Predictive Analysis of Cell Viability, Apoptosis and ADME/Tox Properties Using Multiparametric In Vitro Assays and Human Induced Pluripotent Stem (iPS) Cell-Derived Cardiomyocytes and Hepatocytes

Promega Corporation & Cellular Dynamics International
Optical Assays for In Vitro Toxicity and ADME Analysis
Using Multi-parametric Assays to Enhance Biological Relevance

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Promega Corp.
Madison, WI
Photon Production by Optical Assays

Fluorescence vs. Bioluminescence

- Fluorescence and luminescence differ by the method for creating the excited-state of the photon emitter.
- These differences dictate practical performance differences and provide a basis for **multiplex** combinations.
- Luminescence is not subject to fluorescent interferences.
Bioluminescent Assays

- High sensitivity, large dynamic range
- Quantitative, predictive outcomes.
- Insensitive to interference from fluorescent analytes.
- Simple, rapid protocols for qHTS applications (96, 384, 1536).
  - No sample prep
  - Add and read formats
- Multiplex options for higher content screens.
Improved Data Quality & Clarity from Multiplex Cell-based Assays

Multiplexing: reading multiple end-points from a single well

• Combined **Luminescent** and **Luminescent** assays
  • Alternate limiting assay components
  • Alternate luciferase chemistries

• Combined **Luminescent** and **Fluorescent** assays
  • Sequential non-interfering readouts

• Combined **Fluorescent** and **Fluorescent** assays
  • Non-ovrlapping Ex/Em wavelengths

Quality = reduced variability
Clarity = proper data interpretation
Harnessing the Advantages of Bioluminescence

Assays

Limiting Luciferase
Reagent: Non-limiting ATP & Luciferin
(e.g. reporter assays)

Limiting ATP
Reagent: Non-limiting Luciferase & Luciferin
(e.g. cell viability assay, kinases, transporters)

Limiting Luciferin
Reagent: Non-limiting ATP & Luciferase
(e.g. P450, UGT, GSH/GSSG, MAO, GST)
Cleavable Luciferins and Fluorophores as Enzyme Substrates

Luciferin-R

(Inactive)

Enzyme

R

Luciferin

(active)

Luciferase

ATP

Light

Fluorophore-R

(Inactive)

Enzyme

R

Fluorophore

(active)

Light

Light
Cleavable Luciferins and Fluorophores as Enzyme Substrates

**Luminescent Assays**
- P450 & MAO Assays (phase I)
- UGT Assays (phase II)
- Glutathione Assays
- Protease Assays
  - (apoptosis, viability, toxicity markers)

**Fluorescent Assays**
- Protease Assays
  - (apoptosis, viability, toxicity markers)
**Proteolytic biomarkers of Cell Viability & Cytotoxicity**

<table>
<thead>
<tr>
<th><strong>CellTiterFluor™</strong></th>
<th><strong>CytoToxFluor™</strong></th>
<th><strong>CytoTox-Glo™</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>GlyPhe-AFC</td>
<td>Bis-AlaAlaPhe-R110</td>
<td>AlaAlaPhe-Luciferin</td>
</tr>
</tbody>
</table>
| + Live-cell protease| + Dead-cell protease| + Dead-cell protease |}

Permeable substrate, Protease inactivated upon cell lysis

- **CellTiterFluor™**
  - GlyPhe-AFC
  - Live-cell protease
  - AFC
  - 400Ex/500nm

Impermeable substrate, Protease released by cell lysis

- **CytoToxFluor™**
  - Bis-AlaAlaPhe-R110
  - Dead-cell protease
  - R110
  - 485Ex/520nm

- **CytoTox-Glo™**
  - AlaAlaPhe-Luciferin
  - Dead-cell protease
  - Luciferin
  - Luciferase
  - Light
Viability/Cytotoxicity Response Profiles
MultiTox-Fluor or MultiTox-Glo Multiplex Assays

No effect
- MultiTox-Glo (Viable) EC$_{50}$ = N.D.
- MultiTox-Glo (Non-Viable) EC$_{50}$ = N.D.

