Novel Human-Based In Vitro Liver and Intestinal Technologies for Drug Development: Hepatocytes, intestinal mucosa, IdMOC

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Columbia, MD and Malden, MA
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Cost of a new drug

• 1.5 billion USD
• 15 years
• Reason: >10% clinical success
Species Difference as the Major reason for the Failure of Preclinical Safety Studies to Predict Human Drug Properties

• Species-species differences
  – Metabolism
  – Target organ sensitivity
  – Toxic mechanisms
Reason for Clinical Trial Failure: 
*failure of laboratory animals to predict human drug properties*

- Human-specific pharmacological targets
  - Human-specific receptors
  - Human-specific pathways
  - Human-specific drug metabolism (pro-drugs)

- Human-specific sensitivity to toxicant effects
  - Human-specific drug metabolism
  - Human-specific toxic mechanisms
<table>
<thead>
<tr>
<th></th>
<th>P450</th>
<th>Human</th>
<th>Mouse</th>
<th>Rat</th>
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<td>CYP3A</td>
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<td>3A11/13</td>
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<td>3A12/26</td>
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In vitro in vivo strategy (IVIVS)

• Predict human in vivo drug toxicity with
  – *Human-specific information* developed from *in vitro human-based* experimental systems
  – *Key in vivo parameters* obtained from *early in vivo studies* such as human clinical studies and/or relevant preclinical studies in experimental animals

*Human-based in vitro experimental systems for the evaluation of human drug safety.*
Human-based in vitro experimental systems for the evaluation of human drug safety.

Li AP.

In vitro in vivo strategy (IVIVS)

• Predict human in vivo drug toxicity with
  – *Human-specific information* developed from *in vitro human-based* experimental systems
  – *Key in vivo parameters* obtained from *early in vivo studies* such as human clinical studies and/or relevant preclinical studies in experimental animals
In Vitro In Vivo Strategy (IVIVS)

Li AP (2007). Current Drug Safety
Parallelogram Approach for the Preclinical Assessment of Clinical Drug Properties

**Human in vivo**

**Animal in vivo**

**Human in vitro**

**Animal in vitro**

**Extrapolation**

**IVIVC**

- PBPK
- Genetic polymorphism
- Risk Factors

**Animal Species Selection**

**Species Comparison**
In Vitro ADMET Laboratories (IVAL)

- Locations: Columbia, MD and Malden, MA
- Date of Incorporation: November, 2004
- **Mission**: To provide **products and contract research service** to enhance the efficiency of drug development
  - **Accurate assessment of human drug properties**
IVAL Activity

• Develop and provide *physiologically relevant* in vitro experimental systems
  – *Organ-specific properties*
  – *Species-specific properties*

• Develop experimental approaches to aid *accurate assessment of human drug properties*
IVAL Scientific Team

- David Ho (previously Takeda, Cambridge)
- Qian Yang (previously NCI)
- Ivy Wei (previously NIH)
- Walter Mitchell (previously Pfizer, Groton)
- Carol Loretz (previously University of Washington, Seattle)
- Kirsten Amaral (previously Life Technologies, RTP)
- Albert P. Li (previously Monsanto/G. D. Searle)
Key Components of an *In Vitro* Experimental System for the Evaluation of Human Toxicants: 

*The MTE Requirement*

- Human-specific Metabolism (M)
- Human Targets (T)
- Relevant Endpoints (E)
- Accurate IVIVC
Key IVAL Products: Hepatocytes and Enterocytes

Enterocytes: First-pass metabolism of orally-administered drugs
Hepatocytes: First-pass metabolism of absorbed orally-administered drugs
Key Technical Milestones

- **First demonstration of successful cryopreservation**

- **First demonstration of retention of drug metabolizing enzymes after cryopreservation**

- **First international consensus on utility of cryopreserved human hepatocytes**

- **First demonstration of retention of uptake transporter activities after cryopreservation**

- **First demonstration of effectiveness of CHRM and plateability**

- **Invention of MetMax™ Cryopreserved Human Hepatocytes**

- **First Successful cryopreservation of Human Enterocytes**
Near Perfection of Hepatocyte Cryopreservation
999-Elite™
Cryopreserved Human Hepatocytes

- >90% viability
- >90% confluency
- >9 days culture duration
Applications of 999-Elite™
Cryopreserved Human Hepatocytes in Drug Development

**DMPK**
- Metabolism
- Uptake
- Efflux
- Drug-Drug Interactions (Induction)

**Toxicology**
- Hepatotoxicity screening
- Elimination of sDILI potential
- Species selection for safety studies

**Pharmacology**
- Hepatitis B
- NASH
- Cholesterol synthesis
Plated Hepatocyte Uptake Assay

999Elite™ Human Hepatocytes
Plated Human Hepatocytes Uptake Assay:
Concentration-dependent Pravastatin Uptake in the Presence and Absence of Uptake Inhibitor (6 uM Rifampin)

Rifampin Inhibition of Pravastatin Uptake

\[ y = -0.4631x^2 + 97.358x + 394.13 \]
\[ R^2 = 0.9986 \]

\[ y = -0.1317x^2 + 39.384x + 197.18 \]
\[ R^2 = 0.9991 \]
Time- and Concentration-dependent Pravastatin Uptake in Plated Human Hepatocytes

Rifampin

- 12.5 uM
- 25 uM
- 50 uM
- 100 uM

Michaelis-Menten

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>Best-fit values</strong></td>
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<tr>
<td><strong>Vmax (pmol/million cells)</strong></td>
<td>553.4</td>
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<tr>
<td><strong>Km (uM)</strong></td>
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<td><strong>Std. Error</strong></td>
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<td><strong>Vmax</strong></td>
<td>30.54</td>
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<tr>
<td><strong>Km</strong></td>
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95% Confidence Intervals

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<td><strong>Vmax</strong></td>
<td>422.0 to 684.9</td>
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<tr>
<td><strong>Km</strong></td>
<td>28.26 to 82.01</td>
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</table>
Human Plasma/Human Hepatocytes Uptake Assay

