Novel Human-Based In Vitro Liver and Intestinal Technologies for Drug Development

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lialbert@invitroadmet.com
Cost and Time of a Successful New Drug

• > 1 billion USD
• >10 years
Reason for High Cost of Drug Development:

*Clinical Trial Failure*

Source: Kola and Landis (2004)- Nature Reviews/Drug Discovery

Overall success rate
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
Species-differences in TCDD toxicity
(Kleeman et al., 1988)
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)

Human \textit{in vitro}

Animals \textit{in vivo}

Li AP (2007). \textit{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

**PBPK**

**Genetic polymorphism**

**Risk Factors**

**Animal Species Selection**

**Species Comparison**
Human *in vitro* experimental systems are essential for drug development.
In Vitro ADMET Laboratories Inc.

• 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*
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
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 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)
IVAL Expertise: 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
999-Elite™
Fully Functional Cryopreserved Human Hepatocytes for 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 Human Hepatocytes Uptake Assay:
Time-dependent uptake inhibited by rifampicin

Collaboration with Yuichi Sugiyama (Riken)

Substrate: PTV (0.1 μM), RSV (0.1 μM), CRV (0.1 μM), PRV (2 μM)
Temperature: 37°C
Time point: 0.25, 1.25, 2.5, 5, 15, 45, 90 min
Lot: HH1103

![Graphs showing time-dependent uptake inhibition by rifampicin for different substrates. CRV, PTV, RSV, PRV with control and 100 μM Rifampicin conditions.]
# P450 Induction (Gene Expression)

## CYP1A2 induction fold

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HH1121 Mean</th>
<th>HH1121 SD</th>
<th>HH1142 Mean</th>
<th>HH1142 SD</th>
<th>HH1144 Mean</th>
<th>HH1144 SD</th>
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<tbody>
<tr>
<td>Omeprazole (µM)</td>
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<tr>
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## CYP2B6 induction fold

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<th>HH1121 SD</th>
<th>HH1142 Mean</th>
<th>HH1142 SD</th>
<th>HH1144 Mean</th>
<th>HH1144 SD</th>
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<tbody>
<tr>
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<tr>
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<tr>
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## CYP3A4 induction fold

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<th>HH1142 SD</th>
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<tbody>
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## Human Hepatocyte CYP2C Induction

### Experiment 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP2C8 induction fold</th>
<th>CYP2C9 induction fold</th>
<th>CYP2C19 induction fold</th>
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<td>HH1121</td>
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<tr>
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### Experiment 2

<table>
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<tr>
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<th>CYP2B6 induction fold</th>
<th>CYP3A4 induction fold</th>
<th>CYP2C8 induction fold</th>
<th>CYP2C9 induction fold</th>
<th>CYP2C19 induction fold</th>
<th>UGT1A1 induction fold</th>
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<td>0.52</td>
<td>3.04</td>
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<td>Rifampin</td>
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<td>3.52</td>
<td>0.07</td>
<td>2.84</td>
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<tr>
<td>Phenobarbital</td>
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<td>0.43</td>
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<td>Phenobarbital</td>
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<td>0.13</td>
<td>3.67</td>
<td>0.25</td>
<td>3.33</td>
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</table>
Novel Technologies
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.
MetMax™ Hepatocytes

(Patent Pending)


Drug Metabolism and Disposition, pp.dmd-117.
Suspension Cryopreserved Human Hepatocytes
Cryopreserved Human Hepatocytes in Drug Development

• **Suspension:**
  – *Easier to use than plateable cryopreserved hepatocytes*
  – **Major application:**
    • drug metabolism (metabolic stability screening)
    • Drug-drug interactions (P450 inhibition but mainly done with HLM)
    • Uptake transport (now mainly done with plateable hepatocytes)
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.
## MetMax™ Hepatocytes: Retention of All Organelles

<table>
<thead>
<tr>
<th>Organelles</th>
<th>MetMax™</th>
<th>Intact Hepatocytes</th>
<th>Microsomes</th>
<th>S9</th>
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<tr>
<td>Mitochondria</td>
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<td>Lysosomes</td>
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<td>Golgi</td>
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<td>Plasma Membranes</td>
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<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>😊</td>
<td>😊</td>
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## Ease of use of MetMax™ Hepatocytes

<table>
<thead>
<tr>
<th></th>
<th>MetMax™</th>
<th>Intact Hepatocytes</th>
<th>Microsomes</th>
<th>S9</th>
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<td>Yes</td>
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<tr>
<td>Thaw and Use</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 at equal volume to 2X test article
4. 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*
MetMax™ Human Hepatocytes

Characterization of Drug Metabolizing Enzyme Activities
Characterization of MetMax™ Human Hepatocytes

Pooled Donor Human Hepatocytes (PHS9001) vs.

