Adult Human Primary Cardiomyocytes for Drug-induced Contractility Risk Detection

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A human cardiomyocyte model is urgently needed for detection of drug-induced cardiotoxicity.
Enabling Drug Discovery in Human Healthy and Diseased Tissues

➢ Tissue harvesting methods and solutions are designed to avoid ischemic damage and reperfusion injury
➢ Complete chain of custody, processing methods and rigorous QC ensure excellent tissue quality
➢ Large U.S.A.-based network ensures the availability of samples
➢ Excellent heart quality permits integrated human cardiac drug discovery at the preclinical stages
Comprehensive Drug Discovery
in Ex-Vivo Healthy Human Cardiac Models

> 1000 **ex vivo** human hearts tested

**CELL-BASED ASSAYS**

*Optimization of drugs*

- Pro-arrhythmia & Inotropy
  - Ventricular Myocytes
- Arrhythmia & Inotropy
  - Atrial Myocytes
- Ca\(^{2+}\) Assay
  - Ventricular Myocytes
- Ion Channel
  - Ventricular & Atrial Myocytes
- Action Potential
  - Ventricular & Atrial Myocytes
- Cardiac Fibrosis
  - Cardiac Fibroblasts

**TISSUE-BASED ASSAYS**

*Nomination of drugs*

- Pro-arrhythmia
  - Action Potential
    - Ventricular Trabeculae
- Inotropy
  - Contractility
    - Ventricular & Atrial Trabeculae
- Chronotropy
  - Spontaneous Action Potential
    - Sinoatrial Node
- Vaso-constriction
  - Dilation
    - Coronary Rings
New Isolation Method Provides High Yield of Cardiomyocytes

> 1000 ex vivo human hearts tested
Non-Invasive Measurement of Contraction
Full Retention of Cardiomyocyte Functionality

- Bright-field imaging
- Low technical complexity
- No cytotoxic fluorescent reagents
- High information content

IonOptix: Sarcomere shortening measured by digital cell geometry tracking; stimulation frequency 1Hz
Validating Clinical Relevance of Negative Inotropes

- Validated 33 clinical well characterized controls:

  1) 27 multichannel blockers (mainly $K^+$, $Na^+$ and $Ca^{2+}$ channels) as positive controls

  2) 6 selective hERG blockers as negative controls

  3) Each drug was tested at multiples of the free Effective Therapeutic Plasma Concentration (fETPC, mimic pharmacokinetic aspect)
Verapamil Induces Negative Inotropic Effect
Identification of Negative Inotropic Effects and Determination of Exposure Responses

Table 5. Sarcomere shortening effects for reference drugs measured in adult human primary cardiomyocytes