Rate of Uptake in Human Hepatocytes in Human Plasma

Rifampin Inhibition of Pravastatin Uptake
Plated Hepatocyte Uptake Assay

- Identification of uptake transporter substrates
- Identification of uptake transporter inhibitors
- Human plasma uptake assay may allow direct extrapolation to in vivo
In Vitro Hepatocyte Exposure Assay for Drug Candidates Targeting Hepatocytes
Rat Hepatocytes: TA-4 and TA-5 as preferred NECs (highest transporter-dependent uptake)

<table>
<thead>
<tr>
<th>Rifampin (uM)</th>
<th>AUC (pmol-min/million cells)</th>
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<tbody>
<tr>
<td>Rat 0 5 25 100</td>
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<tr>
<td>TA-1</td>
<td>148.80 140.80 102.10 89.20</td>
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<tr>
<td>TA-2</td>
<td>105.30 88.20 73.60 69.65</td>
</tr>
<tr>
<td>TA-3</td>
<td>116.30 88.00 64.80 60.46</td>
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<tr>
<td>TA-4</td>
<td>193.90 194.30 251.80 249.90</td>
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<tr>
<td>TA-5</td>
<td>285.40 243.20 194.90 139.00</td>
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<tr>
<td>Pravastatin</td>
<td>56.85 45.39 33.36 23.15</td>
</tr>
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</table>

Rat Hepatocytes

![Graph showing AUC values for different drugs and concentrations of Rifampin.]

IRA
In Vitro ADMET Laboratories, Inc.
Similar Results with Human Hepatocytes

<table>
<thead>
<tr>
<th></th>
<th>AUC (pmol-min/million cells)</th>
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<tr>
<td></td>
<td>Rifampin (uM)</td>
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<tr>
<td>Human</td>
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<td>TA-1</td>
<td>23.60</td>
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<tr>
<td>TA-2</td>
<td>22.14</td>
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<td>TA-5</td>
<td>62.77</td>
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<tr>
<td>Pravastatin</td>
<td>7.955</td>
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</table>

![Graph showing AUC values for different Rifampin concentrations and human hepatocytes results.](image-url)
Hepatocyte Exposure Assay

• Efficient assay to select drug candidates based on hepatocyte exposure results:
  – Level of exposure
  – Uptake transporter involvement
P450 Induction Assay

999Elite™ Human Hepatocytes
## P450 Induction (Gene Expression)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP1A2 induction fold</th>
<th>CYP2B6 induction fold</th>
<th>CYP3A4 induction fold</th>
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<tr>
<td></td>
<td>HH1121</td>
<td>HH1142</td>
<td>HH1144</td>
</tr>
<tr>
<td>Omeprazole (µM)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
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<td>1.01</td>
<td>0.12</td>
<td>1.01</td>
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<tr>
<td>20</td>
<td>21.59</td>
<td>2.60</td>
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<td>100</td>
<td>45.03</td>
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<td>250</td>
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<td>18.91</td>
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<tr>
<td>1000</td>
<td>21.58</td>
<td>4.55</td>
<td>20.67</td>
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<tr>
<td>Rifampin (µM)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
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<td>1.09</td>
<td>0.46</td>
<td>1.01</td>
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<td>11.70</td>
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<td>13.56</td>
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<td>20</td>
<td>15.18</td>
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## CYP2C Induction

### CYP2C8 induction fold

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<tbody>
<tr>
<td>Rif (µM)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
<td>0</td>
<td>1.00</td>
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<td>5</td>
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### CYP2C9 induction fold

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<td>1.12</td>
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<td>5</td>
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### CYP2C19 induction fold

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<td>Rif (µM)</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<td>1.01</td>
<td>0.15</td>
<td>1.03</td>
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<tr>
<td>5</td>
<td>14.27</td>
<td>8.62</td>
<td>9.66</td>
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</table>
P450 Induction Assay

- 999Elite™ human hepatocytes as best model to evaluate P450 induction
  - CYP1A2
  - CYP2B6
  - CYP2C8
  - CYP2C9
  - CYP2C19
  - CYP3A4
In Vitro Hepatotoxicity Screening
In Vitro Hepatotoxicity Assay

- Plateable cryopreserved hepatocytes
- 384-well plate format (collagen-coated)
  - Day 1 (0 hr): Plate hepatocytes (10,000 cells/well; 20 μL per well) in Hepatocyte Plating Medium
  - Day 1 (4 hr): Change medium to Hepatocyte Treatment Medium containing test articles
  - Day 2, 3, 4, or longer treatment durations: Evaluate cytotoxicity
    - ATP (mitochondrial impairment; necrosis)
    - ROS (oxidative stress)
    - GSH (reactive intermediate)
    - Caspase 3/7 (apoptosis)
Over-prediction (*left*) and Under-prediction (*right*) of in vitro Human Hepatotoxicity with Nonhuman Hepatocytes
Definition of Metabolism-Dependent Hepatotoxicity
Differential Cytotoxicity Assays for Metabolism-Dependent Hepatotoxicity:


1. Inhibition of Drug Metabolizing Enzymes with 1-aminobenzotriazole (ABT), Nonspecific P450 Inhibitor

2. Comparison to cytotoxicity towards Metabolically Incompetent CHO cells
Clinical implication of metabolism-dependent hepatotoxicity

- Preclinical results with laboratory animals may not predict human clinical hepatotoxicity due to species differences in drug metabolism
- Possible individual differences in hepatotoxicity due to individual differences in drug metabolism
P450 Activity as Risk Factor


<table>
<thead>
<tr>
<th>Donor</th>
<th>Lot</th>
<th>CL_{int, hep} (Intrinsic Hepatic Clearance (pmol/min/million cells))</th>
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<td>1007</td>
<td>2.7</td>
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<td>2</td>
<td>1023</td>
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<td>3</td>
<td>1026</td>
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<td>5</td>
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<td>1.9</td>
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<tr>
<td>6</td>
<td>1036</td>
<td>1.5</td>
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**Acetaminophen**

**Aflatoxin-B1**

**Tamoxifen**

**Cyclophosphamide**

**Ketoconazole**
Identification of sDILI* Drugs

*severe drug-induced liver injuries (liver failure)
Examples of Regulatory Actions on Marketed Drugs due to DILI (1995-2010)
(Source: Mark Avigan, FDA, 2012)

