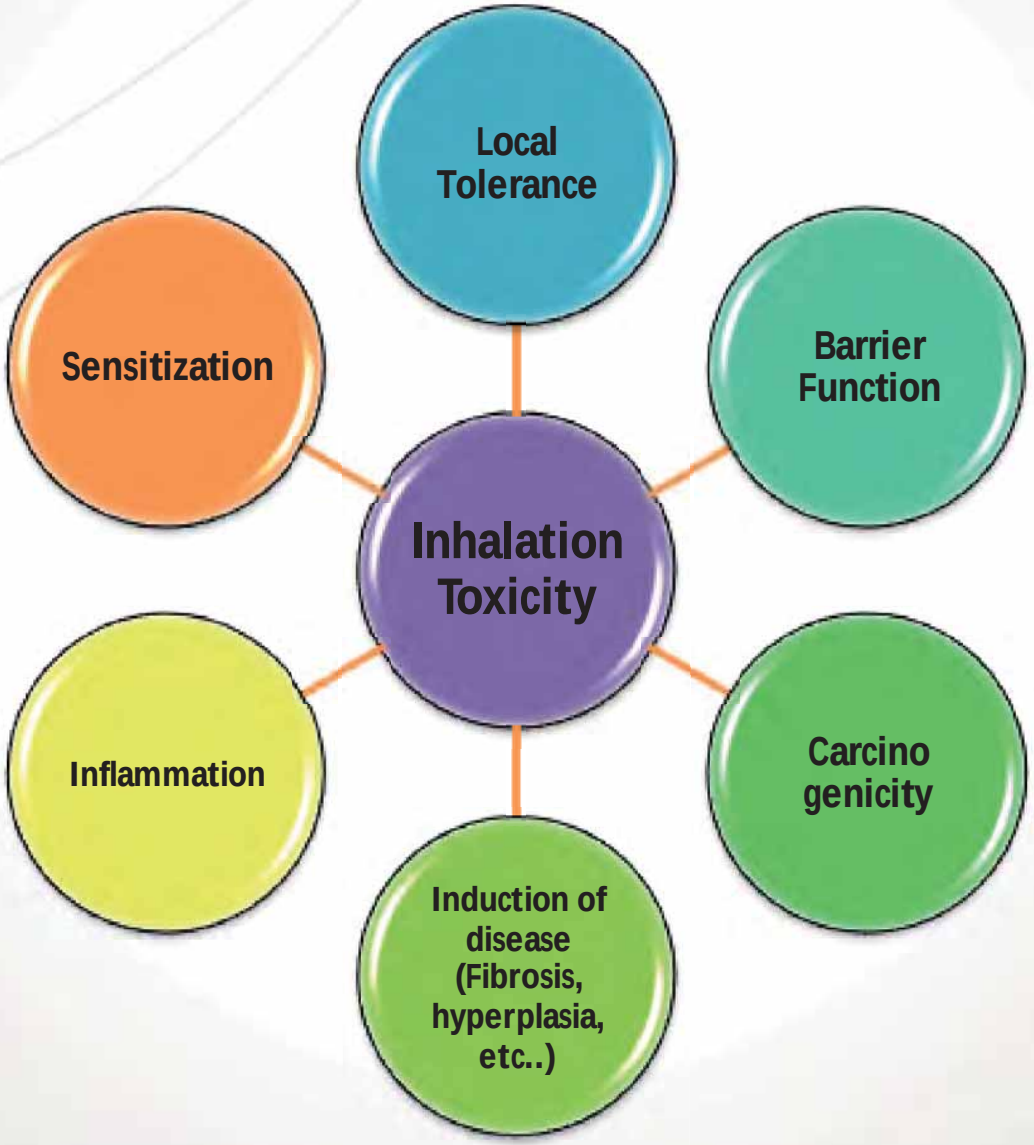
Anti Inflammatory Effect Assessment of drugs

Example of Repeated Dose Treatment



Overcoming Acceptance

Towards regulatory acceptance: Inhalation Toxicity Assessment

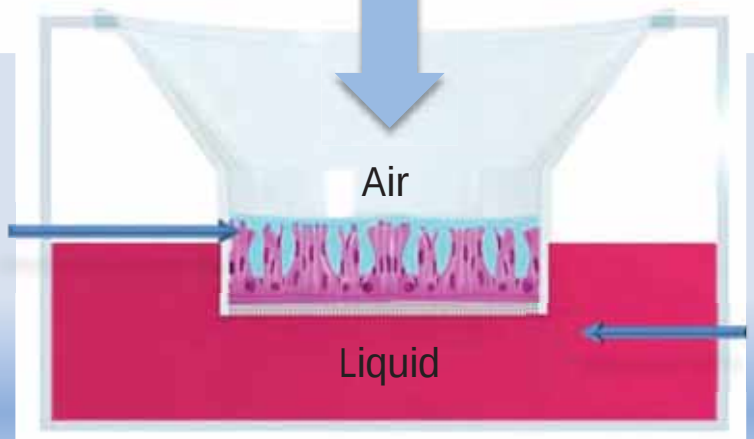


Toxicity Testing

Multiple end-points testing strategy for monitoring acute, chronic and long term effect of molecules/mixtures

Apical Exposure
(e.g. liquids, solids, nanoparticles, gas, smoke, etc.)

Information from Apical Side
- TEER measurement
- Resazurin test
- Cilia beating Monitoring
- Morphology
- Mucin secretion

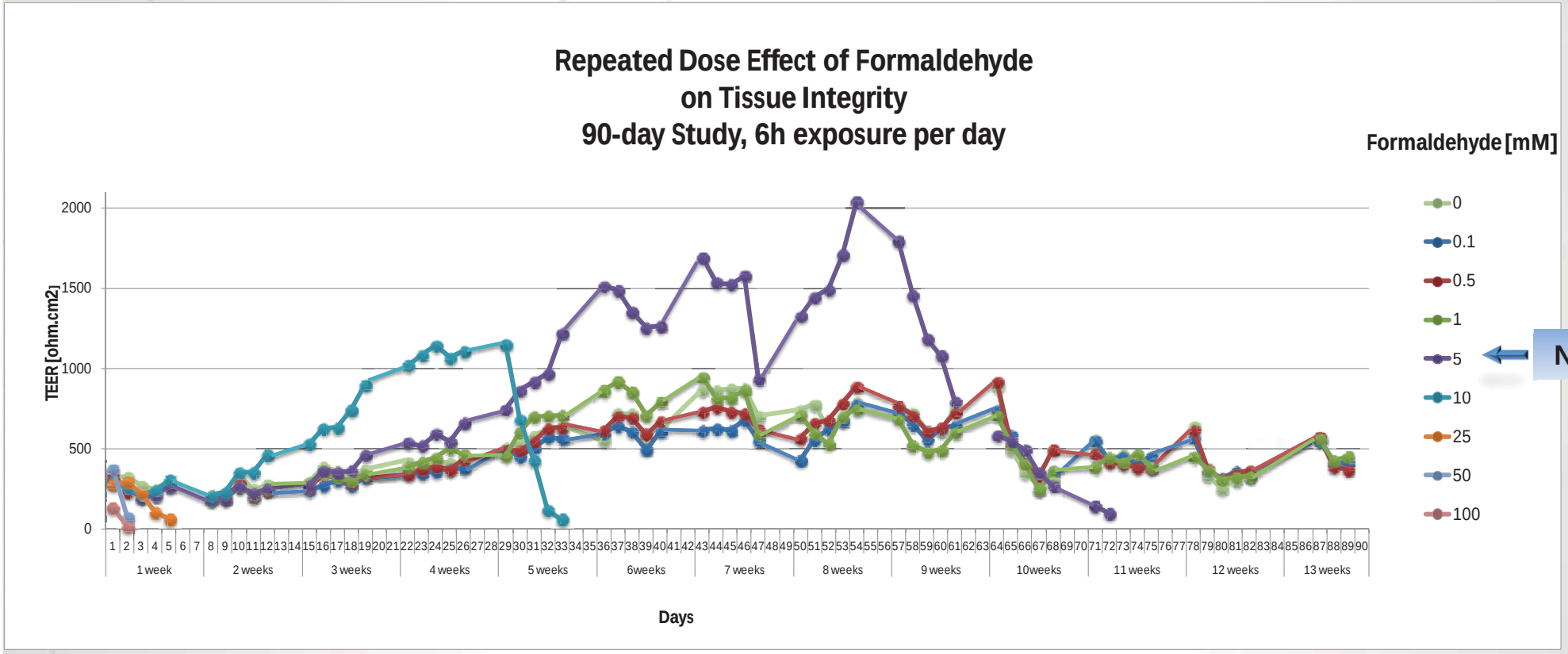


Information from Culture Medium
- Secretion of soluble factors (cytokines/chemokines/ metalloproteinases)
- LDH release