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Top test concentration (μM)</th>
<th>Human myocyte effect</th>
<th>IC$_{50}$ (μM)</th>
<th>Ratio (IC$_{50}$/ETPC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaline</td>
<td>1.53</td>
<td>-ve inotrope</td>
<td>2</td>
<td>31</td>
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<tr>
<td>Azemolol</td>
<td>0.009</td>
<td>No effect</td>
<td>&gt;0.009</td>
<td>30</td>
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<tr>
<td>Azimilide</td>
<td>2.1</td>
<td>-ve inotrope</td>
<td>1.07</td>
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<tr>
<td>Bepridil</td>
<td>0.96</td>
<td>-ve inotrope</td>
<td>0.7</td>
<td>22</td>
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<tr>
<td>Chlorpromazine</td>
<td>1.04</td>
<td>-ve inotrope</td>
<td>1.02</td>
<td>28</td>
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<tr>
<td>Cisapride</td>
<td>0.26</td>
<td>-ve inotrope</td>
<td>0.02</td>
<td>8</td>
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<tr>
<td>Clarithromycin</td>
<td>1.20</td>
<td>-ve inotrope</td>
<td>0.13</td>
<td>13</td>
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<tr>
<td>Clozapine</td>
<td>2.13</td>
<td>-ve inotrope</td>
<td>1.5</td>
<td>21</td>
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<tr>
<td>D, L-Sotalol</td>
<td>4.50</td>
<td>No effect</td>
<td>&gt;450</td>
<td>&gt;30</td>
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<tr>
<td>Diclopinamide</td>
<td>2.1</td>
<td>-ve inotrope</td>
<td>9.3</td>
<td>13</td>
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<tr>
<td>Dofetilide</td>
<td>0.2</td>
<td>No effect</td>
<td>&gt;0.2</td>
<td>&gt;100</td>
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<tr>
<td>Domperidone</td>
<td>2.1</td>
<td>-ve inotrope</td>
<td>0.2</td>
<td>10</td>
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<tr>
<td>Droperidol</td>
<td>0.48</td>
<td>-ve inotrope</td>
<td>0.18</td>
<td>11</td>
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<tr>
<td>Estradiol</td>
<td>5.1</td>
<td>No effect</td>
<td>&gt;5.1</td>
<td>&gt;30</td>
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<tr>
<td>Flecainde</td>
<td>22.6</td>
<td>-ve inotrope</td>
<td>1.1</td>
<td>2</td>
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<tr>
<td>Ibutilide</td>
<td>3</td>
<td>-ve inotrope</td>
<td>2</td>
<td>20</td>
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<tr>
<td>Moxifloxacin</td>
<td>329</td>
<td>No effect</td>
<td>&gt;329</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>11.2</td>
<td>-ve inotrope</td>
<td>14</td>
<td>34</td>
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<tr>
<td>Procainamide</td>
<td>1625</td>
<td>-ve inotrope</td>
<td>2215</td>
<td>38</td>
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<tr>
<td>Quinidine</td>
<td>100</td>
<td>-ve inotrope</td>
<td>3.6</td>
<td>1</td>
</tr>
<tr>
<td>Sematilide</td>
<td>133</td>
<td>No effect</td>
<td>&gt;133</td>
<td>&gt;30</td>
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<tr>
<td>Terodiline</td>
<td>43.5</td>
<td>-ve inotrope</td>
<td>0.7</td>
<td>5</td>
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<tr>
<td>Vaughanetan</td>
<td>9</td>
<td>-ve inotrope</td>
<td>2.7</td>
<td>9</td>
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<tr>
<td>Diltiazem</td>
<td>3.84</td>
<td>-ve inotrope</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Diphenhydrarnine</td>
<td>1.02</td>
<td>-ve inotrope</td>
<td>0.6</td>
<td>17</td>
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<tr>
<td>Loratadine</td>
<td>0.0135</td>
<td>-ve inotrope</td>
<td>0.0135</td>
<td>35</td>
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<tr>
<td>Moxalidine</td>
<td>72</td>
<td>-ve inotrope</td>
<td>0.9</td>
<td>0.4</td>
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<tr>
<td>Mibefradil</td>
<td>0.36</td>
<td>-ve inotrope</td>
<td>0.18</td>
<td>13</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>0.23</td>
<td>-ve inotrope</td>
<td>0.04</td>
<td>5</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>0.091</td>
<td>-ve inotrope</td>
<td>0.06</td>
<td>18</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>200</td>
<td>-ve inotrope</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Tamsulosin</td>
<td>0.063</td>
<td>-ve inotrope</td>
<td>0.99</td>
<td>36</td>
</tr>
<tr>
<td>Verapamil</td>
<td>10</td>
<td>-ve inotrope</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

IC$_{50}$: Concentration inducing 50% decrease in sarcomere shortening. Hill equation using SigmaPlot v13 was fitted to sarcomere shortening concentration-effect curves, assuming drugs would eventually cause complete inhibition of the contractility when they decreased sarcomere shortening by ≥50%. *: CYP3A-selected drug, **: ETPC: free effective therapeutic plasma concentration.