<table>
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<th>Withdrawals (US* &amp;/or other countries**)</th>
<th>Boxed Warnings (US)</th>
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<tr>
<td>troglitazone*</td>
<td>lamivudine</td>
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<tr>
<td>bromfenac*</td>
<td>leflunomide</td>
</tr>
<tr>
<td>trovofloxacin*</td>
<td>propylthiouracil</td>
</tr>
<tr>
<td>gemtuzumab</td>
<td>lapatanib</td>
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<tr>
<td>tilbroquinol**</td>
<td>pazopanib</td>
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<tr>
<td>pemoline**</td>
<td>sunitimib</td>
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<td>tetrabamatate**</td>
<td>tenofovir</td>
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<tr>
<td>ebrotidinet**</td>
<td>tipranavir</td>
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<td>nefazodone**</td>
<td>tolcapone</td>
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<td>niperotidinet**</td>
<td>ambrisentan</td>
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<td>chlormezanone**</td>
<td>acitretin</td>
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<tr>
<td>ximelegatrant**</td>
<td>cytarabine</td>
</tr>
<tr>
<td>lumaricoxibt**</td>
<td>maraviroc</td>
</tr>
<tr>
<td>eltrombopa</td>
<td>acetaminophen (Rx)</td>
</tr>
</tbody>
</table>

Restricted (US)
Trofoloxacin
Felbamate
Pemoline
Annotation of Severe and Overall DILI

- Initial testing set includes 80 marketed drugs commercially available
- A unified list of drugs identified as causes of either overall DILI (oDILI) or drug-induced acute liver failure developed

80 testing drugs

→ 54 oDILI drugs
→ 26 non-DILI drugs
→ 50 non-sDILI drugs
→ 30 sDILI drugs
→ 107 ALF drugs
→ 44 withdrawal

A Unified list (Suzuki et al)

Severe DILI drugs are drugs
1. withdrawn or having Black Box Warning due to DILI
2. Acute liver failure reported in at least two countries

Mindikoglu et al., Liver Transplantation 15:719-729 (2009)
Accurate Prediction of sDILI Potential
Collaboration with Weida Tong et al, FDA-NCTR

ROS: ROS/ATP ratio as most accurate endpoint

MetMax™ Hepatocytes

(Patent Pending)


Drug Metabolism and Disposition, pp.dmd-117.
IVAL Wins EPA/NIH $100K Transform Toxicity Testing Challenge

- **EPA** launched the **Transform Toxicity Testing Challenge** along with the **National Institutes of Health, National Center for Advancing Translational Sciences, and the National Toxicology Program housed within the National Institute for Environmental Health Science.**
- The Transform Toxicity Challenge asked teams of scientists to develop techniques to retrofit existing HTS assays to incorporate processes that reflect how chemicals are broken down and metabolized by the body.

**Winner:** Dr. Albert Li, **In Vitro ADMET Laboratories LLC** developed a novel exogenous metabolic system, **MetMax™ Human Hepatocytes** (MMHH; patent pending), as a convenient experimental reagent to provide hepatic metabolism to in vitro toxicity assays, including cytotoxicity, and genotoxicity. MMHH are cryopreserved permeabilized human hepatocytes supplemented with phase I and II drug metabolizing enzyme cofactors.
Application of Human Hepatocytes in Drug Metabolism

• Advantages (over HLM, S9)
  – Complete drug metabolizing enzyme activities
    • Microsomes lack cytosolic enzymes
    • S9 lacks mitochondrial, lysosomal, plasma membranal enzymes
Application of Human Hepatocytes in Drug Metabolism

• Disadvantages (over HLM, S9)
  – Laborious
    • Storage in LN2
    • Centrifugation and microscopic evaluation of cell viability and cell concentration
    • Sensitive to experimental manipulation (not robot friendly)
    • Metabolism may be hindered by drug toxicity (high drug concentration cannot be used for metabolite profiling)
MetMax™ Human Hepatocytes

- Permeabilized, cofactor supplemented hepatocytes
  - An experimental system with the advantages of hepatocytes and the ease of operation and robustness of cell free systems
MetMax™ Hepatocytes: *Permeabilized Hepatocytes*

Intact Hepatocyte

Hepatocyte In Vivo

*MetMax™ Hepatocyte*
Organelle Composition/DME of Key In Vitro Drug Metabolism Experimental Systems

- **Endoplasmic reticulum:** P450, UGT etc. (**S9; HLM; Hepatocytes**)
- **Cytosol:** SULT, NAT etc. (**S9; Hepatocytes**)
- **Mitochondria:** MAO (**Hepatocytes**)
Organelle Composition/DME of Key In Vitro Drug Metabolism Experimental Systems

• **Plasma membrane:** *Hepatocytes*
  – ATPases, anion transport protein, glyceraldehyde 3-phosphate dehydrogenase, protein kinases, adenylate cyclase, acetylcholinesterase.

• **Lysosome:** *Hepatocytes*
  – glycosidases, proteases, sulfatases

• **Nucleus:** *Hepatocytes*
  – *Nuclear shield enzymes* - glutathione transferase, catalase and glutathione peroxidase; up to seven times higher than in the cytosol.
<table>
<thead>
<tr>
<th>Organelles</th>
<th>MetMax™</th>
<th>Intact Hepatocytes</th>
<th>Microsomes</th>
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<td>Mitochondria</td>
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# Ease of use of MetMax™ Hepatocytes

<table>
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<tbody>
<tr>
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<td>-80 deg. C</td>
<td>LN2</td>
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<td><strong>Centrifugation</strong></td>
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<td><strong>Microscopic Examination</strong></td>
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<td>No</td>
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<td><strong>Cell Counting</strong></td>
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<td>Yes</td>
<td>No</td>
<td>No</td>
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<td><strong>Cofactor Addition</strong></td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<td><strong>Thaw and Use</strong></td>
<td>Yes</td>
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</table>
MetMax™ Hepatocytes
A Thaw and Use Reagent

MetMax™ Hepatocytes

*Freezer to Incubation:*

<5 minutes

1. Retrieve from -80 deg. freezer
2. Thaw in a 37 deg. water bath
3. Add to Cofactor N vial
4. Add at equal volume to 2X test article
5. Incubate

