Novel In Vitro ADMET Hepatic and Enteric Technologies for Drug Metabolism, Drug-drug Interactions, and Enterotoxicity

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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): Analytical Chemistry
- Qian Yang (previously NCI): Molecular Biology
- Ivy Wei (previously NIH): Cell Biology
- Walter Mitchell (previously Pfizer, Groton): Analytical Chemistry
- Novera Alam (previously Tuft’s): Cell Biology/Analytical Chemistry
- Carol Loretz (previously University of Washington, Seattle): Cell Biology
- Kirsten Amaral (previously Life Technologies, RTP): Cell Biology
- Albert P. Li (previously Monsanto/G. D. Searle)
IVAL Focus: Liver and Intestine

Enterocytes: First-pass metabolism of orally-administered drugs
Hepatocytes: First-pass metabolism of absorbed orally-administered drugs
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, inhibition, time-dependent inhibition)

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

**Pharmacology**
- Gene expression modification
- Inhibition of hepatitis viral replication
- NASH
- Cholesterol synthesis
Long-term (>30 days) Culturing of 10 Donor Pooled 999Elite™ Human Hepatocytes

4 hrs  
5 Days  
14 Days  
31 days

Applications:

• DMPK
  • In vitro modeling of clinical P450 Induction
  • metabolism of low turn over compounds

• Toxicology
  • Chronic toxicity

• Pharmacology
  • Stability of gene modification (suppression; expression)
  • Liver disease (hepatis viral propagation)
Novel Technologies
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*
# Ease of use of MetMax™ Hepatocytes

<table>
<thead>
<tr>
<th>Organelles</th>
<th>MetMax™</th>
<th>Intact Hepatocytes</th>
<th>Microsomes</th>
<th>S9</th>
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<tbody>
<tr>
<td>Storage</td>
<td>-80 deg. C</td>
<td>LN2</td>
<td>-80 deg. C</td>
<td>-80 deg. C</td>
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<tr>
<td>Centrifugation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Microscopic Examination</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Cell Counting</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cofactor Addition</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Thaw and Use</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
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</tbody>
</table>
Comparison of 16 Drug Metabolizing Enzyme-Selective Substrates Intact (PHH; blue) Vs MetMax™ (PHHX; red) Hepatocytes:
Cryopreserved Human Intestinal Mucosa (CHIM™; Patent Pending)

Diagram of an Intestinal Villus of the Mucosa
Collagenase Digestion

Gentle Homogenization

Intestinal Villi

Cryopreservation
Time-Dependent Metabolism

CYP3A4 Activity of Cryopreserved Human Intestinal Mucosa

CYP3A4 Activity (luminescence units)