+ Cells information RNA/DNA/Proteins

Repeated Dose Toxicity Testing

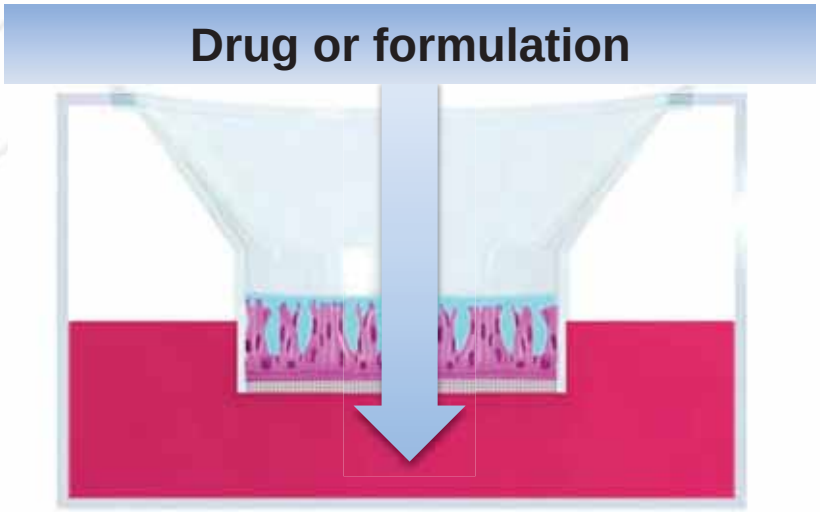
First transposition of the OECD 413 guideline *in vitro*



Example of a 90 days repeated dose exposure study on MucilAir™. 6 hours per day exposure to Formaldehyde for a period of 90 days. Every day, tissue Integrity (TEER) were measured (N=3) then epithelia were reused for the next exposure.

Respiratory Absorption of Drugs/Formulations

Intranasal / intratracheal or intrabronchial drug delivery

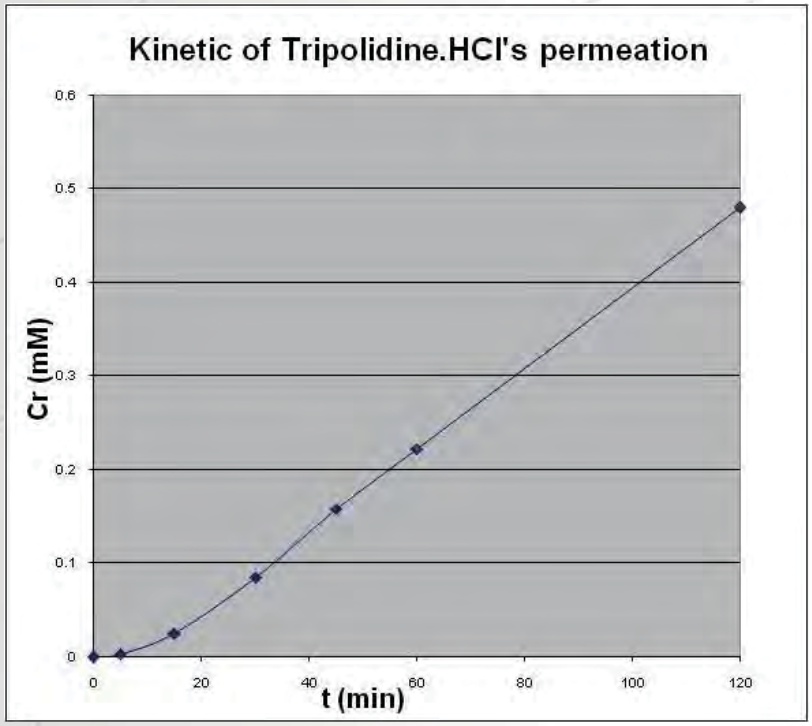


1. What is the amount of active ingredient which can cross the airway epithelium?

2. What are the potential metabolites generated by airway epithelial cells and at which dose can they be found in the blood stream?

3. Ranking of formulation efficiency for drug delivey

Trans epithelial transport of Xenobiotics



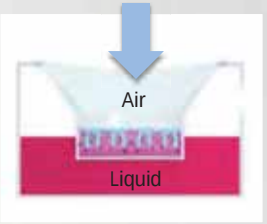
Time course of the rate of permeation of Tripolidine.HCl from the apical to basal lateral side (triplicate)

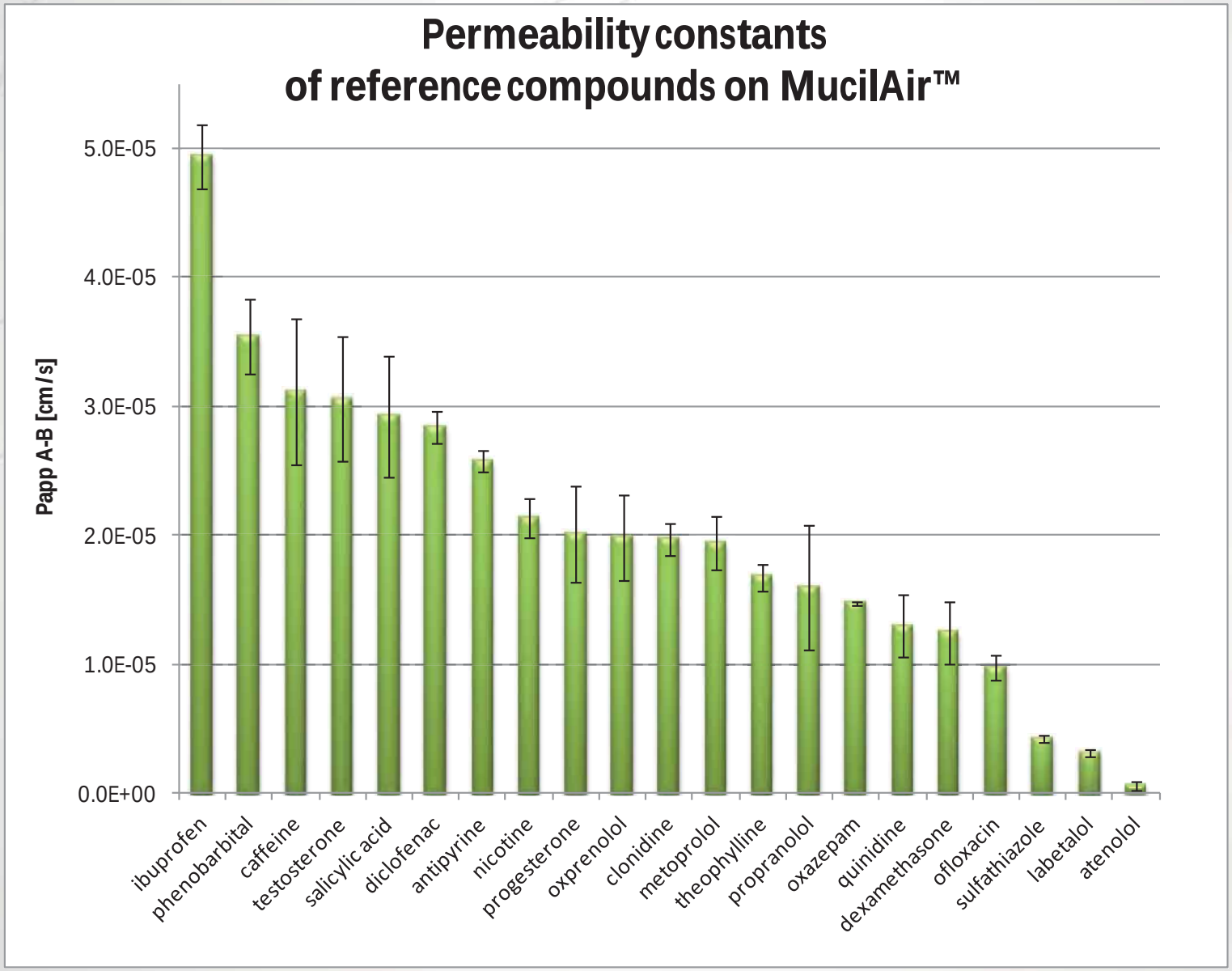
Molecules	Papp (cm/s) A→B	Papp (cm/s) B→A	Asymmetry Ratio
Salicilic Acid	7.7×10^{-5}	1.7×10^{-5}	0.2
Nicotine	2.1×10^{-5}	3.3×10^{-5}	1.6
Propranolol.HCl	1.2×10^{-5}	1.6×10^{-5}	1.3
Ibuprofen	1.1×10^{-5}	1.9×10^{-5}	1.7
Tripolidine.HCl	9.7×10^{-6}	1.2×10^{-5}	1.2
Tetracaïne.HCl	8.0×10^{-6}	1.1×10^{-5}	1.3
Dopamine.HCl	3.0×10^{-6}	2.5×10^{-6}	0.8
Atenolol	2.2×10^{-6}	6.7×10^{-6}	3.0