Cryopreserved Hepatocytes

*Freezer to Incubation:*

>30 minutes

1. Retrieve from LN2 freezer
2. Thaw in a 37 deg. water bath
3. Add to recovery medium
4. Centrifuge
5. Microscopic quantification of viability and cell number
6. Adjust to 2X final cell density
7. Add at equal volume to 2X test article
8. Incubate
MetMax™ Human Hepatocytes

- **Advantages** over human liver S9 and microsomes: *Complete drug metabolism enzyme pathways*

- **Advantages** over intact human hepatocytes for application in drug metabolism studies: *Ease of use; robustness; maximized enzyme activities*
Comparison of MetMax™ and Intact Human Hepatocytes in Drug Metabolizing Enzyme Activities
## Evaluation of 16 Drug Metabolizing Enzyme Substrates

<table>
<thead>
<tr>
<th>Metabolic Pathway</th>
<th>Substrate</th>
<th>Substrate Conc. (µM)</th>
<th>Marker Metabolite</th>
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<td>CYP1A2</td>
<td>Phenacetin</td>
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<td>7-HC, 7-HC-Sulfate, 7-HC-Glucuronide</td>
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<td>Hydroxybupropion</td>
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<td>Dextrophan</td>
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<td>CYP2E1</td>
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<td>CYP3A4-1</td>
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<td>Carbazeran</td>
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<td>4-Hydroxycarbazeran</td>
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</table>
Comparison of 16 Drug Metabolizing Enzyme-Selective Substrates
Intact (PHH; blue) Vs MetMax™ (PHHX; red) Hepatocytes:

Activity (pmol/min/million hepatocytes)

Drug Metabolizing Enzyme Pathway

PHH

PHHX
Comparison of Intact and MetMax™ Human Hepatocytes in DME Activities

• MetMax™ human hepatocytes were similar or higher than intact human hepatocytes in the metabolism of 16 pathway-selective DME pathways

• Results suggest that MetMax™ human hepatocytes can be used for drug metabolism studies performed routinely with intact human hepatocytes
Metabolite Formation at Cytotoxic Drug Concentrations

Acetaminophen
Acetaminophen (APAP) Metabolism Scheme

ultimate hepatotoxicant, NAPQI

[Diagram showing the metabolism scheme of Acetaminophen, including N-hydroxylation by cytochrome P-450, dehydration, glutathione conjugation, and sulfation.]
Acetaminophen Metabolism at Nontoxic (10 mM) and Cytotoxic (100 and 200 mM) Concentrations

Acetaminophen Glucuronidation

Acetaminophen Sulfation

GSH Conjugation of NAPQI
MetMax™ Human Hepatocytes for Metabolite Profiling and Identification

• APAP was incubated at noncytotoxic (10 mM) and cytotoxic (100 and 200 mM) concentrations with intact and MetMax™ human hepatocytes
  – Similar metabolite profiles at the noncytotoxic (10 mM) concentration (glucuronide, sulfate, GSH conjugate)
  – MetMax™ >> intact human hepatocytes in metabolite formation in at cytotoxic concentrations (100 and 200 mM)

• Results suggest that MetMax™ Human Hepatocytes can be incubated with high, cytotoxic concentrations for the generation of metabolites
  – Advantage over intact hepatocytes
Protoxicant Activation Assay

Protoxicant (P) metabolized by MMHH to Toxic Metabolites (TM), causing cytotoxicity to target cells
Protoxicant Activation Assay

with MetMax™ Human Hepatocytes

- **Target cells:** HEK293, devoid of xenobiotic drug metabolism activities
- **Exogenous activation system:** MetMax™ human hepatocytes
- **Metabolic negative control:** Inactivated (boiled) MetMax™ human hepatocytes
- **Pro-toxicants:**
  - Acetaminophen
  - Cyclophosphamide
  - Ifosfamide
  - 2-Naphthylamine
MetMax™ Activation of Acetaminophen

Experiment 1:
0.5 million hepatocytes per mL

Experiment 2:
1.0 million hepatocytes per mL
MetMax™ Activation of Ifosfamide and Cyclophosphamide

**Ifosfamide**

- Without Hepatocytes
- With Boiled MetMax Hepatocytes
- With MetMax Hepatocytes

**Cyclophosphamide**

- Without Hepatocytes
- With Boiled MetMax Hepatocytes
- With MetMax Hepatocytes

Relative Viability vs. uM (microMolar)
MetMax™ Activation of 2-Naphthylamine
MetMax™ Human Hepatocytes
Activation of Prototoxicants

- Cytotoxicity of acetaminophen, cyclophosphamide, ifosfamide, and 2-naphthylamine towards HEK 293 cells were enhanced by MetMax™ human hepatocytes
- Activation was inactivated by boiling of the hepatocytes
- *Results suggest that MetMax™ human hepatocytes can be used as an exogenous activating system for the evaluation of prototoxicants*
MetMax™ Human Hepatocytes: *Cofactor-Directed Metabolic Pathway Selection*
Cofactor-Directed Pathway Selection

• Intact human hepatocytes allow metabolism of a drug by all hepatic pathways, selection of a specific metabolic pathway for evaluation is not easily accomplished

• *In MetMax™ hepatocytes, one can direct metabolism to specific pathways via selection of cofactor contents*
Cofactor-Directed Pathway Selection of Coumarin Metabolism with MetMax™ Human Hepatocytes

Cofactor Directed Pathway Selection of Coumarin Metabolism with MetMax™ Human Hepatocytes

- Cofactor: NADPH
- 7-OH Coumarin
- UDPGA
- PAPS
- Glucuronide
- Sulfate
Cofactor Selection and Expectations for Coumarin Metabolism by MetMax™ Human Hepatocytes

- No cofactors: no metabolism
- NADPH only: $7$-OH coumarin (7-HC) formation; no sulfation or glucuronidation
- NADPH + UDPGA: 7-HC and 7-HC-glucuronide; no 7-HC-sulfate
- NADPH + PAPS: 7-HC and 7-HC-sulfate; no glucuronide
Pathway Selection with Cofactors in MetMax™ Human Hepatocyte: Coumarin Metabolism

- 7-HC
- 7-HCS
- 7-HCG

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>No Cofactors</th>
<th>NADPH</th>
<th>+PAPS</th>
<th>+UDPGA</th>
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<tr>
<td>7-HCS</td>
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<tr>
<td>7-HCG</td>
<td></td>
<td></td>
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</tbody>
</table>