Duration of Metabolism (hours)
Evaluation of Multiple Drug Metabolizing Enzyme Activities
<table>
<thead>
<tr>
<th>Metabolic Pathway</th>
<th>Substrate</th>
<th>Substrate Conc. (µM)</th>
<th>Marker Metabolite</th>
<th>Activity (pmol/min/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ave</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>7-Ethoxyresozufin</td>
<td>20</td>
<td>Resozufin</td>
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<tr>
<td>CYP1A2</td>
<td>Phenacetin</td>
<td>100</td>
<td>Acetaminophen</td>
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<tr>
<td>CYP2A6</td>
<td>Coumarin</td>
<td>50</td>
<td>7-HC, 7-HC-Sulfate, 7-HC-Glucuronide</td>
<td>0.03</td>
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<tr>
<td>CYP2B6</td>
<td>Bupropion</td>
<td>500</td>
<td>Hydroxybupropion</td>
<td>1.99</td>
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<tr>
<td>CYP2C8</td>
<td>Paclitaxel (Taxol)</td>
<td>20</td>
<td>6α-hydroxypaclitaxel</td>
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<tr>
<td>CYP2C9</td>
<td>Diclofenac</td>
<td>25</td>
<td>4-OH Diclofenan</td>
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<tr>
<td>CYP2C19</td>
<td>S-Mephenytoin</td>
<td>250</td>
<td>4-OH S-Mephenytoin</td>
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<tr>
<td>CYP2D6</td>
<td>Dextromethorphan</td>
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<td>Dextrophan</td>
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<tr>
<td>CYP2E1</td>
<td>Chlorozoxzone</td>
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<td>6-OH Chlorozoxane</td>
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<td>CYP3A4-1</td>
<td>Midazolam</td>
<td>20</td>
<td>1-Hydroxymidazolam</td>
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<td>CYP3A4-2</td>
<td>Testosterone</td>
<td>200</td>
<td>6β-hydroxytestosterone</td>
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<tr>
<td>ECOD</td>
<td>7-Ethoxycoumarin</td>
<td>100</td>
<td>7-HC, 7-HC-Sulfate, 7-HC-Glucuronide</td>
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<tr>
<td></td>
<td>7-Ethoxycoumarin</td>
<td>100</td>
<td>7-OH Coumarin</td>
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<tr>
<td>UGT</td>
<td>7-Hydroxycoumarin</td>
<td>100</td>
<td>7-Hydroxycoumarin Glucuronide</td>
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<td>SULT</td>
<td>7-Hydroxycoumarin</td>
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<td>7-Hydroxycoumarin Sulfate</td>
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<td>GST</td>
<td>Acetaminophen</td>
<td>10 mM</td>
<td>Acetaminophen Glutathione</td>
<td>0.50</td>
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<tr>
<td>UGT</td>
<td>Acetaminophen</td>
<td>10 mM</td>
<td>Acetaminophen Glucuronide</td>
<td>5.72</td>
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<tr>
<td>SULT</td>
<td>Acetaminophen</td>
<td>10 mM</td>
<td>Acetaminophen Sulfate</td>
<td>35.07</td>
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<tr>
<td>FMO</td>
<td>Benzydamine HCl</td>
<td>250</td>
<td>Benzydamine-N-Oxide</td>
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<tr>
<td>MAO</td>
<td>Kynuramine HBr</td>
<td>160</td>
<td>4-hydroxyquinoline</td>
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<td>AO</td>
<td>Cabazeran</td>
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<td>4-Hydroxycabazeran</td>
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<tr>
<td>NAT1</td>
<td>4-Aminobenzoic Acid</td>
<td>200</td>
<td>N-Acetyl-p-aminobenzoic acid</td>
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<td>NAT2</td>
<td>Sulfamethazine</td>
<td>100</td>
<td>N-Acetyl-sulfamethazine</td>
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<td>ZJ2</td>
<td>Astemizole</td>
<td>50</td>
<td>O-Demethyl Astemizole</td>
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<tr>
<td>CES2</td>
<td>Irinotecan</td>
<td>50</td>
<td>SN38</td>
<td>3.78</td>
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</table>
**Human Enteric Experimental Models**

- Successful isolation and cryopreservation of enterocytes duodenum, jejunum, and ileum
  - Retention of drug metabolizing enzyme activities
  - DME composition different from hepatocytes
    - Similar CYP3A4, 2C19, NAT-1, and CES-2 activities as hepatocytes
- MetMax™ cryopreserved human enterocytes
  - Enhanced drug metabolizing enzyme activities
- Cryopreserved human intestinal mucosa (CHIM) – Most “complete” in vitro enteric systems
  - Potent drug metabolizing enzyme activities
  - Regional differences in DME activities
  - Application in the evaluation of enteric food drug interaction
    - Herbal supplements as potent inhibitors of enteric CYP3A4 activity
    - Induction of P450 in CHIM
      - CYP24A1 induction by vitamin D3
      - CYP3A4 induction by rifampin and vitamin D3
    - Applications in the evaluation of enterotoxicity
      - Naproxen > APAP in enterotoxicity, similar to clinical findings
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