✓ LC-MS or ICP-MS detection

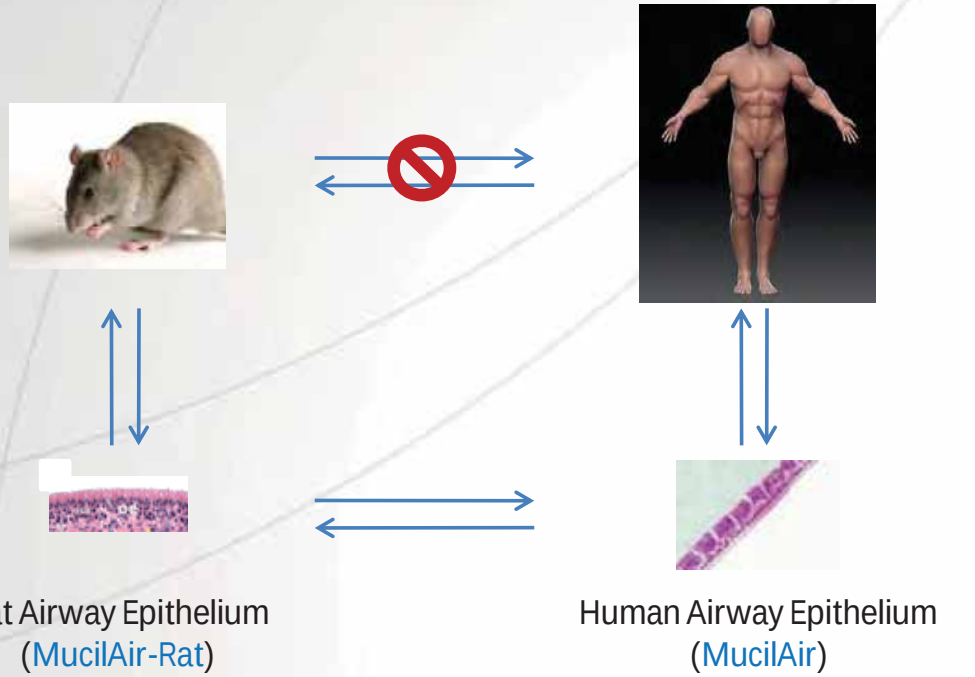
✓ High reproducibility

Trans epithelial transport of Xenobiotics



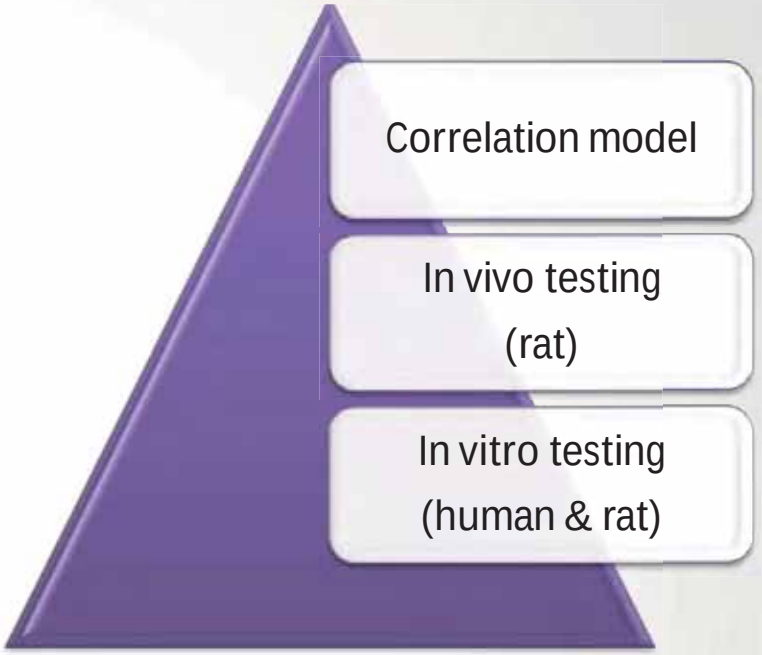


Overcoming barriers: inter-species data transposition



Rat Airway Epithelium
(MucilAir-Rat)

Human Airway Epithelium
(MucilAir)



- Extensive epidemiological and animal data exist
- Difficulty to make extrapolation from animal data to human beings
- Inter species models to bridge the gap for data transposition are needed in certain cases

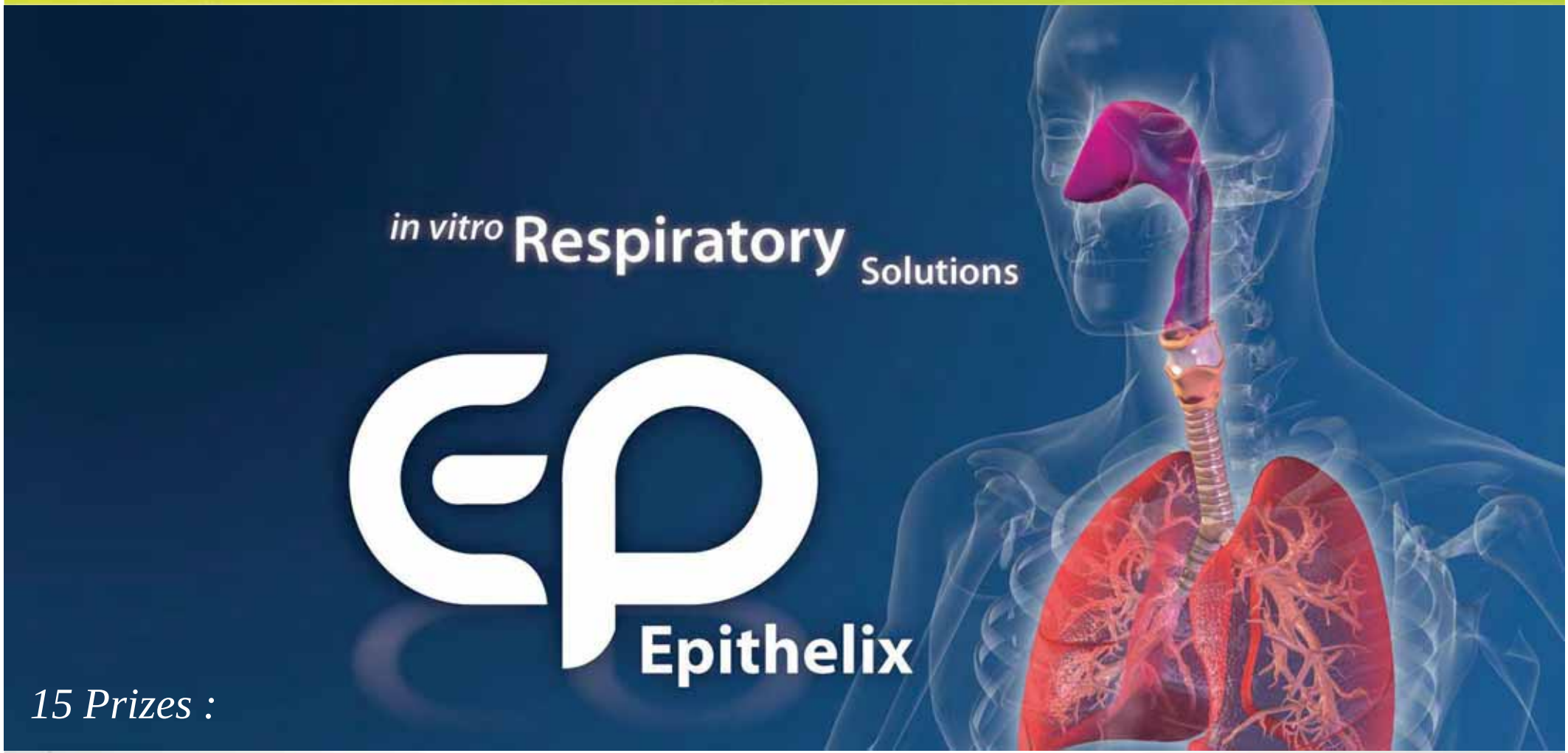
Conclusion: Perceived or real barriers

- Inadequate supply and characterisation of tissues; Practicalities/logistical issues of using fresh tissues; Shelf-life issue, Obtaining ethical documents?
  - ✓ Needs of strong upfront efforts to get ethical approval, optimize supply chain, and transports due to time constraints
- Inter-individual variability?
  - ✓ An advantage: specific tests may benefits of genetic differences in a subpopulation
  - ✓ A problem which can be solved (i.e. Pool of donors)
- Lack of relevant donor information ?
  - ✓ On the tissues: Needs more and more information from the donors (patient stratification)
  - ✓ *In vivo* inhalation data are difficult to obtain (data mainly kept by CROs) – **Needs of reference database**