Cell-cycle Arrest
- Viability EC$_{50}$ = 21nM
- Cytotoxicity EC$_{50}$ = N.D.

Cytotoxicity
- Viability EC$_{50}$ = 25.7nM
- Cytotoxicity EC$_{50}$ = 25.9nM
**Mechanistic Toxicity Assays**

**Viability, Cytotoxicity and Apoptosis:**

**ApoToxGlo™ Multiplex Assay**

- **GF-AFC (Viability)** $EC_{50} = 6.89 \mu M$
- **bis-AAF-R110 (Cytotoxicity)** $EC_{50} = 6.87 \mu M$
- **Caspase-Glo 3/7 (Apoptosis)** $EC_{50} = ND$

![Graphs showing viability, cytotoxicity, and caspase levels](image)

**Primary necrosis**

- Viability ↓
- Cytotoxicity ↑
- Caspase →

**Apoptosis**

- Viability ↓
- Cytotoxicity ↑
- Caspase ↑
Mechanistic Toxicity Assays
Detecting Mitochondrial Toxicity

A549 Cells in Galactose

- CytoxFluor EC$_{50} = $ ND
- CTG EC$_{50} = $ 1.4mM

Short exposure time
Glutathione in Toxicity testing and ADME

GSH depletion indicates:

- ✔ Compound toxicity
- ✔ Reactive metabolites
- ✔ Glutathione S-transferase (GST) substrates
- ✔ Oxidative Stress (GSH→GSSG)

- ✔ Reduced glutathione (GSH) neutralizes reactive metabolites

GSH/GSSG-Glo™ Assay measures oxidative stress by simple addition of two reagents
Total Glutathione and GSSG Reactions

**Total Glutathione**

1. Grow cells
2. Remove media (optional)
3. Treat
4. Lyse Cells
5. Reduce GSSG [add DTT]

**Oxidized Glutathione**

1. Grow cells
2. Remove media (optional)
3. Treat
4. Lyse Cells
5. Block GSH
6. Reduce GSSG [add DTT]

**Chemical Reactions**

- GST
- GS-R
- GSH
- Luciferase, ATP (LDR)

**Output**

Light
Multiplex GSH/Viability/Toxicity Assay

- Viability, EC\textsubscript{50} = 11.3\textmu M
- Glutathione, EC\textsubscript{50} = 1.0\textmu M

MultiTox-Fluor\textsuperscript{TM} Substrates → Record Fluorescence → Remove medium → GSH-detection Reagent → GSH-Glo\textsuperscript{TM} Reagent → Record Luminescence
Menadione (vit K₃) metabolism produces ROS (e.g. by complex I or CYP reductase)
Bioluminescence and Modified Luciferins as Probe Substrates

Luciferin-R (Inactive) $\xrightarrow{\text{P450}}$ Luciferin (active) $\xrightarrow{\text{luciferase}, \text{ATP}}$ Light