Activity (pmol/min/million cells)
Cofactor-Directed Coumarin Metabolism in MetMax™ Human Hepatocytes: 
Summary of Results

• No cofactors: no metabolism
• NADPH only
  – *Mainly 7-OH coumarin (7-HC) formation*;
  – minimum sulfation and glucuronidation
• NADPH + UDPGA:
  – *Mainly 7-HC and 7-HC-glucuronide*;
  – no 7-HC-sulfate
• NADPH + PAPS:
  – *Mainly 7-HC and 7-HC-sulfate*;
  – no glucuronide
MetMax™ Human Metabolism can be directed by specification of cofactor supplements
GSH Rescue Assay

- Reactive metabolite formation is a major mechanism of drug toxicity, especially in drug induced liver injuries (DILI)
- Individual differences in drug metabolizing enzyme activities may lead to individual differences in toxic responses
- Reactive metabolite formation is one of the hypothetical mechanisms for idiosyncratic DILI
- A physiologically-relevant in vitro screening assay for cytotoxic reactive metabolite formation would aid the elimination of drug candidates with hepatotoxic liability from further development
Scientific Rationale

• GSH represents the first line of cellular defense against the toxicity of reactive metabolites.

• Reduction of toxicity via increasing GSH contents would indicate that a toxicant would exhibit its toxicity via the formation of cytotoxic reactive metabolites.
Hypothesis

• GSH attenuation of cytotoxicity of protoxicants in the metabolically incompetent target cell line (HEK293) in the presence of MetMax™ human hepatocytes is an indication of the formation of cytotoxic reactive metabolites
Procedures

• 4 treatment groups:
  – 1. HEK 293 cells without metabolic activation in the absence of GSH;
  – 2. HEK 293 cells without metabolic activation in the presence of GSH;
  – 3. HEK 293 cells with metabolic activation in the absence of GSH; and 4.
  – 4. HEK 293 cells with metabolic activation in the presence of GSH.

• Treatment duration of 24 hrs.

• Cellular ATP as endpoint
Metabolic Activation by MMHH

Acetaminophen

Relative Viability (%)

Concentration (mM)

Cyclophosphamide

Relative Viability (%)

Concentration (mM)
GSH Rescue of Acetaminophen Cytotoxicity in the Presence of MMHH

Acetaminophen with MMHH

Acetaminophen without MMHH

Relative Viability (%) vs. Concentration (mM)
GSH Rescue of Cyclophosphamide Cytotoxicity in the Presence of MMHH

Cyclophosphamide with MMHH

Cyclophosphamide without MMHH
GSH Rescue of Cyclophosphamide Cytotoxicity in the Presence of MMHH
Summary and Conclusion

- Two protoxicants, acetaminophen and cyclophosphamide, that are known to form cytotoxic, reactive metabolites upon hepatic metabolism were evaluated for their cytotoxicity in HEK-263 cells in the presence and absence of GSH with and without exogenous metabolism by MetMax™ Human Hepatocytes
  - The cytotoxicity of both protoxicants was increased by MetMax Human Hepatocytes, confirming the formation of cytotoxic metabolites
  - GSH (20 mM) effectively eliminated the cytotoxicity of the protoxicants in the presence of metabolic activation, thereby confirming MMHH-mediated formation of cytotoxic reactive metabolites
  - GSH (20 mM) did not diminish the cytotoxicity of the protoxicants in the absence of metabolic activation, suggesting that the toxicity was likely due to the inherent cytotoxicity of the parent compounds

*MetMax™ Human Hepatocytes GSH-Rescue Assay can be used to identify xenobiotics with the potential to cause toxicity by forming cytotoxic reactive metabolites*
MetMax™ Hepatocyte/P450 Inhibitory Antibody Kit

Collaboration with Jerome Lasker, CYP450-GP
Identification of P450 Isoform in Drug Metabolism

• P450 isoform pathway identification and fm determination of each isoform in the metabolism of a drug is critical to DDI assessment

• Chemical inhibitors are notoriously non-specific

• Inhibitory antibodies are specific but cannot into hepatocytes

• Hypothesis: Inhibitory antibodies would be effective in MetMax™ Human Hepatocytes due to the permeabilized plasma membranes
Midazolam 1'-hydroxylation was assessed in incubation mixtures containing MetMax™ pooled human hepatocytes (0.5 x 10^5 cells), 20 µM midazolam, anti-CYP3A4 IgG, preimmune (control) IgG and hepatocyte/enterocyte incubation medium. The total amount of immune-specific IgG plus control IgG added was maintained at 75 µg. Hepatocytes were first preincubated with antibodies for 15 min at 37°C, followed by reaction with midazolam for 30 min at 37°C. Individual data points denote the average of triplicate determinations. 100% of control activity was 34.1 ± 0.2 pmol 1'-hydroxymidazolam formed/min/10^6 cells (n = 3). Additional details are given under Methods.
Specific Inhibition of CYP3A4 Activity by Anti-CYP3A4 Antibodies

MetMax™ pooled human hepatocytes (1 x 10⁵ cells) were preincubated with 0.1 mg of either anti-CYP3A4 IgG or preimmune IgG for 15 min at 37°C. Exemplary substrates oxidized by the enzymes shown above were then added to the incubation mixtures, and the reactions were allowed to proceed for an additional 30 min at 37°C. LC/MS-MS was used to determine the amount of specific metabolites formed. The substrates utilized are given under Methods. Enzymatic activities are expressed as % of control, which denote rates of metabolism observed in the presence of anti-CYP3A4 IgG versus those observed in the presence of preimmune IgG.
MetMax™ Human Hepatocytes

- Similar DME activities to intact hepatocytes
- Conjugated phase 1/phase 2 drug metabolism
- Successful application in hepatic clearance prediction
- Metabolite formation at cytotoxic drug concentrations
- Cofactor-mediated pathway selection
- Protoxicant activation
- Reactive cytotoxic metabolite identification
- Compatible with inhibitory P450 antibodies for pathway identification and fm determination
MetMax™ Human Hepatocytes