Conclusion: Perceived or real barriers

- No appropriate models exist in the organ systems needed?
  - ✓ An advantage: for efficacy studies
  - ✓ An inconvenient:Tox data being very different depending on the tested substance (different exposure scenarios, etc..)
  - ✓ Needs of long shelf life 3D human alveolar models made of primary cells
- Perceived regulatory acceptance?
  - ✓ Modelling inhalation toxicology *in vitro* very challenging which may rely on an integrated testing strategy (i.e. Barrier effect; local tolerance, sensitization, carcinogenicity, etc...)
- Lack of specific expertise in human tissue work / Cost / Licensing issues with cells / shipment of tissues to end-user?
  - ✓ Not applicable

Thanks for your attention



15 Prizes :

CTI CERTIFIED

CFSCI

THE WORLD TECHNOLOGY AWARDS 2006
NOVEMBER 3, 2006 • SAN FRANCISCO

RED HERRING WINNER 100 EUROPE

venturelab

Eclosion

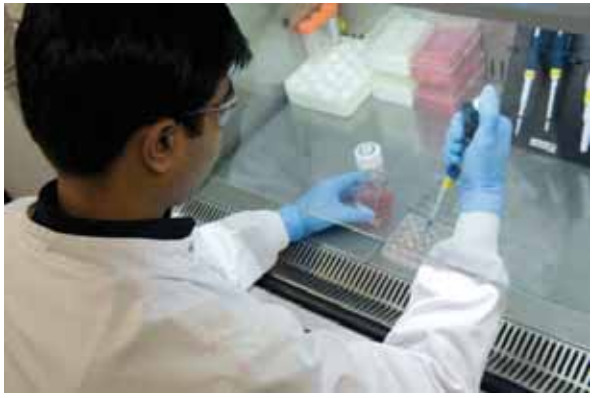
FENRIV

Ligue suisse contre la vivisection

FONDATION W.A.DE VIGIER

Fondation Dr. René Liechti

opi



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Overcoming the barriers to wider uptake of human tissue for safety assessment
15th July, 2014

Fresh, functional human tissues and the prediction of drug safety

Dr David Bunton, CEO, Biopta Ltd

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

MHRA Regulating Medicines and Medical Devices

Human tissue and drug discovery and development

- Human tissue is increasingly in demand during drug R&D
- Data from fresh, functional human tissues is being used to: -

  - Gain confidence about clinical efficacy and understand patient variation (stratified medicine)

  - Identify and understand potential safety pharmacology concerns

- In comparison to animal studies, its use remains relatively infrequent, **but is there scope, and a good reason, to increase its role in safety assessments?**

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Example of a safety pharmacology experiment in fresh human tissue

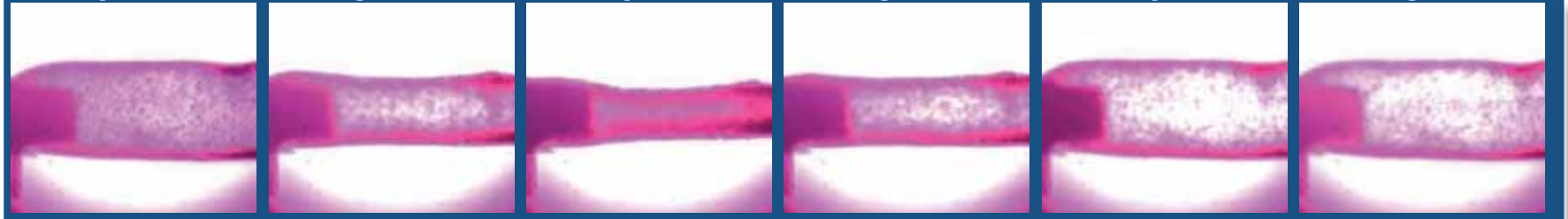
Ethically-donated residual surgical or transplant tissue

Tissue transported immediately to laboratory

Scientist isolates the specific tissues of interest

Multiple tissues e.g. bronchi or pulmonary arteries isolated from sample

The “living” tissues are exposed to drugs



Translational data is generated in the tissue of interest

↑ blood pressure

↓ blood pressure

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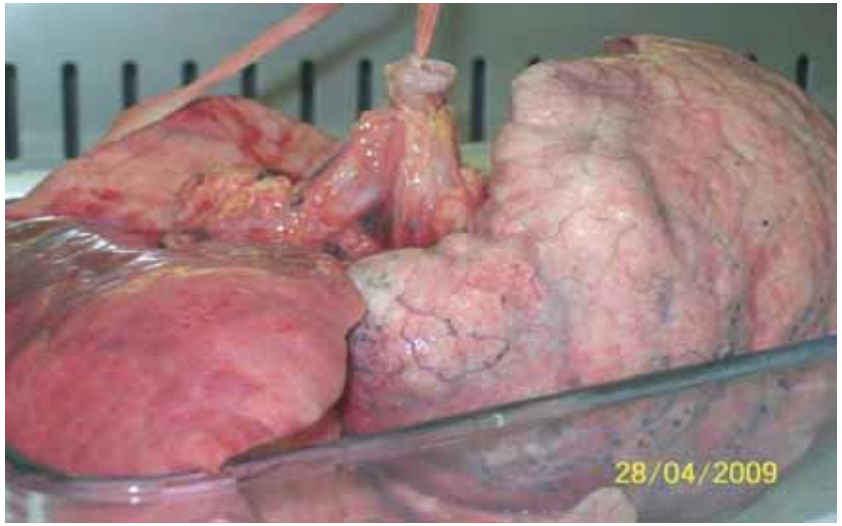
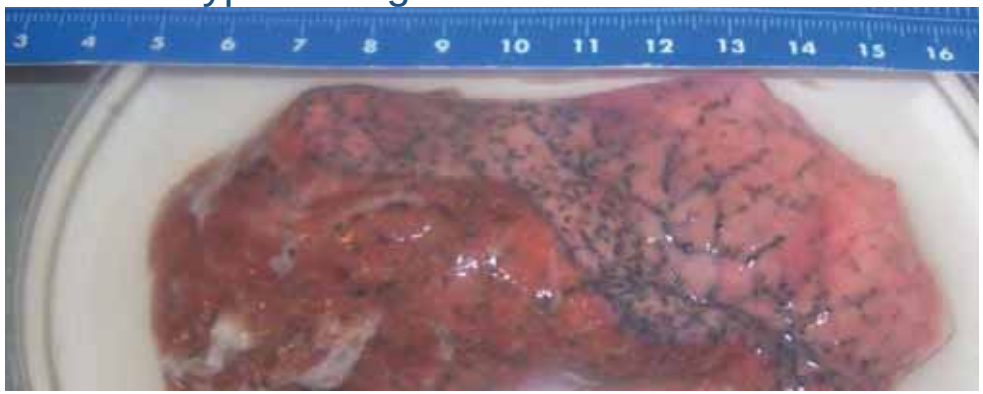
Human *Ex Vivo* Lung Assays and Tissue Availability

- Large lung samples are available from transplant network and surgical resections (healthy and diseased)
- In the US, over 11,000 referrals for transplant organs are made per annum[1]
- In the UK, there are over 650,000 surgical procedures per annum, including over 7,000 lung excisions[2]

Whole lung from transplant network



A typical surgical resection tissue



1. Baldasare, D. *Cell Tissue Bank* (2010)
2. Hospital episode statistics England and Wales 2011-12

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Human tissue experiments relevant to safety pharmacology guidelines (ICH S7A)

Human tissue	Endpoint	Regulatory test	Regulatory Document
Cardiac muscle	Action potential duration	Cardiovascular telemetry core battery	ICH S7A Section 2.7.2
Coronary artery	Coronary artery vasospasm	Cardiovascular telemetry core battery	ICH S7A Section 2.7.2
Resistance arteries	Vasoconstriction/dilatation Control of organ blood flow and main determinant of peripheral vascular resistance	Cardiovascular telemetry core battery in vivo animal: measures of BP and blood flow (by plethysmography)	ICH S7A Section 2.7.2
Bronchi	Bronchoconstriction/dilatation	Respiratory core battery	ICH S7A Section 2.7.3
Stomach or intestinal smooth muscle	GI motility- detection of undesirable side-effects on transit time and fluid secretion	Recommended as a supplementary study as part of ICHS7A section 2.8.2.3- transit time	ICH S7A Section 2.8.2.3

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Why do safety pharmacologists request human fresh lung tissue studies?