Nguyen et al., 2017 FiP
Inhibition of Kinase Activity to Control Tumor Growth Can Lead to Cardiotoxicity

Dysregulation of tyrosine kinases

Progression to many cancers

Tyrosine Kinase inhibitors (TKIs)

effective cancer treatment

TKIs induce cardiotoxicity

heart failure
reduced left ventricular ejection fraction
myocardial infarction
arrhythmias
Validating Clinical Relevance of Cancer Agents

- Validated 9 clinical well characterized controls:
  1) 4 toxic TKIs (Sorafenib, Vandetanib, AZD7762, Imatinib)
  2) 4 non-toxic TKIs (Erlotinib, Dasatinib, Afatinib, Gefitinib)
  3) One toxic anthracycline (Doxorubicin)
  4) Each drug was tested at multiples of the Cmax
  5) Each concentration was perfused for 5 mins
Sorafenib Induces Functional Cardiotoxicity
Sorafenib Induces Structural Cardiotoxicity
Afatinib Induces No Functional or Structural Cardiotoxicity

0.3μM = 3-fold Cmax
1μM = 10-fold Cmax
3μM = 30-fold Cmax
Tyrosine Kinase Inhibitors Affect Human Cardiomyocyte Contractility

Similar human cardiac tissue data recently published by Schneider C et al., 2018 Nature Scientific Reports
*: Limit of solubility
Low Inter- and Intra-Heart Variability

**AZD7762** (µM)
- Heart 1, IC50=1.2µM
- Heart 2, IC50=0.89µM

**Vandetanib** (µM)
- Run 1, IC50=5.6µM
- Run 2, IC50=4.6µM

**Afatinib** (µM)
- Run 1
- Run 2

**Erlotinib** (µM)
- Run 1
- Run 2
Doxorubicin, Anthracycline Agent, Affects Human Cardiomyocyte Contractility

Clinically relevant conc.
Sorafenib Decreases Force in Contracting Human Myocardia

The Anti-Cancer Multikinase Inhibitor Sorafenib Impairs Cardiac Contractility by Reducing Phospholamban Phosphorylation and Sarcoplasmic Calcium Transients

Christopher Schneider1, Markus Wallner2, Ewald Kolenski3, Viktoria Herbst4, Heinrich Mächler5, Martin Pichler6,7, Dirk von Lewinski8, Simon Sedej6,8 & Peter P. Rainer5,7

Developed Force [% of Lmax]

- Control
- Sorafenib

Lmax 1μM 3μM 10μM 30μM Washout

These results indicate myocyte intrinsic cardiotoxicity irrespective of effects on the vasculature and chronic cardiac remodeling.
Protein Kinases in Human Cancer

Cancer-driving kinases

Expression and role of kinases in cancer are well understood

Figure adopted from Cell Signaling Technology, Inc.
Protein Kinases in Human Heart

Heart kinases

- Expression & function of kinases in cardiac tissue are poorly characterized
- Mechanisms of KI-induced cardiotoxicity are not fully understood
Adult Human Heart Kinome Profiling

Phase 1

Gene expression analysis

Full profile of kinase expression in human heart

Enable efficient selection of relevant kinases for selectivity screening to minimize the chance of cardiac side effects

Phase 2

Functional profiling of different kinases

Company KI chemical space
Select 300-400 KIs and/or Single gene knockdown

Contractility assay in human cardiomyocytes

Identify the chemotypes and/or kinases most frequently associated with reduction in cardiac contractility

- Establish a Company Proprietary human-relevant database covering the cardiac kinome
- Enable efficient data-driven selection of leads with lowest cardiotoxicity risk
# Positive Inotropy Assessment - Validation Set