- Similar DME activities to intact hepatocytes
- Conjugated phase 1/phase 2 drug metabolism
- Successful application in hepatic clearance prediction
- Metabolite formation at cytotoxic drug concentrations
- Cofactor-mediated pathway selection
- Protoxicant activation
- Reactive cytotoxic metabolite identification
- Compatible with inhibitory P450 antibodies for pathway identification and fm determination
MetMax™ Human Hepatocytes and Drug Toxicity

• Identification of drugs that are metabolically activated
  – *Enhancement of cytotoxicity by MMHH*

• Identification of drugs forming cytotoxic reactive metabolites
  – *GSH rescue of cytotoxicity in the presence of MMHH*
Cryopreserved Human Enterocytes

Ho, M.C.D., Ring, N., Amaral, K., Doshi, U. and Li, A.P., 2017. Human Enterocytes as an In Vitro Model for the Evaluation of Intestinal Drug Metabolism: Characterization of Drug-Metabolizing Enzyme Activities of Cryopreserved Human Enterocytes from Twenty-Four Donors. Drug Metabolism and Disposition, 45(6), pp.686-691.
Why Enterocytes

• Key cell type for oral bioavailability
• First pass metabolism before the liver
  – Enteric metabolism is a key contributor to oral bioavailability
• Intestinal DDI with orally co-administered substances (foods; nutrient supplements; drugs)
  – Intestinal DDI may not occur in the liver due to lower hepatic exposure (e.g. grapefruit juice)
As of now, primary enterocytes are not commercially available for drug metabolism evaluation.

Current commercially available enterocytes are cultured for multiple passages with little information on drug metabolizing enzyme activities.
Isolation and cryopreservation of enterocytes from human small intestines
Cryopreservation of Human Enterocytes at IVAL

- Successful isolation and cryopreservation of enterocytes with high viability (≥75%) and reproducible yield (1-3 million cells per vial)
Gene Expression
Evaluation of Enterocytes
Cell Specific Marker Gene Expression

• Enterocytes
  – Sucrose Isomaltase (SI)
  – Maltase Glucoamylase (MAGM)

• Hepatocytes
  – Albumin (ALB)
# Cell-type Specific Markers

<table>
<thead>
<tr>
<th>Gene name</th>
<th>$2^{\Delta Ct}$ enterocytes</th>
<th>$2^{\Delta Ct}$ hepatocytes</th>
<th>Ratio of enterocyte to hepatocytes</th>
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</thead>
<tbody>
<tr>
<td>ALB*</td>
<td>ND</td>
<td>34.8693</td>
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<tr>
<td>SI**</td>
<td>48.6312</td>
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<tr>
<td>MAGM**</td>
<td>40.7226</td>
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<td>NA</td>
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</tbody>
</table>

*ALB (Albumin) is a marker gene for hepatocytes  
**SI (Sucrose Isomaltase) and MAGM (Maltase Glucoamylase) are marker genes for enterocytes
## CYP Isoforms

<table>
<thead>
<tr>
<th>Gene name</th>
<th>$2^{ΔCt}$ enterocytes</th>
<th>$2^{ΔCt}$ hepatocytes</th>
<th>Ratio of enterocyte to hepatocytes</th>
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<td>CYP1A2</td>
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<td>CYP2B6</td>
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<td>CYP3A4</td>
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<td>CYP2C8</td>
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Phase II DME

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## Transporters

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<th>$2^{\Delta C_T}_{\text{enterocytes}}$</th>
<th>$2^{\Delta C_T}_{\text{hepatocytes}}$</th>
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Human hepatocytes Lot: PHH8006 (pool hepatocytes); Human enterocytes Lot: HE3005.
Drug Metabolizing Enzyme Activities
## DME Activities of Human Enterocytes

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<th>Gender</th>
<th>Ethnicity</th>
<th>Age</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP3A4</th>
<th>UGT</th>
<th>SULT</th>
<th>2J2</th>
<th>CES2</th>
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<tbody>
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<td>4.32</td>
<td>7.78</td>
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<tr>
<td>HE3010</td>
<td>Male</td>
<td>C</td>
<td>47</td>
<td>1.21</td>
<td>0.62</td>
<td>0.46</td>
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</tr>
<tr>
<td>HE3011</td>
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<td>C</td>
<td>50</td>
<td>0.03</td>
<td>0.01</td>
<td>0.09</td>
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<td>1.70</td>
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<td>HE3013</td>
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<td>57</td>
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<td>0.2</td>
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<td>NA</td>
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<tr>
<td>HE3014</td>
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<td>49</td>
<td>0.44</td>
<td>0.11</td>
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<td>3.55</td>
<td>2.66</td>
<td>1.18</td>
<td>0.17</td>
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<tr>
<td>HE3015</td>
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<td>C</td>
<td>24</td>
<td>2.50</td>
<td>0.49</td>
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<td>32</td>
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<tr>
<td>HE3019</td>
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<td>C</td>
<td>61</td>
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<td>HE3020</td>
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<tr>
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<td>AA</td>
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<td>0.06</td>
<td>0.17</td>
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<td>1.64</td>
<td>0.19</td>
<td>0.08</td>
</tr>
<tr>
<td>HE3027</td>
<td>Female</td>
<td>C</td>
<td>53</td>
<td>2.02</td>
<td>0.31</td>
<td>0.7</td>
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<td>HE3028</td>
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<td>0.68</td>
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<td>0.82</td>
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<td>9.02</td>
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<tr>
<td>HE3029</td>
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<td>0.12</td>
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<td>6.55</td>
<td>3.65</td>
<td>0.76</td>
<td>0.08</td>
</tr>
<tr>
<td>HE3031</td>
<td>Female</td>
<td>C</td>
<td>49</td>
<td>0.34</td>
<td>0.09</td>
<td>0.16</td>
<td>1.60</td>
<td>0.79</td>
<td>0.49</td>
<td>0.18</td>
</tr>
</tbody>
</table>
New Enterocyte Development

• Preparation and characterization of pooled multiple-donor cryopreserved enterocytes

• *Preparation of MetMax™ Cryopreserved Pooled Human Enterocytes (patent pending)*
  – Permeabilized enterocytes supplemented with cofactors
  – Easy to use: Thaw and use – no centrifugation, no cell counting
  – Easy to store: -80 deg. Freezer (Liquid nitrogen not needed)
  – High activity
## Enhanced DME Activities of MetMax™ Pooled Human Enterocytes