- Unexpected observations during clinical trials
- Observations from *in vivo* animal models
- Known species differences for the target/mechanism
- Requests from regulators
- Disconnect between focus of ICH S7A guidelines on pulmonary ventilation and desire to understand drug effects on airway resistance or lung compliance

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Respiratory safety assessment: which endpoints should we consider?

Ventilatory Apparatus

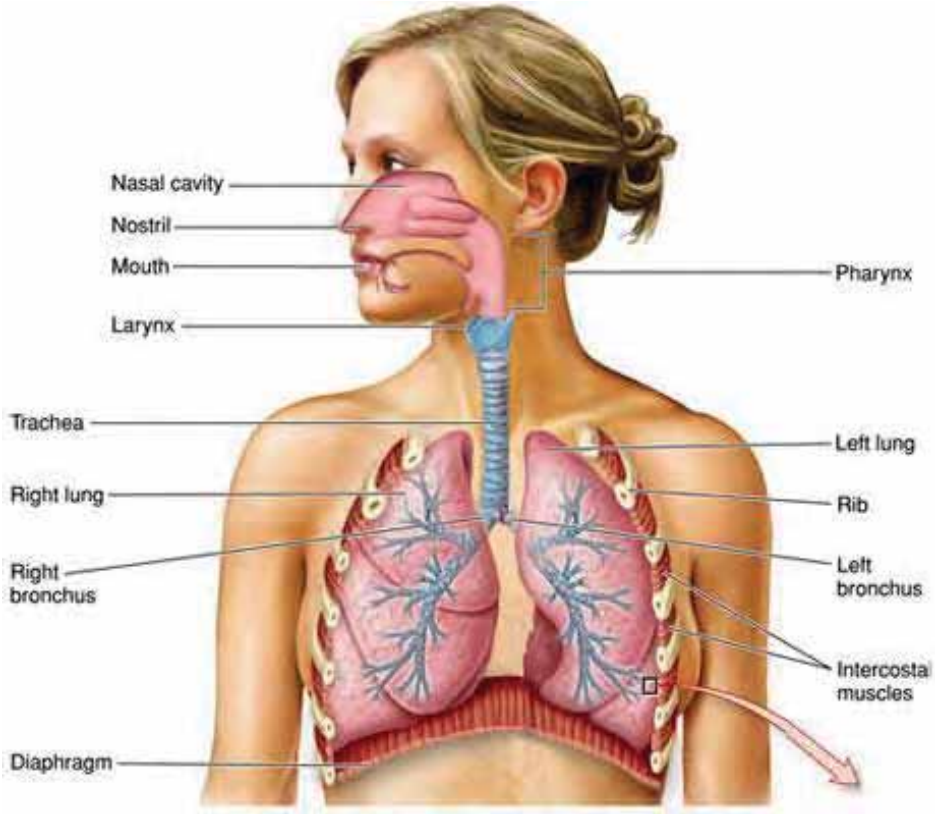
↓

Resp. muscles, CNS, chemo/mechano-receptors

↓

Tidal volume, respiratory rate, minute volume

Main focus of ICHS7A



Gas Exchange Unit

↓

Airways, alveoli, vasculature, parenchyma

↓

Airways resistance, lung compliance, diffusion capacity, P_aO_2/P_aCO_2

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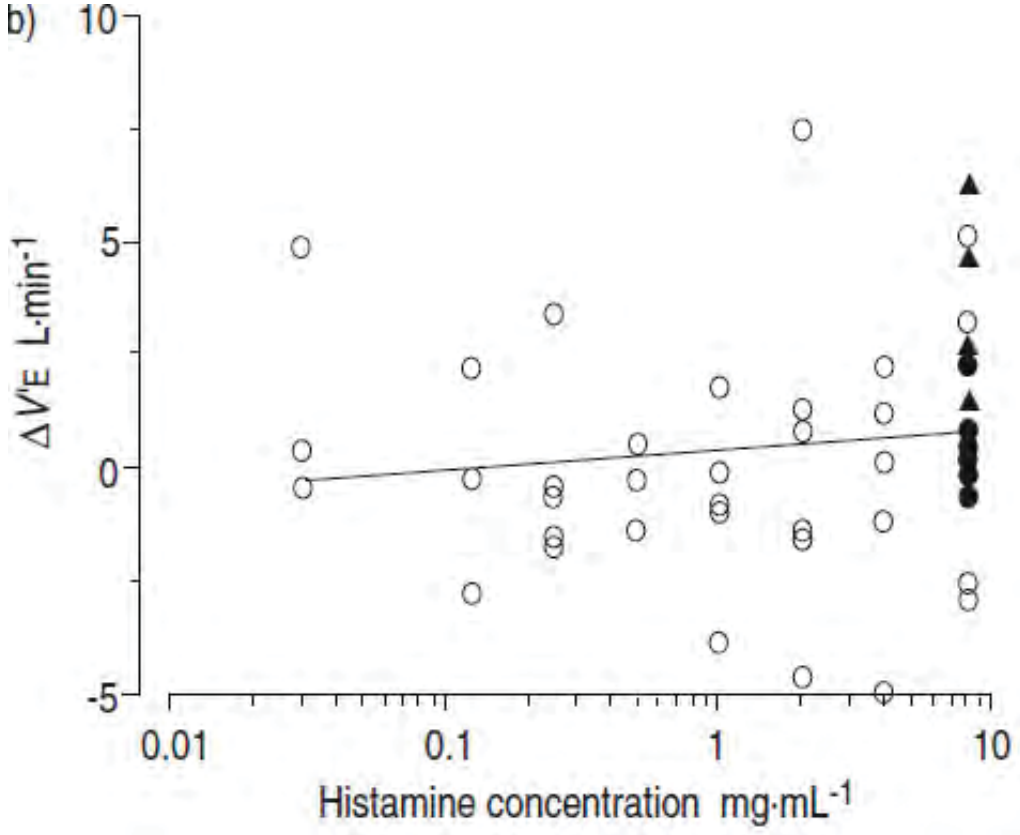
Monitoring ventilation is not a sensitive assessment of the gas exchange unit

- While FEV_1 is a useful parameter clinically, measurements of tidal volume, respiratory and minute volume are not particularly sensitive indicators of an increase in airway resistance or compliance

- Ventilatory responses to induced bronchoconstriction are not consistent in animals or humans [1, 2, 3]

- Therefore, changes in the gas exchange units are not sensitively detected from measurements of ventilation; however,

does this matter?



Meesen *et al.* (1997)

1. Murphy, D.J. (2014) Reg Tox Pharmacol 69: 135-140
2. Meesen, M.E.L. *et al.* (1997) Eur Resp J. 10: 1059-1063
3. Stromberg N.O.T. & Gustafsson, P.M. (1993) Eur Resp J. 6:1126-1131.