Targets 12 Different Mechanisms of Action

<table>
<thead>
<tr>
<th>Mechanism of Action</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺/K⁺ pump inhibition</td>
<td>Digoxin</td>
</tr>
<tr>
<td>Na⁺/K⁺ pump inhibition</td>
<td>Ouabain</td>
</tr>
<tr>
<td>Na⁺/Ca²⁺ exchanger inhibition</td>
<td>SEA-0400</td>
</tr>
<tr>
<td>Myosin activation</td>
<td>Omecamtiv Mecarbil</td>
</tr>
<tr>
<td>Myosin activation</td>
<td>EMD-57003</td>
</tr>
<tr>
<td>Ca²⁺ sensitization</td>
<td>Levosimendan</td>
</tr>
<tr>
<td>Non-selective b-adrenoceptor activation</td>
<td>Isoproterenol</td>
</tr>
<tr>
<td>Non-selective b-adrenoceptor activation</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>β1-adrenoceptor activation</td>
<td>Dobutamine</td>
</tr>
<tr>
<td>PDE3 inhibition</td>
<td>Milrinone</td>
</tr>
<tr>
<td>PDE inhibition</td>
<td>IBMX</td>
</tr>
<tr>
<td>Ca²⁺ channel activation</td>
<td>Bay-K 8644</td>
</tr>
<tr>
<td>Adenylyl cyclase activation</td>
<td>Forskolin</td>
</tr>
<tr>
<td>Adenylyl cyclase activation</td>
<td>NKH-477</td>
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<tr>
<td>SERCA activation</td>
<td>N106</td>
</tr>
<tr>
<td>RyR activation</td>
<td>Caffeine</td>
</tr>
</tbody>
</table>
Isoproterenol Induces Positive Inotropic Effect
Identification of Positive Inotropic Effects and Determination of Exposure Responses
Identification of Positive Inotropic Effects and Determination of Exposure Responses
Differential Effects of Positive Inotropes
Cluster Analysis is Used to Mechanistically Fingerprint Compounds with Inotropic Effects

Heatmap data generated from 4th concentration data. Red and green colors indicate decrease and increase of >25% and 10% change, respectively. Black colors indicate no effect (<-25% < % change < 10%). Numbers in boxes indicate means % change relative to vehicle.
Contraction Failure and Pause-Dependent Arrhythmia Compounds With Na\(^+\) Channel Liability
No Contraction Failure and Pause-Dependent Arrhythmia Compounds With Ca\(^{2+}\) Channel Liability
Segregation of Ca$^{2+}$-Dependent Mechanisms

2D PCA generated from top test concentration data. Blue and red colors indicate increase and decrease in Ca$^{2+}$, respectively. Ellipses show confidence intervals of 0.75.
Simultaneous Ca\textsuperscript{2+} Recording from Multiple Cells (n=26)
Decreased and Increased Ca^{2+} Transient
Non-Cumulative Testing of Verapamil and Isoproterenol

**Baseline** | **5min Vehicle** | **5min Verapamil 10μM**
---|---|---

- **Baseline**
- **5min Vehicle**
- **5min Verapamil 10μM**

**EC_{50}:** 2.5 nM

N= 15, 18 and 18 cells for 0.3, 3 and 30nM, respectively

N= 10, 7 and 9 cells for 0.1, 1 and 10μM, respectively
Adult Human Cardiomyocyte Model: Integrated Evaluation of ALL Human Cardiac Targets

Preclinical

Lead Identification

Clinical Development

Phase 1 - 3

No Risk

Human Tissue-based Cardiac Assessment

Clinical Evaluation

In Vivo Regulatory CVS Assessment

Dial-out Undesired Ion Channel and Unanticipated Effects

Risk
Adult Human Cardiomyocyte Model Can be Deployed to Mechanistically Understand and Dial-Out Contractility Risk

1. Variety of studies
   - Ion channel electrophysiology
   - Action potential electrophysiology
   - Ca\(^{2+}\) imaging
   - Mitochondrial energetics
   - GPCRs, signaling pathways and Secondary Pharmacology
   - Etc.
Adult Human Cardiomyocyte Model
Early Primary Screening Tool for Inotropes

- Provides an integrative assessment with a physiologically functional cell
- Reliable prediction of inotropic risk
- Differentiates cardiotoxic from safe cancer drugs
- Ideal for mechanistic investigations
- Predictive of clinical outcomes
Adult human primary cardiomyocytes can detect contractility risk and provide mechanistic insights.
Thank You!