P450-Glo™
**D-Luciferin: Versatile Enzyme Substrate Scaffold**

![Diagram of D-Luciferin and Enzyme Reaction](image)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Enzymes</th>
<th>Substrates</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luciferin-ME</td>
<td>CYP1A2, 2C8/9, 2J2, 4A11, 4F3B, 19</td>
<td>Luciferin-IPA</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Luciferin-1A2</td>
<td>CYP1A2*</td>
<td>Luciferin-MultiCYP</td>
<td>Most CYP enzymes</td>
</tr>
<tr>
<td>Luciferin-CEE</td>
<td>CYP1A1, 1B1, 3A7</td>
<td>Luciferin-3A7</td>
<td>CYP3A7</td>
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<tr>
<td>Luciferin-H</td>
<td>CYP2C9</td>
<td>Luciferin-4A</td>
<td>CYP4A11 (4A1,2,3)</td>
</tr>
<tr>
<td>Luciferin-ME-EGE</td>
<td>CYP1A1, 1A2, 2D6</td>
<td>Luciferin-2J2/4F12</td>
<td>CYP2J2, 4F12</td>
</tr>
<tr>
<td>Luciferin-H-EGE</td>
<td>CYP1A1, 1A2, 2C19</td>
<td>Luciferin-4F2/3</td>
<td>CYP4F2, 4F3</td>
</tr>
<tr>
<td>Luciferin-BE</td>
<td>CYP3A4, 3A5, 3A7, 4F12</td>
<td>Luciferin-4F12</td>
<td>CYP4F12</td>
</tr>
<tr>
<td>Luciferin-PFBE</td>
<td>CYP3A4, 3A5, 3A7</td>
<td>Luciferin-APE</td>
<td>MAO-A, MAO-B</td>
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<tr>
<td>Luciferin-PPXE</td>
<td>CYP3A4, 3A5, 3A7</td>
<td>Luciferin-NAT2</td>
<td>N-acetyl transferase</td>
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<tr>
<td>aLuciferin-DEVD</td>
<td>Caspase-Glo 3/7</td>
<td>Luciferin-UGTs</td>
<td>Most UGTs</td>
</tr>
<tr>
<td>Luciferin-GSH</td>
<td>GSH quantification</td>
<td>Luciferin-GSTs*</td>
<td>GST enzymes</td>
</tr>
</tbody>
</table>

*prototype*
Selectivity of Luciferin-IPA for CYP3A4

Recombinant CYP Enzyme Activity

Luminescence (RLU/0.1pmol CYP450)

- CYP1A1
- CYP1A2
- CYP1B1
- CYP2A6
- CYP2B6
- CYP2C8
- CYP2C9
- CYP2C18
- CYP2C19
- CYP2D6
- CYP2E1
- CYP2J2
- CYP3A4
- CYP3A5
- CYP3A7
- CYP4A11
- CYP4F2
- CYP4F3A
- CYP4F3B
- CYP4F12
- CYP4F13
- CYP19

control
P450-Glo™ IC₅₀s Correlate with Traditional Assays

IC₅₀s Against Recombinant CYP3A4

- clotrimazole
- ketoconazole
- troleandomycin
- midazolam
- erythromycin
- omeprazole
- α-naphthoflavone
- disopyramide

r²=0.96, P=<0.0001

IC₅₀s Against Human Liver Microsomes (CYP3A4)

- clotrimazole
- nifedipine
- fluvoxamine
- disopyramide
- midazolam
- troleandomycin

r²=0.91, P=0.003
*New* Selective CYP1A2 Assay

P450-Glo CYP1A2-selective Assay

Substrate: Luciferin-1A2
Dialing in selectivity for CYP1A2

CYP1A-Selective Inhibition in Human Liver Microsomes

HLM & P450-Glo CYP1A2 Assay

\[ \text{IC}_{50} = 0.01 \mu M \]

\[ \alpha\text{-Naphthoflavone (\mu M)} \]

HLM & P450-Glo CYP1A2 Assay

\[ \text{IC}_{50} = 0.2865 \mu M \]

\[ \text{Fluvoxamine (\mu M)} \]
P450-Glo™ cell-based assays: rapid method for CYP induction studies

1. Proluciferin CYP substrates cross membrane
2. Luciferin crosses membrane
3. CYP enzyme activity converts proluciferin to luciferin
4. Luciferin is converted to light by Luciferin Detection Reagent

Measure luciferin production in conditioned media or lyse the cells for total luciferin production

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cell-based assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luciferin-IPA</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>Luciferin-1A2</td>
<td>CYP1A2</td>
</tr>
<tr>
<td>Luciferin-4A</td>
<td>CYP4A</td>
</tr>
<tr>
<td>Luciferin-H</td>
<td>CYP2C9</td>
</tr>
</tbody>
</table>
Multiplex CYP3A4/Cell Viability Luminescent/Luminescent Hepatocyte Assay