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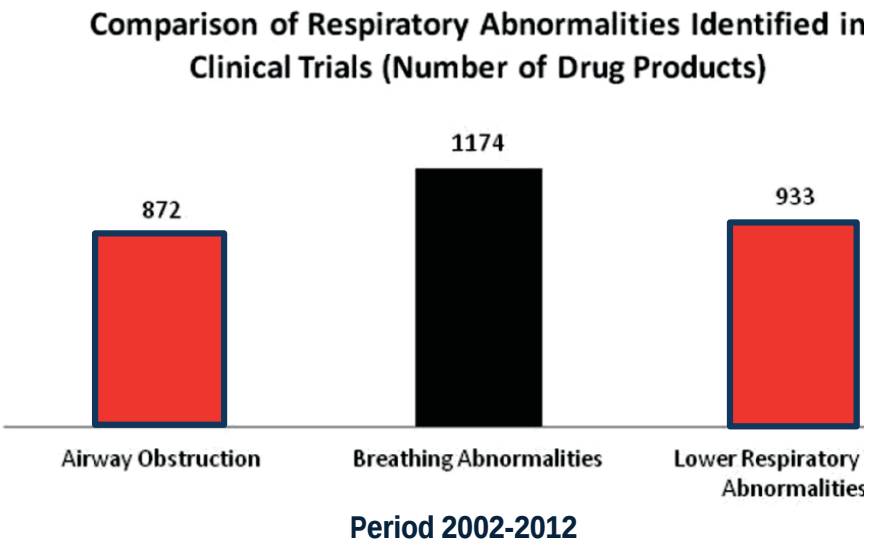
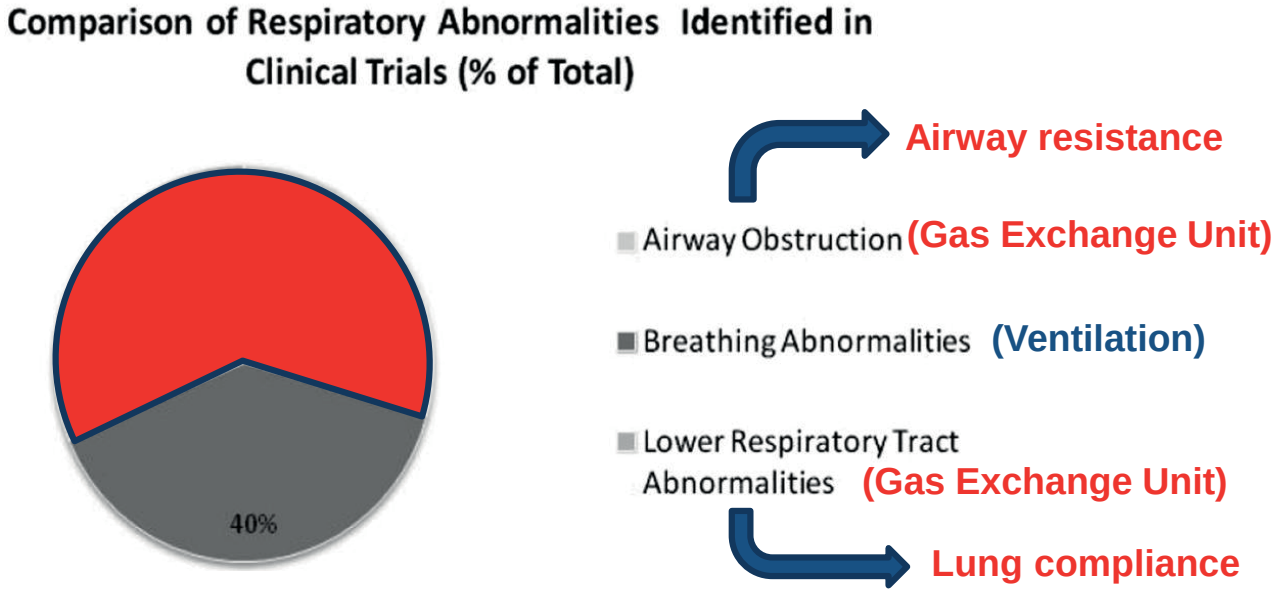
Respiratory safety assessment: which endpoints relate to clinical adverse effects?





Denis J. Murphy
Department of Safety Pharmacology,
GlaxoSmithKline Pharmaceuticals, King of Prussia, PA, USA
Regulatory Toxicology and Pharmacology 69 (2014) 135–140

Why not use *in vitro* animal models?

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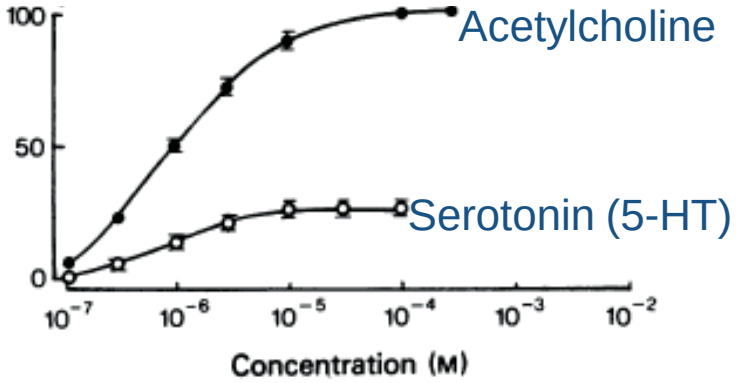
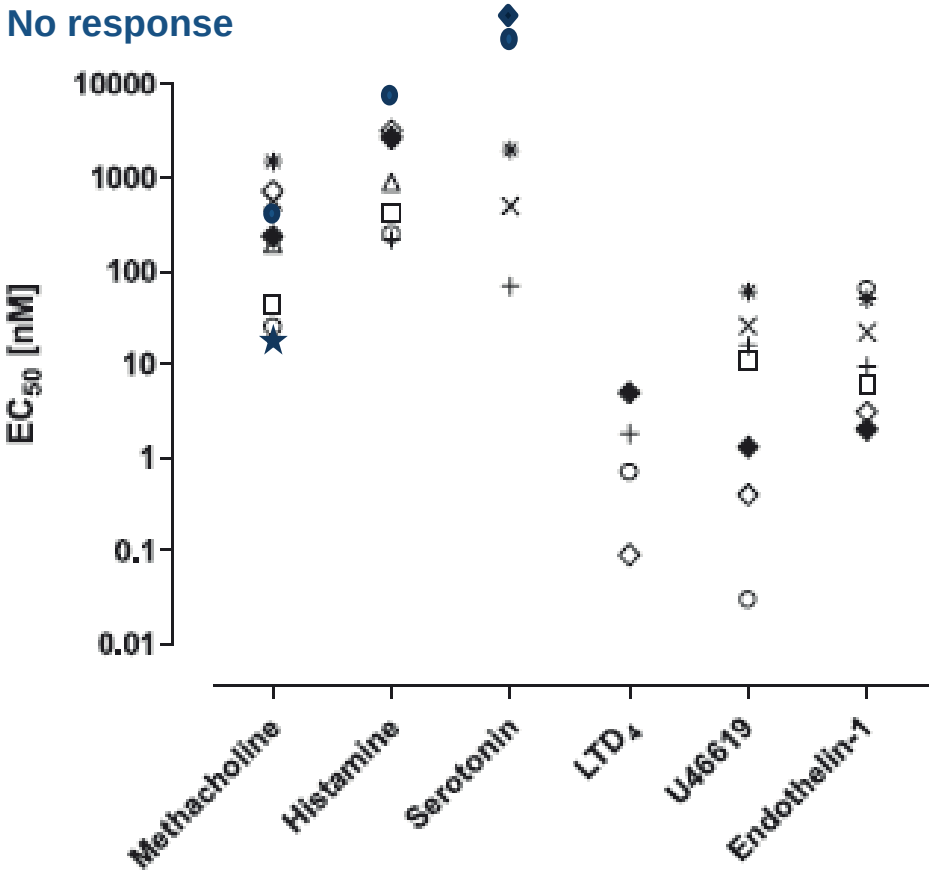
Do species differences exist?

Rat trachea/ bronchi



Human bronchi:
no response to serotonin

No response



- Human
- Marmoset
- Rhesus macaque
- Baboon
- Cyno. macaque
- Rat
- Mouse
- Pig
- Guinea pig
- Dog

Adapted from:
Seehase *et al.* (2011) *J. Appl. Physiol.* 111: 791-798
Downes *et al.* (1986) *J Pharmacol. Exp. Ther.* 237:214-219

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Human fresh lung tissues: measurement of relevant functional endpoints

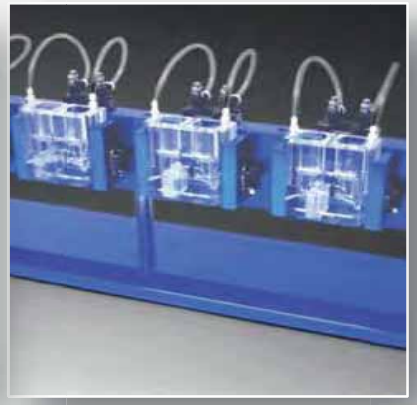
Tissue Baths and Wire Myographs

Bronchoconstriction and dilatation large and small airways (resistance)

Nerve-muscle interaction

Perfusion Myographs

Pulmonary vascular permeability and contractility: oedema (compliance)

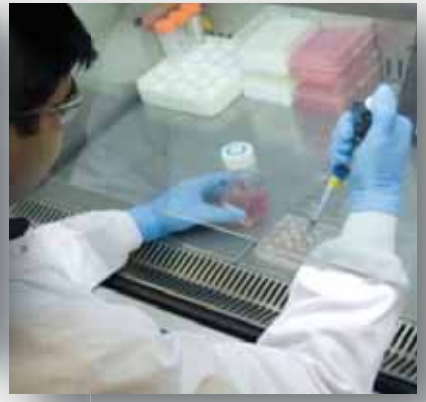


Ussing Chambers

Ion channels
Mucus and serous fluid production (airway resistance)