<table>
<thead>
<tr>
<th>Metabolic Pathway</th>
<th>Substrate</th>
<th>Marker Metabolite</th>
<th>Pooled Enterocytes</th>
<th>MetMax Pooled Enterocytes</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>4-OH Diclofenac</td>
<td>4.05 ± 0.16</td>
<td>5.78 ± 1.13</td>
<td>142%</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin</td>
<td>4-OH S-Mephenytoin</td>
<td>0.55 ± 0.03</td>
<td>3.36 ± 0.32</td>
<td>610%</td>
</tr>
<tr>
<td>CYP3A4-1</td>
<td>Midazolam</td>
<td>1-OH-midazolam</td>
<td>1.21 ± 0.03</td>
<td>4.23 ± 1.22</td>
<td>349%</td>
</tr>
<tr>
<td>CYP3A4-2</td>
<td>Testosterone</td>
<td>6βOH-testosterone</td>
<td>10.6 ± 3.3</td>
<td>147 ± 14.5</td>
<td>1386%</td>
</tr>
<tr>
<td>UGT</td>
<td>7-OH-Coumarin</td>
<td>7-Hydroxycoumarin Glucuronide</td>
<td>16.05 ± 0.32</td>
<td>275 ± 79.5</td>
<td>1713%</td>
</tr>
<tr>
<td>SULT</td>
<td>7-OH-Coumarin</td>
<td>7-Hydroxycoumarin Sulfate</td>
<td>7.24 ± 0.34</td>
<td>13 ± 0.69</td>
<td>179%</td>
</tr>
<tr>
<td>2J2</td>
<td>Astemizole</td>
<td>O-Demethyl Astemizole</td>
<td>0.92 ± 0.43</td>
<td>5.14 ± 1.53</td>
<td>558%</td>
</tr>
<tr>
<td>CES2</td>
<td>Irinotecan</td>
<td>SN38</td>
<td>0.37 ± 0.14</td>
<td>0.38 ± 0.27</td>
<td>102%</td>
</tr>
</tbody>
</table>
MetMax™ Pooled Donor Human Enterocytes

• MetMax™ human enterocytes were prepared from Pooled Donor Human Enterocytes and evaluated for drug metabolizing activities for multiple pathways

• MetMax™ enterocytes were equal or more active than Pooled Donor Human Enterocytes in all pathways evaluated
Cryopreserved Intestinal Mucosal Epithelium
(patent pending)

3-dimensional organoid culture
Cryopreserved Intestinal Mucosal Epithelium

*patent pending*

3-dimensional organoid culture
Diagram of an Intestinal Villus of the Mucosa
Isolated Human Intestinal Mucosa
(multiple villi are shown)
CHIM as an *in vitro* experimental model for Enteric Drug Metabolism
## Drug Metabolism Activities (pmol/min/mg protein) of CHIM

<table>
<thead>
<tr>
<th>Enzyme Type</th>
<th>CHIM6001 (Duodenum)</th>
<th>CHIM6003 (Jejunum)</th>
<th>CHIM6005 (Ileum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>1.46</td>
<td>1.23</td>
<td>3.27</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>5.1</td>
<td>4.99</td>
<td>8.6</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>0.97</td>
<td>1.11</td>
<td>1.84</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>0.08</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>3.51</td>
<td>13.6</td>
<td>14.93</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>4.79</td>
<td>3.39</td>
<td>1.43</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>0.18</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>0.36</td>
<td>0.37</td>
<td>0.66</td>
</tr>
<tr>
<td>CYP3A4-1 (Midazolam)</td>
<td>11.27</td>
<td>18.6</td>
<td>8.4</td>
</tr>
<tr>
<td>CYP3A4-2 (Testosterone)</td>
<td>146.77</td>
<td>195.83</td>
<td>99.43</td>
</tr>
<tr>
<td>ECOD</td>
<td>0.74</td>
<td>5.75</td>
<td>1.56</td>
</tr>
<tr>
<td>UGT (7HC)</td>
<td>6.88</td>
<td>8.6</td>
<td>22.19</td>
</tr>
<tr>
<td>SULT (7HC)</td>
<td>0.07</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>GST (APAP)</td>
<td>0.15</td>
<td>0.25</td>
<td>0.69</td>
</tr>
<tr>
<td>UGT (APAP)</td>
<td>1.07</td>
<td>1</td>
<td>7.41</td>
</tr>
<tr>
<td>SULT (APAP)</td>
<td>4.39</td>
<td>5.31</td>
<td>9.71</td>
</tr>
<tr>
<td>FMO</td>
<td>7.06</td>
<td>6.02</td>
<td>8.87</td>
</tr>
<tr>
<td>MAO</td>
<td>303.64</td>
<td>480.99</td>
<td>740.74</td>
</tr>
<tr>
<td>AO</td>
<td>0.03</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>NAT1</td>
<td>5.45</td>
<td>10.87</td>
<td>36.61</td>
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<tr>
<td>NAT2</td>
<td>3.38</td>
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<td>5.6</td>
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<tr>
<td>2J2</td>
<td>3.49</td>
<td>2.71</td>
<td>5.73</td>
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<tr>
<td>CES2</td>
<td>1.63</td>
<td>1.22</td>
<td>2.56</td>
</tr>
</tbody>
</table>
Time-Dependent Metabolism

CYP3A4 Activity of Cryopreserved Human Intestinal Mucosa

Duration of Metabolism (hours)
CHIM as an in vitro experimental model for Enteric P450 Induction
Importance of Enteric P450 induction

• Vitamin D Receptor (VDR): regulator of enteric transporters and drug metabolism enzymes
  – Transporters: P-gp, MRP2, MRP4, OATP1A2, renal OAT3
  – DME: SULT2A1, and CYP3A4, CYP24A1