CYP Enzyme Assay:
Transfer medium to a 2nd plate with P450-Glo™ Assay Reagent

1. Record Luminescence
CYP enzyme Activity

2. Record Luminescence
Cell viability

Normalize CYP activity to cell viability

ATP Viability Assay:
Add CellTiter-Glo® Assay Reagent to 1st plate

Incubate Hepatocytes with the P450-Glo™ CYP Enzyme Substrate in 1st plate
Concentration-dependent CYP3A4 Induction in fresh Human Hepatocytes (Celsius/IVT)

- EC$_{50}$ = 0.16 µM for Hyperforin
- EC$_{50}$ = 0.62 µM for Rifampicin
- EC$_{50}$ = 0.31 µM for SR12813
- EC$_{50}$ = 0.82 µM for Forskolin
- EC$_{50}$ = 38.87 µM for Phenylbutazone
- EC$_{50}$ = 35.86 µM for Sulfinpyrazone

70 year old male donor
CYP3A4 Induction in Human Hepatocytes
Luciferin-IPA Assay vs. TS6β-OH Assay

- Cryopreserved hepatocytes from Celsis/IVT.
- CYP3A4 Rifampicin induction (25μM)
- Testosterone assay (TS-6βOH) vs Luciferin-IPA Assay.
- Fold induction = induced ÷ basal activity.

Data adapted from Moeller et al, Celsis/IVT.
P450-Glo™ CYP1A2Selective Assay with Human Hepatocytes

Cell-Based Induction Assay

- Control
  - Basal activity
- Omeprazole
  - CYP1A inducer
  - AHR agonist
- Rifampicin
  - CYP3A/2C inducer
  - PXR agonist
- Phenobarbital
  - CYP2B6/A6 inducer
  - CAR agonist

Activity with Luciferin-1A2

Luminescence (RLU)

control Omeprazole Rifampicin Phenobarbital

0 400000 300000 200000 100000 0

Cells
Thank You for Your Attention!
Predictive Analysis of Cell Viability, Apoptosis and ADME/TOX Properties Using Multiparametric in vitro Assays and Human iPSC-derived Cardiomyocytes and Hepatocytes
• CDI overview and iPS cell technology
• CDI iPS cell-derived cell types
  • iCell™ Cardiomyocytes
  • iCell™ Hepatocytes (*in development*)
• Toxicity testing and cell-specific effects
• The world’s largest producer of human iPS cells and iPS cell-derived cell types

• Core industrial competencies
  • Creation and culture of human iPSC lines
  • Genetic engineering of iPSC lines
  • Manufacture of human tissue cells

• Founded in 2004 by Dr. James Thomson—1st human ES cells lines in 1998

• Currently employs ~107 people at its location in Madison, WI
Key Characteristics for Adoption

Quality

- Exhibit key cellular characteristics
- Recapitulate normal human biology
- Reproducible
- Known and relevant genotype

Quantity

- Sufficient to support HTP drug screening and safety testing
  ~ $1 \times 10^9$ pure cardiomyocytes/day

Purity
High Level Process Summary

1. CREATE iPS cell line from a single individual
2. EXPAND iPS cell line -> 2 billion cells per day
3. DIFFERENTIATE iPS cells into tissue specific cells
4. PURIFY* cell type of interest

- ISO and GMP processes ensure highest quality
- Industrial quantities of iPS cells and differentiated cell types
- Highly pure populations of differentiated cell types
  - Differentiation, selection, maintenance
- Manufacturing process is translatable, scalable and IP protected
• CDI overview and iPS cell technology
• CDI iPS cell-derived cell types
  • iCell™ Cardiomyocytes
  • iCell™ Hepatocytes (in development)
• Toxicity testing and cell-specific effects
iCell™ Cardiomyocytes

- Human iPS cell-derived
- 99% pure, cryopreserved, ready to use.
- Available in virtually unlimited quantities
- Full product solution; cells, media, protocols
- Demonstrate normal human cardiac biology, electrophysiology, and toxicity responses
- Broad platform utility for discovery and preclinical development
iCell Cardiomyocytes
Genomic and Transcript Characterization