Precision-Cut Lung Slices

Fibrosis- changes in lung compliance
Immunotoxicity, chemotoxicity



Ex vivo cultures

Mediator release assays (e.g. cyokines, mucus)

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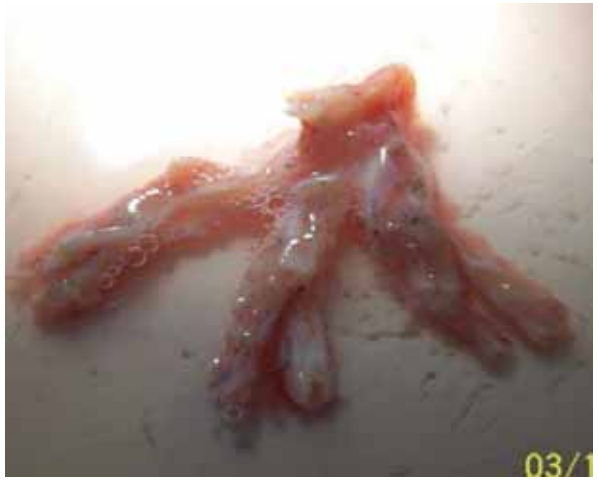
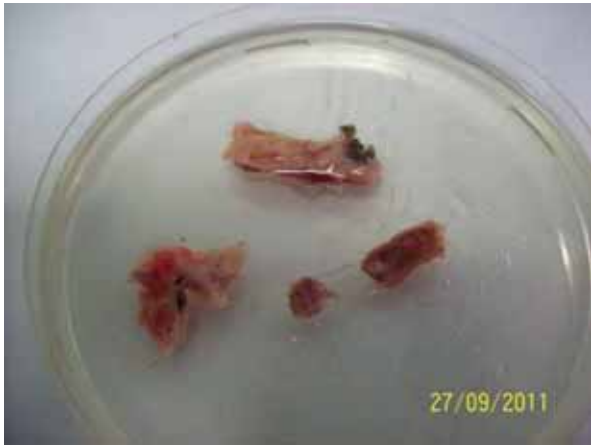
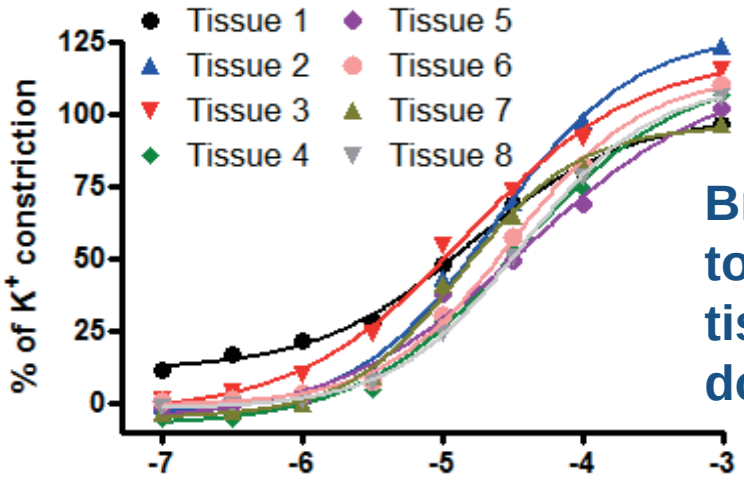
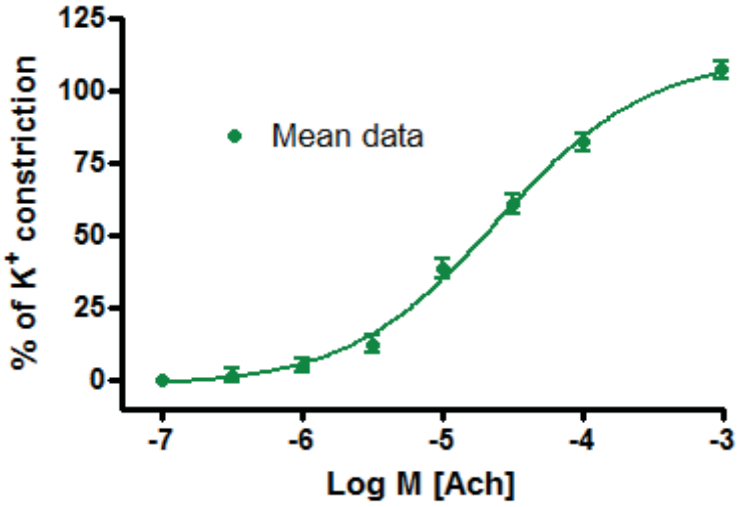
Airway resistance and compliance can be measured in human isolated bronchi









Bronchoconstriction to acetylcholine: 8 tissues from the same donor

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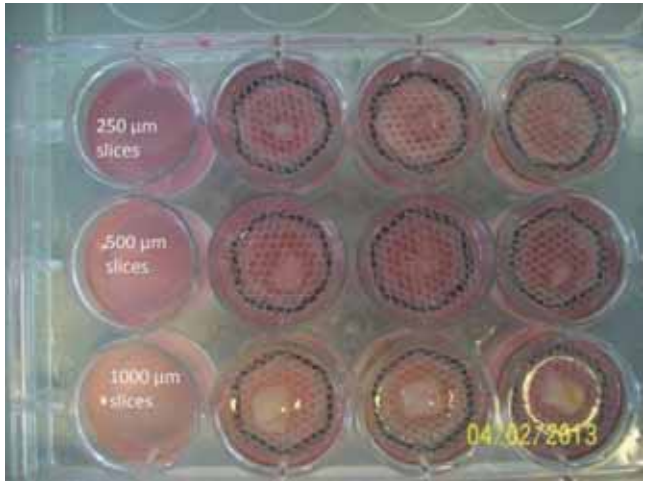
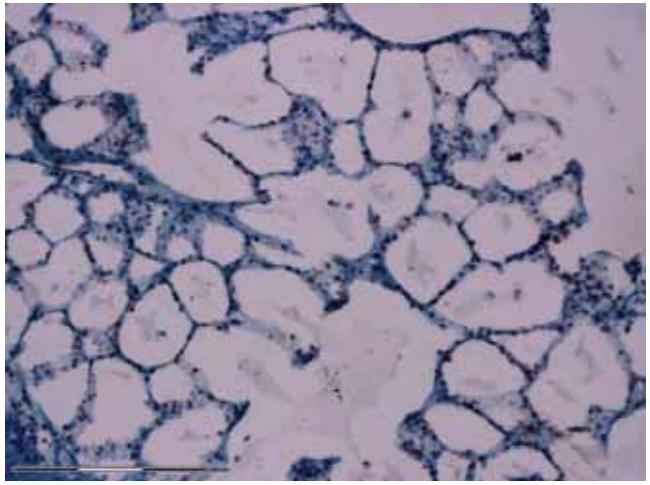
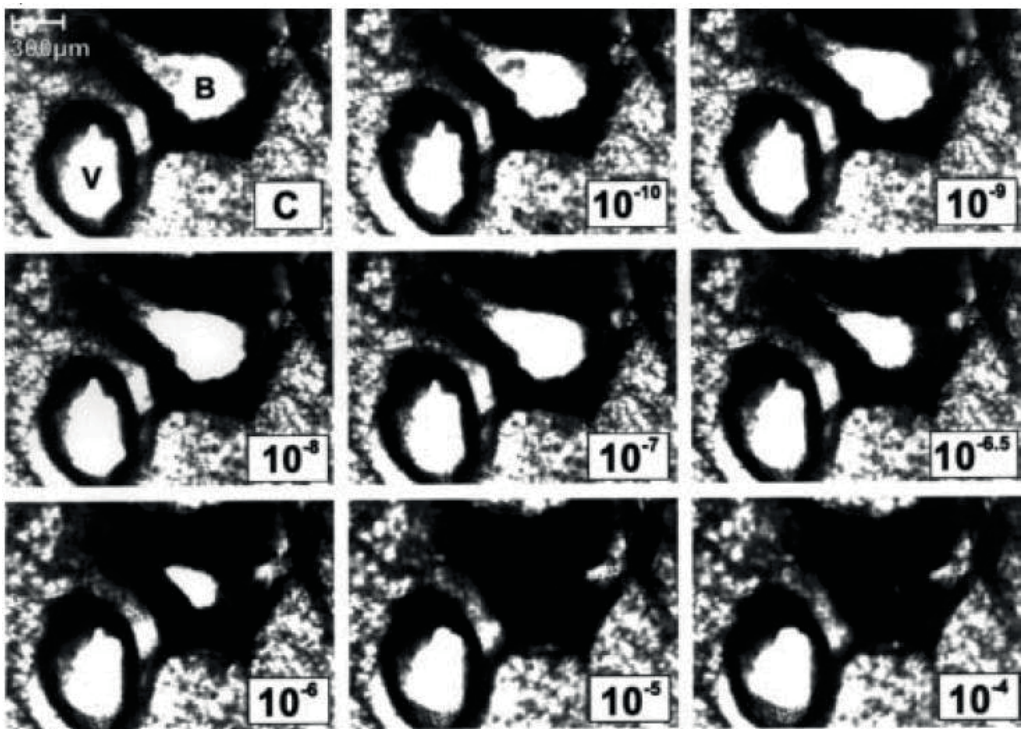
Precision-Cut Lung Slices (PCLS)