• CYP3A4: Key contributor to oral bioavailability of highly permeable drugs
Vitamin D Induction of P450 Gene Expression in CHIM (Duodenum; 24 hr treatment)
Rifampin Induction of CYP3A4 Gene Expression in CHIM (Duodenum; 24 hr treatment)
CHIM as an in vitro experimental model for
Enterotoxicity
Acetaminophen Enterotoxicity in CHIM (4 hr treatment)
Naproxen Enterotoxicity in CHIM (4 hr treatment)
Acetaminophen (APAP) and Naproxen Enterotoxicity in Cryopreserved Human Intestinal Mucosa from Three Donors: IC50 Values (mg/mL)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Duodenum (Donor 1)</th>
<th>Jejunum (Donor 1)</th>
<th>Duo+Juj+Ile (Donor 2)</th>
<th>Duodenum (Donor 3)</th>
<th>Jejunum (Donor 3)</th>
<th>Ileum (Donor 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APAP</td>
<td>0.90</td>
<td>0.64</td>
<td>1.17</td>
<td>1.18</td>
<td>0.99</td>
<td>0.92</td>
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<tr>
<td>Naproxen</td>
<td>0.53</td>
<td>0.20</td>
<td>0.48</td>
<td>0.35</td>
<td>0.39</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Human Enterocytes

- Successful isolation and cryopreservation of enterocytes and intestinal mucosa from duodenum, jejunum, and ileum
  - Retention of drug metabolizing enzyme activities
- MetMax™ cryopreserved human enterocytes
  - Enhanced drug metabolizing enzyme activities
- Enteric food drug interaction
  - Green tea extract, grapefruit juice as potent inhibitors of enteric CYP3A4 activity
- Induction of P450 in CHIM
  - CYP24A1 induction by vitamin D3
  - CYP3A4 induction by rifampin and vitamin D3
- Successful application of cryopreserved human intestinal mucosa in the evaluation of enterotoxicity
  - Naproxen > APAP in enterotoxicity
Integrated Discrete Multiple Organ Co-culture (IdMOC™)

Patent allowed in U. S., Japan, Korea, China, EU countries
IdMOC™
Integrated
discrete
Multiple
Organ
Culture

Inner well
Outer well
IdMOC: A practical co-culture system

• Wells-in-a well concept allowing co-culturing of multiple cell types as physically separated (discrete) entities but interconnected (integrated) by a common overlying medium
  – Specially manufactured plates with shallow inner wells to minimize dilution
  – Identical foot-prints as regular 24- and 96-well plates, thereby compatible with routinely used lab equipment such as multichannel pipettes and plate readers
Key IdMOC Publications

• Li AP, Bode C, Sakai Y. A novel in vitro system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast cancer cells. Chem Biol Interact. 2004; 150:129-36.


• Li, AP, LaForge, Y, Uzgare A. Definition of metabolism-dependent drug toxicity with the novel integrated discrete multiple organ co-culture: Results with model toxicants tamoxifen, aflatoxin B1 and cyclophosphamide. Chem Biol Interact 2012; 199(1): 1–8
IdMOC: Demonstration of Exogenous Hepatic Metabolism

3T3/Human Hepatocyte IdMOC
IdMOC with 3T3 cells (metabolically incompetent) and human hepatocytes (metabolically competent)

- Medium (no cells)
- 3T3 cells (metabolically incompetent)
- Human hepatocytes (metabolically competent)
P450 CYP3A Activity (Luciferin-IPA Metabolism) in IdMOC with 3T3/Hepatocytes: Effects of Varying Number of Wells with Human Hepatocytes

![Graph showing P450 CYP3A Activity](image-url)
IdMOC: Demonstration of Metabolism-dependent Toxicity

*Cyclophosphamide cytotoxicity* towards 3T3 cells in 3T3/Human Hepatocyte Co-culture
IdMOC with metabolically incompetent (3T3) and competent cells (human hepatocytes)

- **Medium (no cells)**
- **3T3 cells (metabolically incompetent)**
- **Human hepatocytes (metabolically competent)**
Cyclophosphamide Cytotoxicity to 3T3 Cells in IdMOC: Effects of Varying Number of Wells with Human Hepatocytes

Plate 1

- No Hepatocytes
- 1 Hepatocyte (1 well)
- 2 Hepatocytes (2 wells)
- 3 Hepatocytes (3 wells)

Plate 2

- No hepatocytes
- with hepatocytes (1 well)
- With hepatocytes (2 wells)
- With hepatocytes (3 wells)

Plate 3

- No hepatocytes
- with hepatocytes (1 well)
- With hepatocytes (2 wells)
- With hepatocytes (3 wells)
IdMOC 3T3/Hepatocytes:
MTT Metabolism after Cyclophosphamide Treatment
IdMOC 3T3/Hepatocytes:
MTT Metabolism after Cyclophosphamide Treatment

Cyclophosphamide

0 mM  2.5 mM  5 mM  10 mM

No Hep 1 Hep 2 Hep 3 Hep
Application of IdMOC for Metabolism-based Classification of Cytotoxicity
Significance of Metabolism-based Classification

- **Type I: Direct-acting (Tamoxifen):** Organ-specific toxicity dependent of route of exposure to parent toxicant

- **Type II: Metabolically activated, localized toxicity (Aflatoxin-B1):** Organ-specific toxicity dependent on site of metabolic activation

- **Type III: Metabolically activated, diffusible toxic metabolites (Cyclophosphamide):** Organ-specific toxicity dependent on metabolite distribution
Next Generation IdMOC
Multiple organs with key cell types of each major organ
Parallelogram Approach for the Preclinical Assessment of Clinical Hepatotoxicity

Human Hepatotoxicity in vivo

Extrapolation

PBPK
Genetic polymorphism
Risk Factors

Animal Hepatotoxicity in vivo

Animal Species Selection

Human hepatocytes in vitro

Species Comparison

Animal Hepatocytes in vitro
Parallelogram Approach for the Preclinical Assessment of Clinical Toxicity of Nonhepatic Organs

Human Enterotoxicity in vivo

Extrapolation

PBPK
Genetic polymorphism
Risk Factors

Human CHIM

Species Comparison

Animal CIM

Extrapolation

Animal Toxicity in vivo

Animal Species Selection
Parallelogram Approach for the Preclinical Assessment of Clinical Toxicity of Multiple Organs

Human Multiple Organ Toxicity in vivo

Extrapolation

PBPK
Genetic polymorphism
Risk Factors

Human IdMOC

Animal Toxicity in vivo

Animal Species Selection

Species Comparison

Animal IdMOC