Transcriptome Analysis

Gene Category
Stem cell
Transition
Cardiomyocyte

Comparative Analysis

iCell Cardiomyocytes

Novartis GNF expression atlas
Heart expression ≥ 10X median tissue expression
GO analysis confirms Cardiac-specific enrichment

‘Primary’ Cardiomyocytes

“More human than human”
**iCell Cardiomyocytes**

**Electrophysiology Characterization**

### Ionic Currents

- **Inward Sodium Current (INa)**
- **Calcium Current (ICa-L)**
- **Potassium Inward Current (IK)**
- **Potassium Delayed Rectifier Current (IKr)**
- **Potassium Flicker Current (Ifunny)**
- **Potassium Delayed Rectifier Current (IK1)**

### Spontaneous Action Potentials

- **Atrial like**
- **Nodal like**
- **Ventricular like**

### Table

<table>
<thead>
<tr>
<th>Currents</th>
<th>Example Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>INa</td>
<td>26.7±1.4</td>
</tr>
<tr>
<td>ICa-L</td>
<td>73.8±1.5</td>
</tr>
<tr>
<td>IKr</td>
<td>286.2±21.2</td>
</tr>
<tr>
<td>IK1</td>
<td>414.7±21.8</td>
</tr>
<tr>
<td>Ifunny</td>
<td>2.5±0.2</td>
</tr>
</tbody>
</table>

### Drug Effects

- **Isoproterenol**
- **Phenylephrine**
- **Carbachol**

### GPCR Activity

- **G_αs – β1**
- **G_αq – α1**
- **G_αi – m2**
• CDI overview and iPS cell technology
• CDI iPS cell-derived cell types
  • iCell™ Cardiomyocytes
  • iCell™ Hepatocytes (in development)
• Toxicity testing and cell-specific effects
### Key Hepatocyte Characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Adherent monolayer; polarized phenotype; functional bile canalicul network; polynucleation</td>
</tr>
<tr>
<td><strong>Molecular Markers</strong></td>
<td>Albumin; α-1-antitrypsin; HNF family transcription factors</td>
</tr>
<tr>
<td><strong>Intrinsic Metabolism</strong></td>
<td>Glycogen storage; lipid metabolism; insulin responsiveness; urea synthesis</td>
</tr>
</tbody>
</table>
| **Phase I & II Metabolism**| - CYP3A4, 2C9, 2C19, 2D6, 1A2, 2B6, 2C8  
|                           | - UGT, ST, GSTα activity                                                    |
| **Transporter Function**  | Transport via uptake (e.g. OATP, NTCP) and efflux transporters (e.g. MDR-1/P-gp, BCRP, BSEP, MRP2) |
iCell Hepatocytes
Intrinsic Function

**Bar Graph**

<table>
<thead>
<tr>
<th></th>
<th>Albumin (pg/ALB+cell/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHH</td>
<td>40</td>
</tr>
<tr>
<td>HepG2</td>
<td>1 (low)</td>
</tr>
<tr>
<td>iCell Hepatocytes</td>
<td>30 (high)</td>
</tr>
</tbody>
</table>

**Images**

- **PAS**
  - 50.0 μm
- **Oil Red**
  - 50.0 μm
- **BODIPY**
  - 50.0 μm
ECOD Assay

CYP3A Basal Metabolism (Testosterone)

CYP3A4 Induction

7-EC: 7-Ethoxycoumarin
7-HC: 7-Hydroxycoumarin
7-HCG: 7-Hydroxycoumarin glucuronide
7-HCS: 7-Hydroxycoumarin sulfate
iCell Hepatocytes
Transporter Function

CDFDA

Taurocholate (NTCP/BSEP)

Rosuvastatin (OATP/MRP2)

NTCP
BSEP
OATP
MRP2

Accumulation (pmol/mg protein)

BEI (%)

Accumulation (pmol/mg protein)

BEI (%)

B-CLEAR® is covered by US Pat. No. 6,780,580 and other US and International patents both issued and pending.
CDI overview and iPS cell technology
CDI iPS cell-derived cell types
- iCell™ Cardiomyocytes
- iCell™ Hepatocytes (in development)
Toxicity testing and cell-specific effects
Drug induced Arrhythmias