- Increased throughput
- Reproducibility
- Ability to investigate bronchioles, alveoli
- Showing promise for chemical tox studies

Ref: Martin *et al.* (1996)

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Precision-cut lung slices used to predict chemical immunotoxicity

Table 1
Human PCLS were exposed to 20 chemicals including 4 respiratory allergens, 11 contact allergens, and 5 non-sensitizing irritants under submerged conditions for 24-h in serum-free DMEM. EC_{25} (effective concentration at 25% reduction of cell viability) and EC_{50} (effective concentration at 50% reduction of cell viability) values of chemicals were calculated on the basis of dose–response curves assessed by WST-1 reduction (Supplement 2). EC_{25} values published for a human promyelocytic cell line (THP-1) and a human keratinocyte cell line (NCTC), and LD_{50} values from published data of inhalation studies with rats exposed to the same chemicals were found (online databanks: ChemIDplus Lite, TOXNET, and NIOSH Pocket Guide to Chemical Hazards). The deposited doses were calculated. abbr: abbreviation; inhal: inhalation.

Chemical abbr.	Chemical name	Category	Human PCLS EC_{25} (μg/mL)	Human PCLS EC_{50} (μg/mL)	THP-1 EC_{25} (μg/mL)	NCTC EC_{25} (μg/mL)	Rat inhal LD_{50} (mg/m^3)	Rat inhal deposited dose (mg)
HCIPt	Ammonium hexachloroplatinate	Respiratory allergen	66	193	15	15	565/8 h	1.36
GA	Glutaraldehyde	Respiratory allergen	19	58	Unknown	Unknown	480/4 h	1.15
TMA	Trimellitic anhydride	Respiratory allergen	256	761	>500	>500	>2330/4 h	5.60
MA	Maleic anhydride	Respiratory allergen	188	576	Unknown	Unknown	>4350/4 h	10.45
2-Bro	1,2-Dibromo-2,4-dicyanobutane	Contact allergen	8.1	24	Unknown	Unknown	Unknown	Unknown
CinAld	Cinnamaldehyde	Contact allergen	48	147	20	40	Unknown	Unknown
CinOH	Cinnamyl alcohol	Contact allergen	97	286	Unknown	300	Unknown	Unknown
DNCB	2,4-Dinitrochlorobenzene	Contact allergen	1.7	5.2	5	5	Unknown	Unknown
Eug	Eugenol	Contact allergen	55	159	Unknown	225	2580/4 h	6.20
PPD	p-Phenylenediamine	Contact allergen	12	36	22	108	920/4 h	2.21
Res	Resorcinol	Contact allergen	1895	5625	Unknown	>1000	>7800/4 h	18.75
TMTD	Tetramethylthiuram disulfide	Contact allergen	6	19	5	32	500/4 h	1.20
Glyo	Glyoxal	Contact allergen	0.051	0.16	Unknown	Unknown	2.440/4 h	0.01
2-Mer	2-Mercaptobenzothiazole	Contact allergen	32	93	Unknown	125	1270/4 h	3.05
4-Nit	4-Nitrobenzyl bromide	Contact allergen	0.6	1.9	Unknown	2.5	Unknown	Unknown
Phe	Phenol	Irritant	>250	>250	70	70	316/4 h	0.76
LA	Lactic acid	Irritant	256	761	Unknown	>500	7940/4 h	19.10
SA	Salicylic acid	Irritant	200	585	250	250	900/1 h	2.16
SLS	Sodium dodecyl sulfate	Irritant	19	55	30	30	Unknown	Unknown
Glyc	Glycerol	Irritant	25	77	Unknown	>1000	570/1h	1.37

Lauenstein *et al.* (2014)
Toxicol in vitro 28: 588-599

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Key Points

- There exists a sufficient supply of human tissue to allow the conduct of studies in a timely manner and a positive impact on the 3Rs
- Avoids species differences
- Most importantly, such studies have the potential to more closely predict respiratory adverse effects in the clinic

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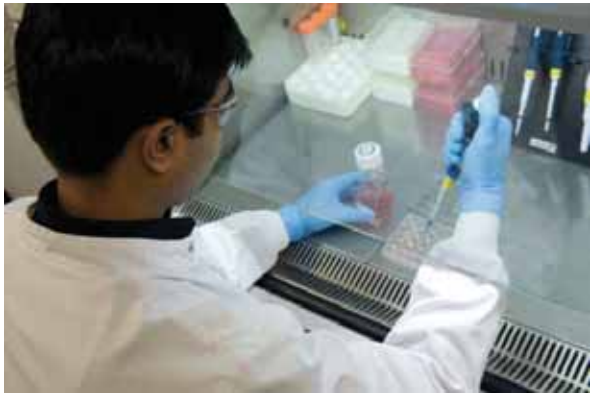

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Thank You

Email: davidbunton@biopta.com

100 years of life-changing discoveries

MRC Medical Research Council
CENTENARY 1913-2013

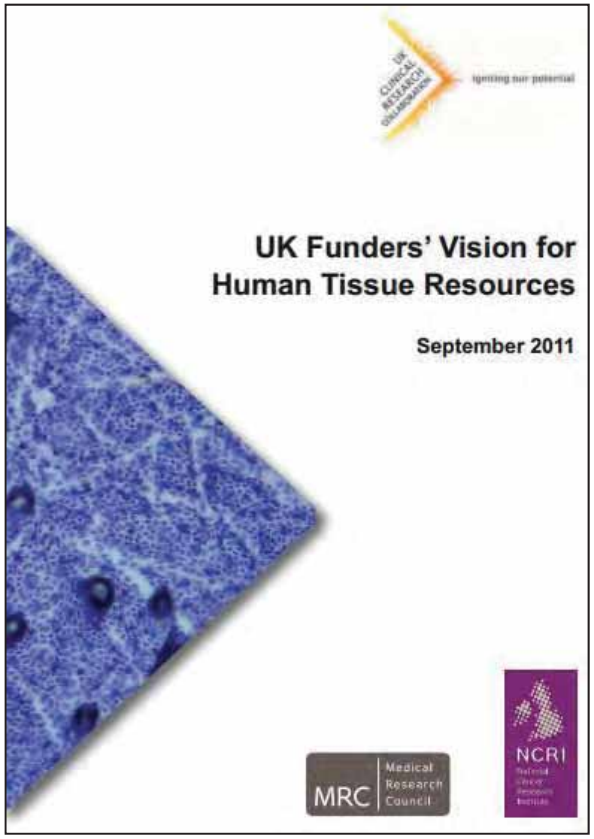
UKCRC Joint Funders Tissue Directory Call

Dr Jon Fistein

Background

- The UKCRC Experimental medicine Funders Group vision for funded collections of human tissue and biosamples was published in September 2011.
- Among other recommendations, it identified the need for systems to make collections more discoverable (Action 9).

- See: http://www.ukcrc.org/research-infrastructure/experimental-medicine/funders-vision-for-human-tissue-resources/



Aims

- To develop and deliver a functional Resource Finder/Directory to enable researchers to discover, search across and contact multiple human tissue and biosample collections via a unified interface (taking account of existing systems) on order to facilitate sample access. The Centre will be expected to provide an evaluation of the system, in terms of usability, effectiveness in providing the ability to locate relevant samples, a demonstration of the benefits of the chosen approach, and an appraisal of potential options for a second phase of development, for example with increased metadata content;
- To provide coordination and guidance to increase harmonisation of standards at specific points across the entire biosample lifecycle;
- To build and manage engagement between researchers, biosample collections, the public, regulators and policy makers supporting evidence based approaches to sample collection, governance and public engagement.

Logistics

Evaluation Criteria:

- Data and informatics vision
- Knowledge of and engagement with the current research data environment
- Relationship management
- Project management
- Cost

Funding & Timetable:

- £905k over three years, for a single centre.
- Funding decision early August 2014
- Award to start Autumn 2014

Questions

Further information:

http://www.mrc.ac.uk/funding/browse/ukcrc-joint-funders-tissue-directory-and-coordination-centre/