MetMax™ Pooled Human Hepatocytes (PHHX8011; derived from PHS9001)
Cell concentration vs. activity
Linear P450 Activity vs. Cell Concentration in MetMax Human Hepatocytes

- CYP1A2
- CYP2B6
- CYP2C9
- CYP2C19
- CYP2D6
- CYP2E1
- CYP3A4 (Midazolam)
- CYP3A4 (Testosterone)
Activity vs. Cell Concentration

• Linear activity vs. cell concentration using cell concentrations of 0.25, 0.5, 1.0, and 2 million cells per mL
  – 1 million cells per mL chosen as the cell concentration for subsequent characterization
Time Course:
(1 million cells/mL)
**Time Course: P450 Isoform-Selective Substrate Metabolism**

**Human Hepatocytes (PHS9001; Blue) vs. MetMax Human Hepatocytes (PHHX8011; Red)**

**CYP1A2**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2A6**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2B6**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2C8**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2C9**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2C19**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2D6**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2E1**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP2J2**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP3A4-Testosterone**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**CYP3A4-Midazolam**

- 0
- 20
- 40
- 60
- 30
- 60
- 120
- 240

**Incubation Duration (minutes)**
Time Course: Non-P450 Pathway-Selective Substrate Metabolism

Human Hepatocytes (PHS9001; Blue) vs.
MetMax Human Hepatocytes (PHHX8011; Red)
Time Course

• Linear time-course for most drug metabolism enzyme pathways at incubation times of 30, 60, 120, and 240 minutes

• Similar trend of metabolite formation versus time for intact and MetMax™ human hepatocytes

• MetMax™ hepatocytes similar or higher than intact hepatocytes in rates of metabolite formation
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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<tbody>
<tr>
<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>CYP2B6</td>
<td>Buproprion</td>
<td>500</td>
<td>Hydroxybuproprion</td>
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<td>CYP2C8</td>
<td>Paclitaxel (Taxol)</td>
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<td>6α-hydroxypaclitaxel</td>
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<td>25</td>
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<td>1-Hydroxymidazolam</td>
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<td>Carbazeran</td>
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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

CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4-1, CYP3A4-2, ECOD, UGT, SULT, FMO, MAO, AO
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**
MetMax™ Human Hepatocytes as Exogenous Metabolic Activation System for the Evaluation of Pro-toxicants

SOT 2018 Late Breaking Poster 3365
Thursday, March 15, P147
Metabolite Formation at Cytotoxic Drug Concentrations

Acetaminophen
Acetaminophen (APAP) Metabolism Scheme

*ultimate hepatotoxicant, NAPQI*

- **Acetaminophen**
  - $N$-hydroxylation by cytochrome P-450
  - Dehydration

- **NAPQI**
  - Glutathione conjugation
  - Conjugation with proteins, nucleic acids

- **Glucuronidation**
  - Sulfation

- Toxicity
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 (T), 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
Acetaminophen (APAP) Metabolism Scheme

*ultimate hepatotoxicant, NAPQI*

-N-hydroxylation by cytochrome P-450

-glucuronidation

-sulfation

-dehydration

-glutathione conjugation

-NAPQI conjugation with proteins, nucleic acids

-toxicity
MetMax™ Activation of Acetaminophen

Experiment 1:
0.5 million hepatocytes per mL

Experiment 2:
1.0 million hepatocytes per mL
Pro-toxicant Activation: Cyclophosphamide

Cyclophosphamide

M = (ClCH₂CH₂)₂N⁻

4-Hydroxycyclophosphamide

4-Ketocyclophosphamide

Carboxyphosphamide

Phosphoramidemustard

Aldehydedehydrogenase

Aldophosphamide

Nonenzymatic

Acrolein

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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
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

1. Coumarin
2. NADPH
3. UDPGA
4. PAPS
5. 7-OH Coumarin
6. Glucuronide
7. 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

![Graph showing the effect of cofactors on metabolite activity](graph.png)
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 prototoxicants in the metabolically incompetent target cell line (HEK263) 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**

- **Without MMHH**
- **With MMHH**

**Cyclophosphamide**

- **Relative Viability (%)**
- **Concentration (mM)**
- **0** 3.12 6.25 12.5 25 50
- **0** 3.12 6.25 12.5 25 50
GSH Rescue of Acetaminophen Cytotoxicity in the Presence of MMHH

![Graph showing relative viability of Acetaminophen with and without MMHH and GSH.](image)
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™ 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
MetMax™ Human Hepatocytes and Drug Toxicity

• Identification of drugs that are metabolically activate
  – 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)
## DME Activities of Human Enterocytes

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<tr>
<th>Lot Number</th>
<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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<td>0.79</td>
<td>0.49</td>
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</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
Donors Used for Pooling

10 donors (5 female; 5 male)
20 million cells per donor

<table>
<thead>
<tr>
<th>Lot No.</th>
<th>Gender</th>
<th>Race</th>
<th>Age (Years)</th>
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Drug Metabolizing Enzyme Activities of Pooled Cryopreserved Human Enterocytes
# Enhanced DME Activities of MetMax™ Pooled Human Enterocytes