Biochemical induced events
- Cell Permeability
- Cell energetics
- Oxidative stress
- Mitochondrial dysfunction
- Necrosis
- Apoptosis

The two toxicities can be species specific and may not be causal or linked

The ideal test system is species specific and biologically relevant.
### iCell Cardiomyocytes
**Safety Pharmacology - APD Assay**

#### iNa Block – Decreased dv/dt

![Graph showing iNa block with TTX variations](image)

#### ICa Block – shortened APD

![Graph showing ICa block with Nifedipine variations](image)

#### IKr Block – prolonged APD

![Graph showing IKr block with E4031 variations](image)

<table>
<thead>
<tr>
<th>% of control</th>
<th>Dose</th>
<th>Peak</th>
<th>MDP</th>
<th>APD10</th>
<th>APD50</th>
<th>APD90</th>
<th>dV/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTX N=5</td>
<td>1μM</td>
<td>103.8±3.0</td>
<td>100.3±0.9</td>
<td>107.1±2.5</td>
<td>103.1±1.9</td>
<td>101.7±2.0</td>
<td>76.7±7.4</td>
</tr>
<tr>
<td></td>
<td>3μM</td>
<td>100.0±1.3</td>
<td>99.5±0.8</td>
<td>105.7±4.3</td>
<td>100.6±2.5</td>
<td>98.8±0.8</td>
<td>41.2±11.2*</td>
</tr>
<tr>
<td></td>
<td>10μM</td>
<td>99.0±2.7</td>
<td>97.6±1.1</td>
<td>108.8±6.6</td>
<td>98.2±4.3</td>
<td>96.4±3.7</td>
<td>16.7±1.8*</td>
</tr>
<tr>
<td></td>
<td>30μM</td>
<td>99.1±3.6</td>
<td>96.2±2.1</td>
<td>112.4±6.3</td>
<td>102.0±3.4</td>
<td>100.2±3.1</td>
<td>16.8±2.0*</td>
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<tr>
<td>E4031 N=5</td>
<td>3nM</td>
<td>99.0±1.0</td>
<td>99.8±0.6</td>
<td>94.8±3.4</td>
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<td>98.7±2.0</td>
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<td></td>
<td>10nM</td>
<td>100.5±1.2</td>
<td>98.3±0.8</td>
<td>92.2±2.8</td>
<td>100.5±1.6</td>
<td>112.8±2.8</td>
<td>98.2±5.9</td>
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<td></td>
<td>30nM</td>
<td>99.3±1.0</td>
<td>97.4±1.2</td>
<td>90.1±3.0</td>
<td>109.1±3.7*</td>
<td>140.3±7.6*</td>
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<tr>
<td></td>
<td>100nM</td>
<td>101.1±1.4</td>
<td>94.2±2.6</td>
<td>83.0±10.2</td>
<td>113.4±3.9*</td>
<td>170.4±13.6*</td>
<td>69.4±17.1</td>
</tr>
<tr>
<td>Nifedipine N=5</td>
<td>3nM</td>
<td>89.5±4.9</td>
<td>99.5±0.4</td>
<td>83.3±7.1*</td>
<td>84.6±2.4*</td>
<td>89.4±1.9*</td>
<td>83.9±14.5</td>
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<tr>
<td></td>
<td>10nM</td>
<td>82.2±9.0</td>
<td>98.7±0.8</td>
<td>65.0±12.3*</td>
<td>70.3±6.1*</td>
<td>78.4±4.4*</td>
<td>91.3±4.4</td>
</tr>
<tr>
<td></td>
<td>30nM</td>
<td>87.2±6.9</td>
<td>97.3±1.8</td>
<td>60.9±7.6*</td>
<td>65.7±3.0*</td>
<td>74.0±2.3*</td>
<td>84.8±11.2</td>
</tr>
<tr>
<td></td>
<td>100nM</td>
<td>73.6±12.2</td>
<td>96.6±2.0</td>
<td>31.7±11.1*</td>
<td>45.4±4.5*</td>
<td>58.2±5.4*</td>
<td>87.7±8.6</td>
</tr>
</tbody>
</table>
iCell Cardiomyocytes: Toxicity Testing – Cell-based Assays