Here is the table for metabolite activity:

<table>
<thead>
<tr>
<th>Metabolic Pathway</th>
<th>Substrate</th>
<th>Marker Metabolite</th>
<th>Pooled Enterocytes (pmol/10⁶/min)</th>
<th>MetMax Pooled Enterocytes (pmol/10⁶/min)</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>
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<td>142%</td>
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<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>
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<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>
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<tr>
<td>CYP3A4-2</td>
<td>Testosterone</td>
<td>6βOH-testosterone</td>
<td>10.6 ± 3.3</td>
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<td>UGT</td>
<td>7-OH-Coumarin</td>
<td>7-Hydroxycoumarin Glucuronide</td>
<td>16.05 ± 0.32</td>
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<td>SULT</td>
<td>7-OH-Coumarin</td>
<td>7-Hydroxycoumarin Sulfate</td>
<td>7.24 ± 0.34</td>
<td>13 ± 0.69</td>
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<td>2J2</td>
<td>Astemizole</td>
<td>O-Demethyl Astemizole</td>
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<td>5.14 ± 1.53</td>
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<tr>
<td>CES2</td>
<td>Irinotecan</td>
<td>SN38</td>
<td>0.37 ± 0.14</td>
<td>0.38 ± 0.27</td>
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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
Enteric Food-Drug Interactions
Grapefruit Juice

• Clinically significant DDI with orally administered drugs
  – Inhibition of intestinal CYP3A4
  – Inhibition of P-gp efflux
  – Minimal hepatic effects
384-well HTS Enteric Herb-Drug Interaction Assay with MetMax™ Enterocytes

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<th>3.12%</th>
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### 384-well HTS Enteric Herb-Drug Interaction Assay with MetMax™ Enterocytes

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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 of Isolated Human Intestinal Mucosa

CYP3A4 Activity of Cryopreserved Human Intestinal Mucosa

![Graph showing CYP3A4 activity over time.]

- CYP3A4 Activity (luminescence units)
- Duration of Metabolism (hours)
<table>
<thead>
<tr>
<th></th>
<th>CHIM6001 (Duodenum)</th>
<th>CHIM6003 (Jejunum)</th>
<th>CHIM6005 (Ileum)</th>
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<td>CYP2E1</td>
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<td>CYP3A4-1 (Midazolam)</td>
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<td>CYP3A4-2 (Testosterone)</td>
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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)

CYP3A4 Induction by 1,25(OH)2D3

CYP24A1 Induction by 1,25(OH)2D3
Rifampin Induction of CYP3A4 Gene Expression in CHIM (Duodenum; 24 hr treatment)
## Time-Course of P450 Induction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP3A4 Induction fold (normalized to each day)</th>
<th>CYP3A4 Induction fold (normalized to day 1)</th>
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<td></td>
<td>Day1</td>
<td>Day2</td>
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<tr>
<td>Vitamin D3</td>
<td>(nM)</td>
<td>Mean</td>
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<td>0.32</td>
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<tr>
<td>0.41</td>
<td>2.46</td>
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<th>CYP24A1 Induction fold (normalized to each day)</th>
<th>CYP24A1 Induction fold (normalized to day 1)</th>
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<td>Day1</td>
<td>Day2</td>
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<tr>
<td>(nM)</td>
<td>Mean</td>
<td>SD</td>
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<td>0.41</td>
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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)

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<tr>
<th>Drug</th>
<th>Duodenum (Donor 1)</th>
<th>Jejunum (Donor 1)</th>
<th>Duo+Juj+Ile (Donor 2)</th>
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<th>Jejunum (Donor 3)</th>
<th>Ileum (Donor 3)</th>
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<td>APAP</td>
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<td>Naproxen</td>
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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
In Vitro Liver and Intestine Models

• Liver
  – Fully functional plateable cryopreserved human hepatocytes (*999Elite™*)
  – MetMax™ cryopreserved hepatocytes

• Intestine
  – Cryopreserved enterocytes
  – MetMax™ cryopreserved enterocytes
  – Cryopreserved intestinal mucosa
Human In Vitro Experimental Systems:

*Critical Tools for Preclinical Assessment of Clinical Drug Properties*

- Human *in vivo*
- Animal *in vivo*
- Human *in vitro*
- Animal *in vitro*

**IVIVC**
- PBPK
- Genetic polymorphism
- Risk Factors

**Extrapolation**
- Species Comparison
- Animal Species Selection
Happy Hepatocytes
Happy Enterocytes
Happy Scientists!

Please join our 2016 IVAL Hepatocyte/Enterocyte Workshop and Symposium in Boston
Contact Information

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- Nozomi Mizuno, Japanese Sales and Marketing: n.mizuno@genomembrane.com
Thank you for your attention