Cell Titer-Glo

- Staurosporine
- Doxorubicin
- Doxazosin mesylate
- Valinomycin
- Gleevec
- Isoproterenol

GSH-Glo

- Gleevec
  \( EC_{50} = 3.37 \times 10^{-5} \)

Proteasome-Glo

- Epoxomicin
  \( EC_{50} = 1.70 \times 10^{-9} \)

Caspase-Glo 3/7

- Staurosporine

HDAC-Glo

- SAHA
  \( EC_{50} = 2.68 \times 10^{-7} \)
iCell Cardiomyocytes
Toxicity Testing - Mechanisms

Mechanistic Information
Single-endpoint Assays

CellTiter-Glo 24 hr

- Staurosporine
- Caspase-Glo 3/7

CellTiter-Glo 24 hr

- imatinib

Caspase-Glo 3/7

- Staurosporine
- imatinib

Multiplexed Assays
Apo Tox-Glo™

- Cytotoxicity EC₅₀ > 100µM
- Viability EC₅₀ > 100µM
- Caspase EC₅₀ = ND

- Cytotoxicity EC₅₀ = 12µM
- Viability EC₅₀ = 3.5µM
- Caspase EC₅₀ ~ 5µM
Transcending Proteasome Inhibition for the Treatment of Cancer

Summer 2008

By Dr. Mark Rose

Healthy cellular function requires a carefully controlled balance between protein synthesis and protein degradation, a process known as protein homeostasis. The major intracellular pathway for controlling protein degradation is the ubiquitin proteasome system (UPS). Specific pharmacological inhibition of the UPS with a potent small molecule inhibitor of the proteasome...

Figure 1 The ubiquitin-proteasome pathway (UPS) controls the destruction of most short-lived cellular proteins and is involved in the regulation of many different signalling pathways. The Nedd 8 activating enzyme (NAE) is a target within the pathway.

Related Articles

Proteasome-Glo

![Proteasome-Glo Graph]

iCell™ Cardiomyocytes

![Graph showing iCell™ Cardiomyocytes]

HDAC-Glo

![HDAC-Glo Graph]

Epoxomicin

EC$_{50}$ = 1.7 x 10$^{-9}$

SAHA

EC$_{50}$ = 2.68 x 10$^{-7}$
Cell Specific Effects
Proteasome Inhibitor Velcade (24hr Exposure)

iCell Cardiomyocyte

- Cytotoxicity EC$_{50}$ = ND
- Viability EC$_{50}$ = ND
- Caspase EC$_{50}$ = ND

K562

- Cytotoxicity EC$_{50}$ ~15nM
- Viability EC$_{50}$ = 20nM
- Caspase EC$_{50}$ = 16nM

No apparent cytotoxicity* or caspase activation

Cytotoxicity by apoptosis

*Bortezomib (and other proteasome inhibitors) are known to partially inhibit the viability assay protease biomarker at concentrations greater than 1µM; >50 fold the EC$_{50}$ for K562.
Cell Specific Effects

Histone Deacetylase Inhibitor (24hr Exposure)

**iCell Cardiomyocyte**

- Cytotoxicity EC$_{50}$ = ND
- Viability EC$_{50}$ = ND
- Caspase EC$_{50}$ = ND

No apparent cytotoxicity or caspase activation

**K562**

- Cytotoxicity EC$_{50}$ = 4.5µM
- Viability EC$_{50}$ = 1.7µM
- Caspase EC$_{50}$ = 3.3µM

Cytotoxicity by apoptosis
Summary

- Human iPS derived cell types are produced in pure, industrialized quantities
- Show relevant human biology
- Demonstrate typical toxic endpoints
- Can be used in conjunction with several Promega cell-based assays to provide rapid, cell-specific and mechanistic information.

Together the technologies provide a means to generate relevant human-based data early in preclinical development